2-16-2018 10:30 AM

Functional and Structural Mimicry of A-Kinase Anchoring Proteins by Human Adenovirus E1A

Cason R. King, *The University of Western Ontario*

Supervisor: Mymryk, Joe S, *The University of Western Ontario*

A thesis submitted in partial fulfillment of the requirements for the Doctor of Philosophy degree in Microbiology and Immunology

Abstract

As an obligate intracellular parasite, human adenovirus (HAdV) must utilize host factors for survival and replication. Early during infection, its multifunctional E1A protein interacts with an impressive range of cellular target proteins to exert control over the cellular environment. Through these virus-host interactions, E1A massively reprograms both viral and cellular transcription to activate the other HAdV genes, downregulate the host's immune response, and induce the cell cycle. Consequently, E1A converts the infected cell into a compliant state more amenable for HAdV replication, resulting from its numerous protein-protein interactions. I sought to examine E1A's interaction with cellular protein kinase A (PKA), a well-studied component of host cAMP signaling that plays a critical role in cellular transcription. While characterizing the molecular determinants of the E1A-PKA interaction, I showed that E1A uses mimicry of cellular A-kinase anchoring proteins (AKAPs) to usurp PKA for the benefit of HAdV. This mechanism allowed E1A to outcompete endogenous AKAPs and induce an E1A-mediated relocalization of PKA into the nucleus of infected cells. Here, PKA was recruited to viral gene promoters. This ultimately resulted in increased viral transcription from these loci as well as enhanced protein synthesis and higher levels of viral replication. This mechanism of mimicry was conserved in E1A proteins across various, distinct species of HAdV, indicating its evolutionary importance. Interestingly, different E1A proteins displayed a preference for manipulating one type of PKA isoform over another. Despite mechanistic differences, all species of HAdV that commandeered PKA via their E1A proteins required this action for wild-type levels of both viral genome replication and infectious progeny production. Additionally, these E1A proteins could induce transcription from a cAMP- and PKA-regulated reporter gene suggesting that this interaction may modulate host genes in a manner similar to viral ones. Together, these studies show that manipulation of PKA by E1A is a conserved feature of diverse HAdVs. This allows these viruses to subvert cellular cAMP signaling and use a host factor to enhance multiple aspects of their replication cycles. Furthermore, this mechanistically demonstrates HAdV E1A as the first known viral AKAP – a unique form of viral mimicry.

Keywords

Human adenovirus, HAdV, Early region 1A, E1A, Protein kinase A, PKA, A-kinase anchoring protein, AKAP, Cyclic AMP, cAMP, Viral mimicry, Viral AKAP, Protein-protein interaction, Gene expression, Subcellular localization, Transcription, Viral replication, Oncoprotein, Hub protein

Co-Authorship Statement

Sections of Chapter 1 of this thesis were published in Trends in Microbiology, 2016; 24:323–4 or submitted to mBio (mBio00390-18). I was the primary author of both these manuscripts.

Chapter 2 of this thesis was published in PLoS Pathogens, 2016; 12:e1005621. I performed all the experiments with the exception of the molecular modelling in Figures 2.3A–G and 2.4 which was carried out by Brennan Dirk in the laboratory of Dr. Jimmy Dikeakos.

Chapter 3 of this thesis was published in Journal of Virology, 2018; 92(8):e01902-17. I was the primary author and performed all the experiments.

Chapter 4 of this thesis contains unpublished data. The RNAseq experiment and initial analysis was carried out by Drs. Mike Cohen and Greg Fonseca. I performed all subsequent data analyses described in this section.

Acknowledgments

I am faced with the daunting task of succinctly thanking a massive number of terrific people without whom none of this would have been possible. Rest assured the names that appear below deserve far more gratitude from me than a few pages could convey.

First and foremost I am immensely thankful for my boss, mentor, and friend, Dr. Joe Mymryk. Years ago he sparked my interest in virology research by being a charismatic and approachable undergraduate lecturer. From there, my journey through science has been guided by his incredible knowledge, brilliant ideas, and wonderful personality. I am truly lucky to have spent my formative scientific years in his lab learning not only the ins-and-outs of molecular biology, but also the finer points of what makes a truly good person. The selfless way Joe finds happiness in helping his students succeed is a lesson I can never forget.

I would like to thank my other professional mentors, Drs. Lakshman Gunaratnam and Greg Dekaban for years of valuable advice and criticism of my work, culminating in this thesis. I am also grateful to other members of the Department of Microbiology & Immunology, particularly Drs. Jimmy Dikeakos and Kelly Summers who evolved from being superiors and examiners into collaborators and friends.

At the bench, I had an abundance of riches when it came to senior students willing to teach me. Drs. Matt Miller, Greg Fonseca, and Mike Cohen stand out both as teachers of key techniques and examples of personal success I've tried to emulate. London family and friends (both in and out of the department) are too numerous to list, but I feel compelled to mention how their longtime presence in my professional and personal life motivated me even when experiments failed. From my cousin Danny to my friends Tanner, Ali, Steve, Jeremy, Kyle, Rod, Lee, Jordan, Sam, Joe, Alan, Stu, and especially Kris James: thanks for everything.

My family is an unrelenting source of encouragement and I am blessed to have an amazing support network. My parents, Sherry and Doug, mean more to me than the

deepest, most heartfelt words could explain. Thank you for always enabling my endeavours and helping me make good decisions. Lastly, the man who taught me to keep my stick on the ice both in hockey and in life; thank you for showing all of us what true strength and character are. While I miss you terribly and wish you were here, I know I have made you proud…

This one's for you, Grandpa.

Table of Contents

List of Tables

List of Figures

List of Appendices

List of Abbreviations

Ad Pol	Adenovirus DNA-dependent DNA polymerase
ADP	Adenovirus death protein
AKAP	A-kinase anchoring protein
ANOVA	Analysis of variance
AP	Activator protein
ARD	Acute respiratory disease
ATCC	American Type Culture Collection
ATF	Activating transcription factor
ATP	Adenosine triphosphate
BAK	Bcl-2 antagonist/killer
BAX	Bcl-2 associated X protein
Bcl-2	B-cell lymphoma 2
Bre1	E3 ubiquitin-protein ligase BRE1
BSA	Bovine serum albumin
BS69	Zinc finger MYND domain-containing protein 11
cAMP	Cyclic adenosine monophosphate
CAR	Coxsakievirus and adenovirus receptor
CBP	CREB-binding protein
Cdk	Cyclin-dependent kinase
cDNA	Complementary DNA
cGMP	Cyclic guanosine monophosphate
ChIP	Chromatin immunoprecipitation
CKII	Casein kinase II
CMV	Cytomegalovirus
CNB	Cyclic nucleotide binding domain
CoIP	Co-immunoprecipitation
CR	Conserved region
CRE	cAMP response element
CREB	CRE-binding protein

CREM	CRE modulator
CtBP	C-terminal binding protein
DAPI	4',6-diamidino-2-phenylindole
DAVID	Database for Annotation, Visualization, and Integrated Discovery
DBP	Adenovirus ssDNA-binding protein
DCAF7	DDB1- and CUL4-associated factor 7
D/D	Docking/dimerization domain
DE	Differentially expressed
DMEM	Dulbecco's modified eagle media
DNA	Deoxyribonucleic acid
DSBR	DNA double-stranded break response
dsDNA	Double-stranded DNA
DYRK	Dual-specificity tyrosine-regulated kinase
EBV	Epstein-Barr virus
EDTA	Ethylenediaminetetraacetic acid
EGTA	Egtazic acid
EGFP	Enhanced green fluorescent protein
EGS	Ethylene glycol bis(succinimidyl succinate)
ER	Endoplasmic reticulum
E1A	Early region 1A
E1B	Early region 1B
E2	Early region 2
E3	Early region 3
E4	Early region 4
FBS	Fetal bovine serum
FOXK	Forkhead box K
Fsk	Forskolin
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GCN5	Histone acetyltransferase GCN5
GO	Gene Ontology
GPCR	G protein-coupled receptor

GSK-3	Glycogen synthase kinase-3
HA	Hemagglutinin
HAdV	Human adenovirus
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HDAC	Histone deacetylase
HEK	Human embryonic kidney
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HIPK2	Homeodomain-interacting protein kinase 2
HIV	Human immunodeficiency virus
hPTM	Histone post-translational modification
HPV	Human papillomavirus
HPyV	Human polyomavirus
HSV	Herpes simplex virus
H2B	Histone 2B
H3K9	Histone 3 lysine 9
H3K18	Histone 3 lysine 18
H3K36	Histone 3 lysine 36
IAV	Influenza A virus
IF	Immunofluorescence
IFN	Interferon
IgG	Immunoglobulin G
IS	Inhibitory site
ISG	Interferon-stimulated gene
ITR	Inverted terminal repeat
KAT	Lysine acetyl transferase
kbp	Kilobase pairs
kDa	Kilo Dalton
KEGG	Kyoto Encyclopedia of Genes and Genomes
KSHV	Kaposi’s sarcoma-associated herpes virus
KSR	Kinase suppressor of ras

LDS	Lithium dodecyl sulfate
MAPK	Mitogen-activated protein kinase
MED	Mediator complex subunit
MeV	Measles virus
MHC I	Major histocompatibility complex class I
MOI	Multiplicity of infection
mTOR	Mechanistic target of rapamycin
MuV	Mumps virus
NES	Nuclear export signal
NFAT	Nuclear factor of activated T cells
NF-κB	Nuclear factor of Kappa light polypeptide gene enhancer in B-cells
NGS	Next-generation sequencing
NLRP	NACHT, LRR and PYD domains-containing protein
NLS	Nuclear localization signal
NP40	Nonidet P-40
NXF1	Nuclear RNA export factor 1
NPC	Nuclear pore complex
ONPG	Ortho-nitrophenyl-β-galactoside
ORF	Open reading frame
Paf1	RNA polymerase II-associated factor 1
PBS	Phosphate-buffered saline
pCAF	p300/CBP-associated factor
PCNA	Proliferating cell nuclear antigen
PCR	Polymerase chain reaction
PDB	Protein data bank
PDE	Phosphodiesterase
PDK1	3-phosphoinositide-dependent protein kinase 1
PEPCK	Phosphoenolpyruvate carboxykinase
Pfu	Plaque-forming unit
PHK	Phosphorylase kinase
PIC	Preinitiation complex

PI3K	Phosphatidylinositol 3-kinase
PKA	Protein kinase A
PKC	Protein kinase C
PKR	Protein kinase R
PML	Promyelocytic leukemia nuclear body
PP	Protein phosphatase
PVDF	Polyvinylidene difluoride
p53	Cellular tumor antigen p53
p107	Retinoblastoma-like protein 1
p130	Retinoblastoma-like protein 2
p300	Histone acetyltransferase p300
p400	E1A-binding protein p400
RACK1	Receptor of activated protein C kinase
Rb	Retinoblastoma protein
RGD	Arginine-arginine-aspartic acid
RIAD	RI anchoring disruptor
RIDαβ	Receptor internalization and degradation complex αβ
RNA	Ribonucleic acid
RNAPII	RNA polymerase II
RNAPIII	RNA polymerase III
RNAseq	RNA sequencing
RT-qPCR	Quantitative reverse transcription PCR
SAdV	Simian adenovirus
sAKAPis	SuperAKAP-in silico
SD	Standard deviation
SDS	Sodium dodecyl sulfate
SEC	Super elongation complex
SEM	Standard error of the mean
siRNA	Silencing RNA
SLiM	Short linear interaction motif
SUMO	Small ubiquitin-like modifier

TAD	Transactivation domain
TBP	TATA-binding protein
TBS	Tris-buffered saline
TF	Transcription factor
TMAdV	Titi monkey adenovirus
TNF	Tumor necrosis factor
TP	Terminal protein
TR	Thyroid hormone receptor
TRAIL	TNF-related apoptosis-inducing ligand
TRRAP	Transformation/transcription domain-associated protein
TSS	Transcriptional start site
USF	Upstream stimulatory factor
UXP	U-exon protein
VA RNA	Virus-associated RNA
VACV	Vaccinia virus
vAKAP	Viral AKAP
VZV	Varicella-zoster virus
WDSV	Walleye dermal sarcoma virus
WT	Wild-type

Chapter 1

1 Introduction

"Not explaining science seems to me perverse. When you're in love, you want to tell the world."

– Carl Sagan

1.1 General Introduction

As obligate intracellular parasites, all viruses are critically dependent on their infected hosts for survival. Due to physical constraints and limited coding capacity, no known viruses are capable of independently producing either their own protein or metabolic energy. As a result of coevolution with their hosts, viruses have instead adapted to take advantage of machinery and factors from within the cell by engaging in a wide array of virus-host interactions (Rozenblatt-Rosen et al., 2012). Infected cells will typically be drastically altered into environments more favourable for viral progeny production. By teasing out the virus-host interactions that drive these changes, we gain greater understanding of not only viral pathogenesis, but also basic cellular functions such as gene expression, cell division, or immunological responses (Ferrari et al., 2014, 2008; Fonseca et al., 2012; Miyake-Stoner and O'Shea, 2014; Shah and O'Shea, 2015; Soria et al., 2010).

Human adenovirus (HAdV) has served as a prime example of the utility for using viruses to study the molecular biology of eukaryotic cells (Berk, 2005; Cohen et al., 2013; Pelka et al., 2008). Scientific breakthroughs related to cell cycle control, DNA replication, mRNA splicing, gene expression, interferon (IFN) signaling, and cancer can all be attributed to studies involving HAdV (Bayley and Mymryk, 1994; Berget et al., 1977; Chow et al., 1977; Flint and Shenk, 1997; Gallimore and Turnell, 2001). The virus was originally discovered and characterized in 1953 as a causative agent for respiratory infections (Hillerman and Werner, 1954; Rowe et al., 1953). HAdVs are non-enveloped viruses containing a linear double-stranded DNA (dsDNA) genome of approximately 36

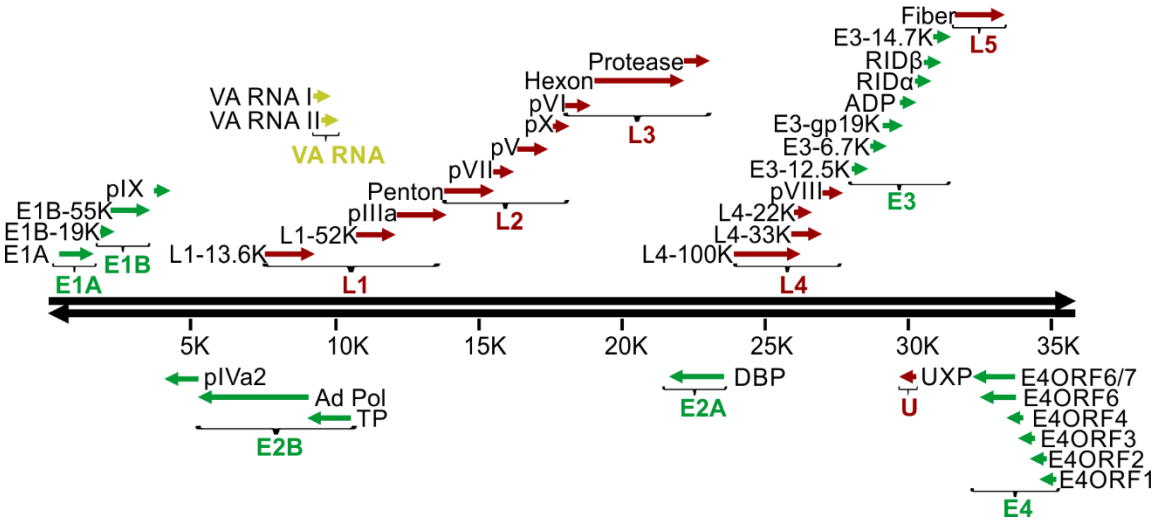


Figure 1.1. Schematic representation of the HAdV-5 double-stranded DNA genome.

The HAdV-5 genome is approximately 36kbp in length and composed of both early (E) and late (L) transcription units. Arrows depict the directionality of individual transcripts with their protein products labelled accordingly. Early genes are depicted in green while late genes are displayed in red. VA RNA is shown in yellow. Adapted from NCBI sequence NC_001405.1.

kilobase pairs (kbp) (Davison et al., 2003) (Figure 1.1). There are at least 57 distinct HAdV types which are divided across 7 unique species (A-G) based on various biological properties (Aoki et al., 2011; Jones et al., 2007; Walsh et al., 2011) (Figure 1.2). While broadly similar, these species have evolved molecular differences that confer specific tissue and organ tropisms including the respiratory tract, gastrointestinal tract, ocular tissue, and blood (Ghebremedhin, 2014). Clinically, most HAdV infections result in mild symptoms that resolve without treatment; however, disease can occasionally progress beyond a subclinical presentation, especially in immunocompromised hosts (Lion, 2014).

HAdVs are considered part of the genetically-distinct but functionally-similar family of DNA tumour viruses, which include other well-studied pathogens such as human papillomavirus (HPV) and polyomavirus (HPyV) (Howley and Livingston, 2009; Yaniv, 2009). All of these viruses can cause cancer in either animal or human systems (DiMaio and Fan, 2013). Indeed, in 1962 HAdV type 12 was the first human virus ever shown to be tumourigenic, albeit in a rodent model (Trentin et al., 1962). Research interest in the DNA tumour viruses exploded after this seminal discovery, with particular focus on the activities of the viral oncoproteins produced by these pathogens. Although not all species of HAdV can cause tumours in rodents, all viruses tested can transform cells in tissue culture (Berk, 2013; Freeman et al., 1967; Gallimore, 1972; McBride and Wiener, 1964) (Figure 1.2). Differences in pathogenicity and tumourigenicity between various HAdV types can largely be attributed to the radical changes to the cellular environment conferred by virus-host interactions. For HAdV, its early region 1A (E1A) protein is largely responsible for these pathophysiological changes and is the primary focus of this thesis.

1.2 Adenovirus

1.2.1 Physical properties of adenoviruses

Adenoviruses are composed of a non-enveloped, icosahedral protein capsid that houses a linear, dsDNA genome (Ginsberg et al., 1966; Russell, 2009). The capsid shell ranges in size between 60-90 nm in diameter, depending on the species. It consists of 252 capsomeres, 240 of which are hexons while 12 are pentons. Pentons form the base for a

protruding fiber protein and together these capsid components mediate attachment and entry of HAdV particles into targeted cells (Norrby, 1966; Norrby and Skaaret, 1967; San Martín, 2012). For some HAdV species, the fiber interacts with the coxsakievirus and adenovirus receptor (CAR) which is commonly found on the surface of epithelial cells (Bergelson et al., 1997; Devaux et al., 1987; Roelvink et al., 1998; Tomko et al., 1997). This is followed by an interaction of the arginine-glycine-aspartic acid (RGD) motif in the penton base with cell surface integrins (αvβ3 and αvβ5 for HAdV-5), resulting in endocytic uptake of the viral particle (Meier and Greber, 2003; Wickham et al., 1993). Notably, not all HAdV bind CAR (species B and D HAdVs can bind cellular CD46 instead) or contain an RGD motif, contributing to altered specificity for cellular receptors (Albinsson and Kidd, 1999; Gaggar et al., 2003; Lenman et al., 2015; Segerman et al., 2003; Sirena et al., 2004; Wickham et al., 1995; Wu et al., 2004).

The genomes of HAdVs are linear, dsDNA ranging in size between 30-36 kbp (Davison et al., 2003; Roberts et al., 1984) (Figure 1.1). Genomic organization is highly conserved and contains inverted terminal repeats (ITRs) at each end, as well as a covalently-attached 55 kilo Dalton (kDa) terminal protein (TP) which functions as a primer for viral genome replication (Challberg et al., 1980; Rekosh et al., 1977; Steenbergh et al., 1977). TP may also shield viral genomes from being recognized as free, damaged DNA by the host's DNA damage response machinery (Karen et al., 2009; Stracker et al., 2002; Weitzman and Ornelles, 2005). HAdV genomes have been assigned arbitrary directionality, the 'left' end of which contains repeats of cis-acting packaging sequences that direct proper packaging of viral DNA into infectious progeny virions (Gräble and Hearing, 1992; Hearing et al., 1987; Ostapchuk and Hearing, 2003). Viral genes are encoded on both strands of the genome and are broadly separated into two classes, early or late (Berk, 2013; Fessler and Young, 1998; Tollefson et al., 2007). Early genes are transcribed before the onset of viral genome replication and serve to reorganize the cell into a suitable environment for virus replication and suppress host immune responses. Late genes are expressed after viral genome replication and encode the structural proteins that make up the HAdV capsid. Depending on the species, HAdVs also encode one or two virus-associated RNAs (VA RNA) (Reich et al., 1966; Weinmann et al., 1974). These do not encode for viral proteins but instead act to antagonize the host's IFN-

Species	Hemagglutination Group	Adenovirus Types	Oncogenic potential: Tumours in Animals	Oncogenic potential: Transformation in Tissue Culture	%G-C in genome	Tissue tropism
A	IV (little or no agglutination	12, 18, 31	High	+	48-49	Gastrointestinal, respiratory tract
B	I (complete agglutination of monkey erythrocytes)	3, 7, 11, 14, 16, 21, 34, 35, 50	Moderate	+	50-52	Gastrointestinal, respiratory tract, ocular tissue
C	III (partial agglutination of rat erythrocytes)	1, 2, 5, 6	Low or none	+	57-59	Gastrointestinal, respiratory tract
D	II (complete agglutination of rat erythrocytes)	8, 9, 10, 13, 15, 17, 19, 20, 22-30, 32, 33, 36-39, 42-49, 51	Low or none	+	57-61	Gastrointestinal, ocular tissue
E	III	4	Low or none	+	57-59	Respiratory tract, ocular tissue
F	III	40, 41	Unknown	Unknown	Unknown	Gastrointestinal
G	Unknown	52	Unknown	Unknown	55	Gastrointestinal

Figure 1.2. Classification and properties of HAdV species.

Human adenoviruses are separated into 7 distinct species comprising over 50 unique types based on sequence similarity of their variable hexon epitopes. Various characteristics are shown here, including hemagglutination group, tumourigenicity, transformation in tissue culture, percent of G-C in genome, and tissue tropisms. Figure adapted from Field's Virology, 7th edition with additional information from (Ghebremedhin, 2014).

induced activation of protein kinase R (PKR) (Andersson et al., 2005; Reichel et al., 1985.; Schneider et al., 1984). Both early and late HAdV transcripts are produced by the host RNA polymerase II (RNAPII) with the exception of VA RNA (transcribed by RNAPIII) (Mathews and Shenk, 1991; Weinmann et al., 1974).

1.2.2 HAdV life cycle

Internalization of HAdV occurs after viral attachment to a host cell receptor via the knob of the fiber protein (Devaux et al., 1987). Following binding of the RGD sequence within the penton base to αvβ3 and αvβ5 integrins, receptor-mediated endocytosis ensues (Meier and Greber, 2003; Wickham et al., 1993). Acidification of the endosome causes partial disassembly of the viral capsid and release of the core viral particle into the cytosol through an undefined mechanism (Greber et al., 1993; Mellman, 1992; Svensson, 1985; Varga et al., 1991). Partially-uncoated virions are subsequently trafficked to the nucleus via an interaction between hexon and the host microtubule network (Dales and Chardonnet, 1973; Leopold et al., 2000; Mabit et al., 2002). At nuclear pore complexes (NPC), final uncoating occurs, allowing viral DNA bound by HAdV protein VII to be actively transported into the nucleus (Greber et al., 1997, 1996; Karen and Hearing, 2011; Spector et al., 2003). The attachment, entry, and subcellular trafficking phase of HAdV infection is rapid, taking place in under two hours (Martin-Fernandez et al., 2004; Trotman et al., 2001). In the nucleus, the viral genome becomes decorated by cellular histones and the transcription of early genes begins (Chen et al., 2007; Komatsu et al., 2011; Trotman et al., 2001). It is this early transcriptional phase of the viral life cycle that is a molecular focus of the thesis and will be discussed in more detail in subsequent sections.

As HAdV gene products encoding viral genome replication machinery accumulate, the infection enters its late phase (Berk, 2013; Nevins et al., 1979). This can begin as soon as six hours post-infection, depending on the type of infected cells and multiplicity of infection (MOI). The ITRs of the HAdV genome serve as the origin of replication for the virally-encoded DNA polymerase (Lechner and Kelly, 1977). New genomes are replicated via a strand-displacement mechanism not involving Okazaki fragments as

present in cellular DNA replication intermediates. This is concomitant with the production of viral capsid proteins and the assembly of capsomeres in the cytoplasm (Horwitz et al., 1969; Velicer and Ginsberg, 1970). These are subsequently transported into the nucleus where the assembly of progeny virions occurs. An association between the HAdV late proteins and the packaging signal in newly-synthesized genomes facilitates the assembly of infectious particles (Daniell, 1976; Hearing et al., 1987; Ostapchuk and Hearing, 2005). Final release of potentially 10,000 – 100,000 mature HAdV progeny occurs as the infected cell lyses (Green and Daesch, 1961; Tollefson et al., 1996a).

1.2.3 HAdV transcripts

Once an incoming viral genome reaches the cell nucleus, the first gene transcribed is the early region 1A gene (E1A), driven by a strong enhancer upstream of the E1A promoter (Hearing and Shenk, 1983; Nevins et al., 1979) (Figure 1.1). E1A is the main focus of this thesis and will be discussed later in more detail. Briefly, E1A performs key functions that suppress the cell's immune response, push the infected cell into S-phase, and drive expression of other HAdV genes (Bayley and Mymryk, 1994; Berk, 2005; Ferrari et al., 2014, 2008; Flint and Shenk, 1997; Fonseca et al., 2012; Gallimore and Turnell, 2001; Pelka et al., 2008; Schaack et al., 2004). Transcription of the other early genes follows closely after E1A and includes E1B, E2, E3, and E4. The rest of the late proteins are encoded mainly by the Major Late transcription unit (Ahi and Mittal, 2016; Shaw and Ziff, 1980; Tollefson et al., 2007). Most HAdV transcripts undergo extensive splicing, increasing the potential number of protein products upon translation (Berget et al., 1977; Berk, 2013).

Along with E1A, the E1 region of HAdV-5 includes the E1B gene encoding for two proteins named for their molecular weights: E1B-55k and E1B-19k, both of which are anti-apoptotic (Cuconati and White, 2002; Yew and Berk, 1992). E1B-55k functions as an E3 ubiquitin-ligase complex promoting the ubiquitination and subsequent degradation of the p53 tumour suppressor (Blanchette et al., 2004; Harada et al., 2002; Querido et al., 2001). E1B-19k functions as a viral mimic of cellular Bcl-2 by binding to BAK and BAX, blocking apoptosis via the release of mitochondrial cytochrome-c (Chiou et al.,

1994; Farrow et al., 1995; Rao et al., 1992). E1B-55k also functions as a SUMO (small ubiquitin-like modifier) ligase to modify p53 and force its nuclear export through an interaction with promyelocytic leukemia nuclear bodies (PML) and works in conjunction with HAdV open reading frame (ORF) E4-ORF6 to export viral mRNA into the cytoplasm via recruitment of the Nxf1/Tap export machinery (Gonzalez et al., 2006; Gonzalez and Flint, 2002; Muller and Dobner, 2008; Pennella et al., 2010; Yatherajam et al., 2011). The combination of both HAdV E1A and E1B is also capable of transforming primary cells in culture, demonstrating E1B's functionality as an oncoprotein (Bayley and Mymryk, 1994; Subramanian et al., 1991; Van den Elsen et al., 1983).

The E2 region contains three genes encoding proteins required for viral genome replication. These include a DNA-dependent DNA polymerase (Ad Pol), terminal protein (TP), and a single-stranded DNA binding protein (DBP) (Challberg and Kelly, 1979; Levine et al., 1976; van der Vliet and Levine, 1973). As mentioned earlier, TP serves as a protein primer for viral genome replication (Kelly and Lechner, 1979). DNA replication occurs in the standard 5' to 3' direction by Ad Pol, which contains exonuclease activity for proofreading capability (Field et al., 1984; King et al., 1997). As single-stranded intermediates are produced by strand displacement, they are bound by DBP to prevent hairpin formation and/or destruction. Newly-synthesized genomes, whether double or single stranded, serve as templates for additional rounds of replication (Challberg and Kelly, 1981; Kelly and Lechner, 1979; van Breukelen et al., 2003; van der Vliet and Levine, 1973).

The E3 region encodes for one transcript that is differentially spliced to code for several unique protein products, including receptor internalization and degradation complex αβ (RIDαβ), gp19k, 14.7k, 12.5k, 6.7k, and adenovirus death protein (ADP) (Berk, 2013; Lichtenstein et al., 2004). E3 proteins are all involved in modulating cell death and immune signaling. In conjunction with other E3 proteins, RIDαβ blocks extrinsic death signal ligands such as TNF (tumour necrosis factor), Fas ligand, and TRAIL (TNF-related apoptosis-inducing ligand) (Benedict et al., 2001; Friedman and Horwitz, 2002; Shisler et al., 1997; Tollefson et al., 2001). RIDαβ downregulates the proapoptotic receptors of these ligands by promoting internalization and subsequent lysosomal

degradation. E3-gp19k is a transmembrane glycoprotein that inhibits the cell-surface function of major histocompatibility complex class I (MHC I) (Burgert et al., 1987; Burgert and Kvist, 1985; Wold et al., 1985). It promotes immune evasion by binding and retaining MHC I molecules in the endoplasmic reticulum (ER) and also blocks the tapasin-mediated peptide loading of MHC (Bennett et al., 1999). E3-14.7k binds and inhibits the apoptotic signaling activities of TNF, NF-κB, and Caspase 8 (Carmody et al., 2006; Gooding et al., 1990, 1988; Kim and Foster, 2002). E3-6.7k works with RIDαβ to downregulate TRAIL and TNF signaling via blockage of arachidonic acid release (Benedict et al., 2001; Moise et al., 2002). Despite its genomic locus, ADP is predominantly produced later during infection and serves a proapoptotic role mediating release of progeny virus via cell lysis (Tollefson et al., 1996b, 1992). Lastly, E3-12.5k has no known function and is not required for viral replication, yet its presence is highly conserved among various HAdV species suggesting it may play an important, yet undiscovered, role (Hawkins and Wold, 1992).

Similar to E3, the HAdV E4 region encodes one mRNA that is differentially-spliced to encode many protein products. These include E4ORF1, E4ORF3, E4ORF4, E4ORF6, and E4ORF6/7 and are involved in a wide array of biological functions (Berk, 2013; Täuber and Dobner, 2001). Sequences encoding for additional ORFs have also been predicted but have either no known function or are undetectable during infection. Furthermore, the deletion of these ORFs via mutagenesis does not impair HAdV replication. Both E4ORF1 and E4ORF4 enhance viral replication by modulating metabolism. E4ORF1 activates the mechanistic target of rapamycin (mTOR) pathway by activating phosphatidylinositol 3-kinase (PI3K) (Frese et al., 2003; O'Shea et al., 2005). It also modulates glycolysis through an interaction with MYC resulting in cellular metabolites being rerouted for nucleotide biosynthesis (Miyake-Stoner and O'Shea, 2014; Thai et al., 2014). E4ORF4 also activates mTOR independently of E4ORF1, by binding cellular phosphatase PP2A (O'Shea et al., 2005; Zhang et al., 2011). Though the mechanistic details are unclear, this function increases viral replication under nutrient-limiting conditions. E4ORF3 inhibits the cell's DNA double-stranded break response (DSBR) by sequestering the MRN DNA damage complex (MRE11/RAD50/NBS1) (Araujo et al., 2005; Evans and Hearing, 2005; Sohn and Hearing, 2012; Stracker et al.,

2002). It also inhibits antiviral functions of PML bodies mediated through the PML-associated protein, Daxx, and inhibits p53 (in an E1B-independent manner) by inducing heterochromatin at p53-targeted promoters (Soria et al., 2010; Ullman and Hearing, 2008). E4ORF6 works mainly in a complex with E1B-55k to either degrade p53 (described earlier) (Dobner et al., 1996; Harada et al., 2002; Querido et al., 2001) or perform various other tasks to enhance viral DNA replication, nuclear export of viral mRNA, and blocking host protein synthesis (Bridge and Ketner, 1990; Halbert et al., 1985; Yatherajam et al., 2011). Finally, E4ORF6/7 binds cellular E2F transcription factors (TFs) and recruits them to the HAdV E2 promoter, inducing transcription of the aforementioned E2 genes (Hardy et al., 1989; Helin and Harlow, 1994; Huang and Hearing, 1989).

Overall, the early gene products serve to reprogram/reorganize the cell into an optimal environment for HAdV replication, by inducing DNA replication, and protecting progeny from destruction by immune responses (Berk, 2013). The late products then form the basis for assembling and egressing mature virions for infecting new hosts. Along with the U-exon protein (UXP), the major late transcription unit codes for both the structural and non-structural proteins required for virion assembly and packaging (Ahi and Mittal, 2016; Shaw and Ziff, 1980; Tollefson et al., 2007). These arise from differential splicing of the major late mRNA into a plethora of products, which are further classified into 5 regions (L1-L5). Penton, hexon, and fiber (described earlier) are encoded on L2, L3, and L5 respectively.

1.2.4 HAdV diversity and virulence

Historically, HAdV types were categorized into various serotypes by their resistance or sensitivity to neutralization by antisera against other HAdV types (Wold and Ison, 2013). More recently, molecular techniques utilizing amino acid or nucleotide sequence similarities within the main neutralizing epitopes (the hexon capsid protein) have been adopted to classify different types (Aoki et al., 2011; Jones et al., 2007; Madisch et al., 2005). Consequently, there are currently seven accepted HAdV species (A-G) encompassing over 50 distinct HAdV types (Adams et al., 2017) (Figure 1.2). All HAdVs are quite species-specific in their ability to infect and replicate within hosts, as is

the case for non-human adenoviruses as well. Even nonhuman primates make typically poor hosts for HAdV and these viruses are generally non-pathogenic in animals (Wold and Ison, 2013). In cell culture models, modest replication will occur in cells from rats, mice, dogs, pigs, and hamsters (Jogler et al., 2006; Ternovoi et al., 2005; Thomas et al., 2006; Toth et al., 2005). However, human-derived immortalized cell lines (such as HEK293, HeLa, and A549) are by far the best culture system for abundant production of HAdV.

Epidemiologically, HAdVs are ubiquitous in the human population (Ghebremedhin, 2014; Lion, 2014; Wold and Ison, 2013). The lower-numbered HAdV types (such as HAdV-5) are especially seroprevalent, particularly in children and adolescents. Transmission occurs via the fecal-oral route or aerosols, leading to viral entry through the mouth, nasopharynx, and ocular tissues (Couch et al., 1966; Fox et al., 1977; Russell et al., 2006). Most HAdVs share similar cellular receptors (discussed earlier), however the mechanisms underlying preferred organ/tissue tropisms are not well understood. HAdVs primarily infect a wide array of tissue sites, depending on the species (Ghebremedhin, 2014; Law and Davidson, 2005; Lenman et al., 2015; Nicklin et al., 2005; Wold and Ison, 2013; Zhang and Bergelson, 2005). These include respiratory tissues (species A, B, C, E), gastrointestinal tract (species A, B, C, D, and especially F and G), and eye (B, D, E).

As human pathogens, HAdVs are well-adapted to their hosts and typically cause asymptomatic or mild infections (Ghebremedhin, 2014; Lion, 2014; Wold and Ison, 2013). However, these infections can also cause acute respiratory disease (ARD), most commonly in children and military recruits. HAdV is thought to contribute to approximately 8% of the global viral disease burden, including 10% of all febrile illnesses in children. More severe disease can include pharyngitis, gastroenteritis, and conjunctivitis depending on the site of infection and HAdV species responsible (Binder et al., 2017). In young children or immunocompromised individuals, HAdV infection can cause pneumonia, fulminant hepatitis, or encephalitis (Krilov, 2005; Walls et al., 2003). Occasionally, severe outbreaks have occurred, such as the emergence of fatal pneumonia cases linked to HAdV-14 across multiple American states in 2005 (Kajon et al., 2010; Lewis et al., 2009; Metzgar et al., 2007; Seto et al., 2009).

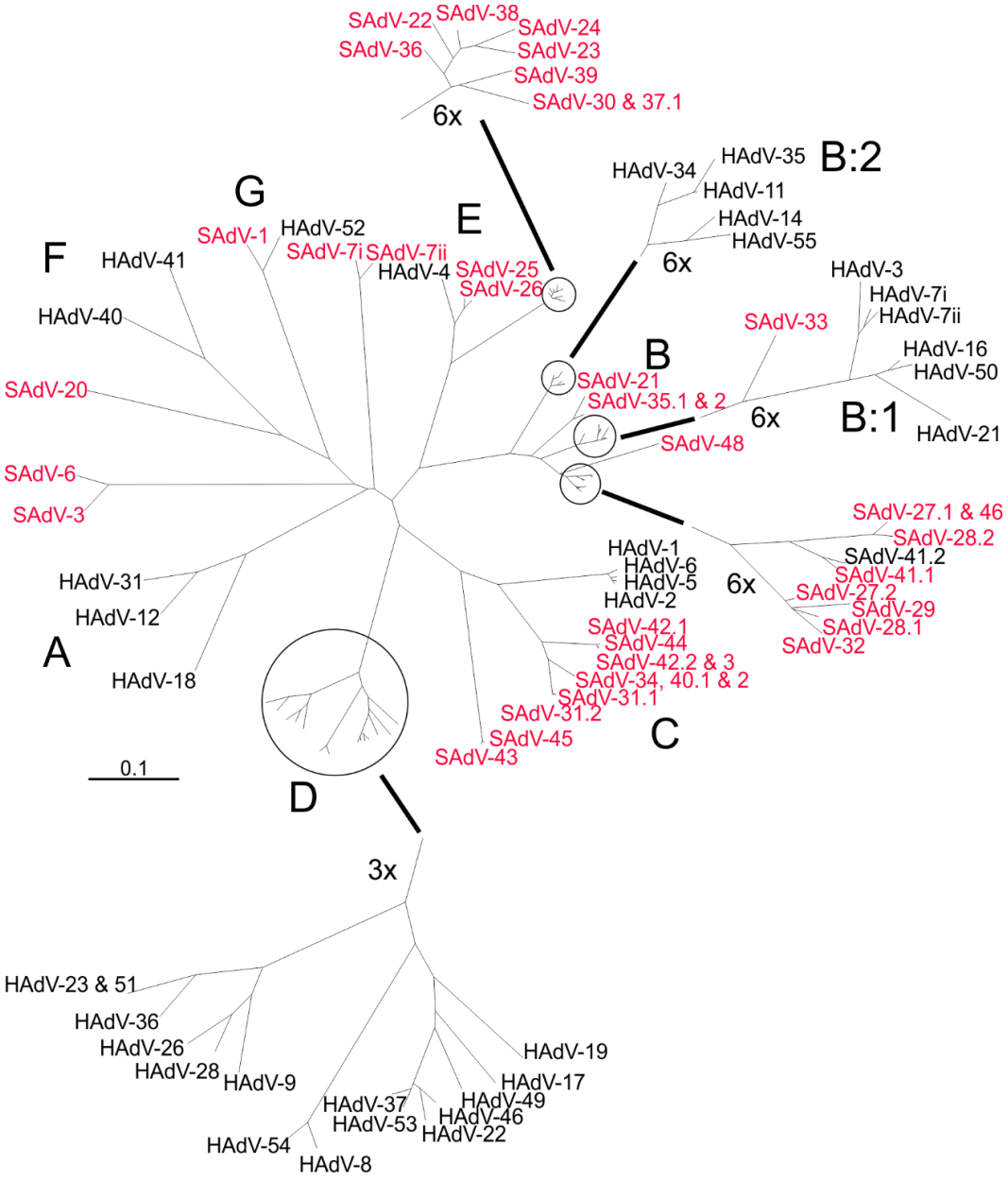


Figure 1.3. Comparison of human and simian adenoviruses.

Phylogenetic analysis of HAdVs and SAdVs based on the amino acid sequence of the viral E1A proteins. E1A sequences were imported into the PHYLIP suite of software to calculate phylogenetic distance using the programs PROTDIST.EXE and NEIGHBOR.EXE (Dr. Joe Felsentein, University of Washington, Seattle, WA) and visualized with TreeView (Page, 1996). Branch lengths are proportional to genetic distance and some regions are magnified by the indicated factor for better visualization.

At the cellular level, HAdV infection causes characteristic cytopathic effects (CPE), such as enlarged cell nuclei and formation of inclusion bodies, eventually leading to cell death (Boyer et al., 1959; Kasel, 1979; Wold and Ison, 2013). Host cell synthesis of DNA, RNA, and protein are also broadly inhibited. The viral replication cycle in primary sites of infection is typically rapid and cytolytic; however, virus can be shed at low levels for months after visible symptoms have passed, often from secondary tissue sites (King et al., 2016c; Lion, 2014). HAdV can sometimes establish persistent-style infections in humans (Abken et al., 1987; Garnett et al., 2009; Rowe et al., 1953). While some recent studies have revealed molecular details on how HAdV can re-task the host cell's own antiviral response to establish persistence, these have been difficult to reproduce in cell culture and are poorly understood (Zheng et al., 2016). Persistent infections are characterized as long-term with occasional and low-level shedding of progeny virus that may cause little or no harm to host tissue (Zhang et al., 2010). Persistence is an effective and common strategy for maintaining virus production beyond the primary infection, while simultaneously avoiding immune surveillance. This alternative lifestyle likely benefits the virus by allowing the continuous production of new progeny at intermediate levels over a vastly extended timeframe. It also partially explains why HAdV was originally isolated from the secondary lymphoid tissues (adenoids and tonsils) of visibly healthy individuals in 1953 (Rowe et al., 1953).

Despite the label of being a DNA tumour virus, HAdV does not cause cancer in humans (Mackey et al., 1976; Mende et al., 2004). Some HAdVs are, however, tumourigenic in rodent species and can stably transform primary rodent or human cells in culture (Berk, 2013; Freeman et al., 1967; Gallimore, 1972; McBride and Wiener, 1964). These oncogenic properties were initially observed in 1962 when injection of HAdV-12 into newborn hamsters induced malignant and aggressive tumours (Trentin et al., 1962). This was the first demonstration of a human virus causing cancer (albeit in a rodent model) and caused a burgeoning interest in the field of adenovirology research. Transformation by HAdV requires the activities of both its major oncoproteins, E1A and E1B, a process driven largely by the many protein-protein interactions made by these viral antigens with host target proteins (Graham et al., 1977; Houweling et al., 1980; Subramanian et al., 1991; Van den Elsen et al., 1983; Whittaker et al., 1984). Following these initial

discoveries, researchers have used HAdV as a model for uncoupling the molecular pathways that lead to cancer when dysregulated. The E1A protein itself has undoubtedly had the largest impact on these findings and continues to be utilized as a molecular tool today.

While considered rare, cross-species transmission events of HAdV have evidently occurred between humans and nonhuman primates (Ersching et al., 2010; Hoppe et al., 2015; Robinson et al., 2013; Roy et al., 2009; Wevers et al., 2011). Adenovirus zoonosis (the ability to transmit from human to non-human and vice versa) has given rise to multiple HAdV types now endemic in humans. HAdV-4 (the only member of species E) is a recombinant virus comprised of hexon epitopes of HAdV-16 (encompassing 2.5% of the whole genome) within the backbone of simian adenovirus-26 (SAdV) (Dehghan et al., 2013). The newly-classified and lone member of species G (HAdV-52) has extremely low detection rates in humans and is most closely related to SAdV-1, suggesting the virus has recently crossed into the human population (Jones et al., 2007; Lenman et al., 2015). The closest HAdV relatives to HAdV-52 are those of species F (HAdV-40 and -41) as they have the highest amino acid similarity (Figure 1.3). Species F and G contain the only HAdV types with two distinct fiber genes, and all readily infect the GI tract (Kidd et al., 1993). Cross-talk between adenoviruses of humans and nonhuman primates is still ongoing, as recently a novel titi monkey adenovirus (TMAdV) caused a deadly pneumonia outbreak in a New World monkey colony in California (Chen et al., 2011). Most of the monkeys died, one of the human workers became seriously ill, and the virus was also transmitted to a family member of this worker. The fact that TMAdV could readily transmit between both monkeys and humans demonstrates that zoonotic adenoviruses need to be monitored as potentially deadly causes of viral disease outbreaks.

1.3 HAdV Early Region 1A (E1A)

1.3.1 E1A gene, transcripts, and protein products

The HAdV E1A gene is found at the very left end of the viral genome, downstream of a strong transcriptional enhancer and the left ITR (Hearing and Shenk, 1983; Nevins et al.,

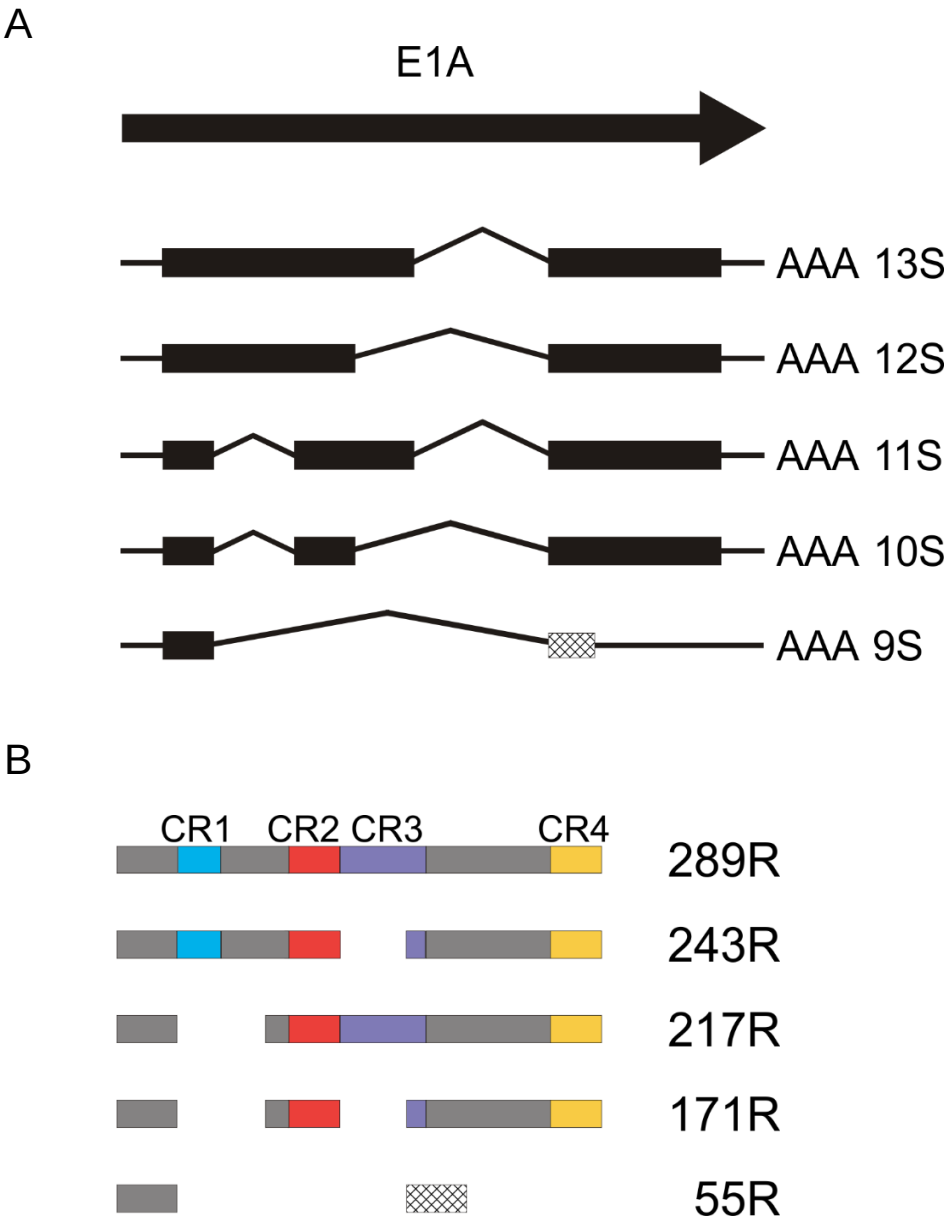


Figure 1.4. HAdV-5 E1A splice products.

(A) The E1A transcript is differentially spliced into 5 products, each named for their sedimentation coefficients (13S, 12S, 11S, 10S, and 9S). Black bars represent coding RNA while the lines represent excised sequence. All splicing events maintain the same translational reading frame except for the C-terminus of the 9S mRNA (denoted by the checkered bar). (B) Diagram of the E1A protein products resulting from differential splicing. Conserved regions 1 – 4 are denoted as is each protein's length in amino acid residues. The unique sequence in the 55R product is denoted by the checkered bar.

1979) (Figure 1.1). E1A is the first viral gene expressed upon HAdV infection and is essential for productive viral replication unless extremely high multiplicities of infection are used (Jones and Shenk, 1979). Broadly, E1A-encoded products function to activate expression of the other HAdV early genes (Berk et al., 1979; Montell et al., 1984, 1982; Winberg and Shenk, 1984) and condition the cellular environment into a compliant state amenable to an efficient and successful HAdV infection (Bayley and Mymryk, 1994; Berk, 2005; Flint and Shenk, 1997; Gallimore and Turnell, 2001; Pelka et al., 2008). The E1A transcript is differentially spliced into five isoforms, denoted by their sedimentation coefficients (13S, 12S, 11S, 10S, and 9S in HAdV-5) (Berk, 2013) (Figure 1.4A). All splicing events preserve the reading frame except for the 9S product, which has a unique 29 amino acid stretch in its C-terminus (Virtanen and Pettersson, 1983) (Figure 1.4B). The two largest mRNAs yield proteins of 289 and 243 amino acids respectively and are expressed highly during the early phase of infection (Perricaudet et al., 1979). These differ only by the absence of an internal 46 amino acid stretch in the smaller isoform. Both localize to the nucleus and the cytoplasm and carry out the majority of E1A's known functions (Rowe et al., 1983; Turnell et al., 2000). The three smaller isoforms (encoding proteins of 217, 171, and 55 amino acids) are produced at later times post-infection (Spector et al., 1978; Stephens and Harlow, 1987; Svensson et al., 1983; Ulfendahl et al., 1987). While they can enhance the HAdV life cycle, their precise roles are far less-characterized than their larger and more highly-expressed counterparts (Miller et al., 2012). Sequence comparisons of the largest E1A protein across different HAdV species revealed four regions of sequence similarity termed conserved region (CR) 1 through 4 (Avvakumov et al., 2004, 2002b; Kimelman et al., 1985; van Ormondt and Hesper, 1983) (Figure 1.5). The highly conserved nature of these sequences suggests they play crucial roles in the viral life cycle.

1.3.2 E1A is a viral hub protein

E1A's crucial biological role is a direct result of its numerous protein-protein interactions within the host cell. In contrast to other well-studied viral hub proteins, E1A has no intrinsic DNA-binding or enzymatic activities (Avvakumov et al., 2002a; Chatterjee et al., 1988; Zu et al., 1992); instead it acts in a modular fashion by binding to and altering

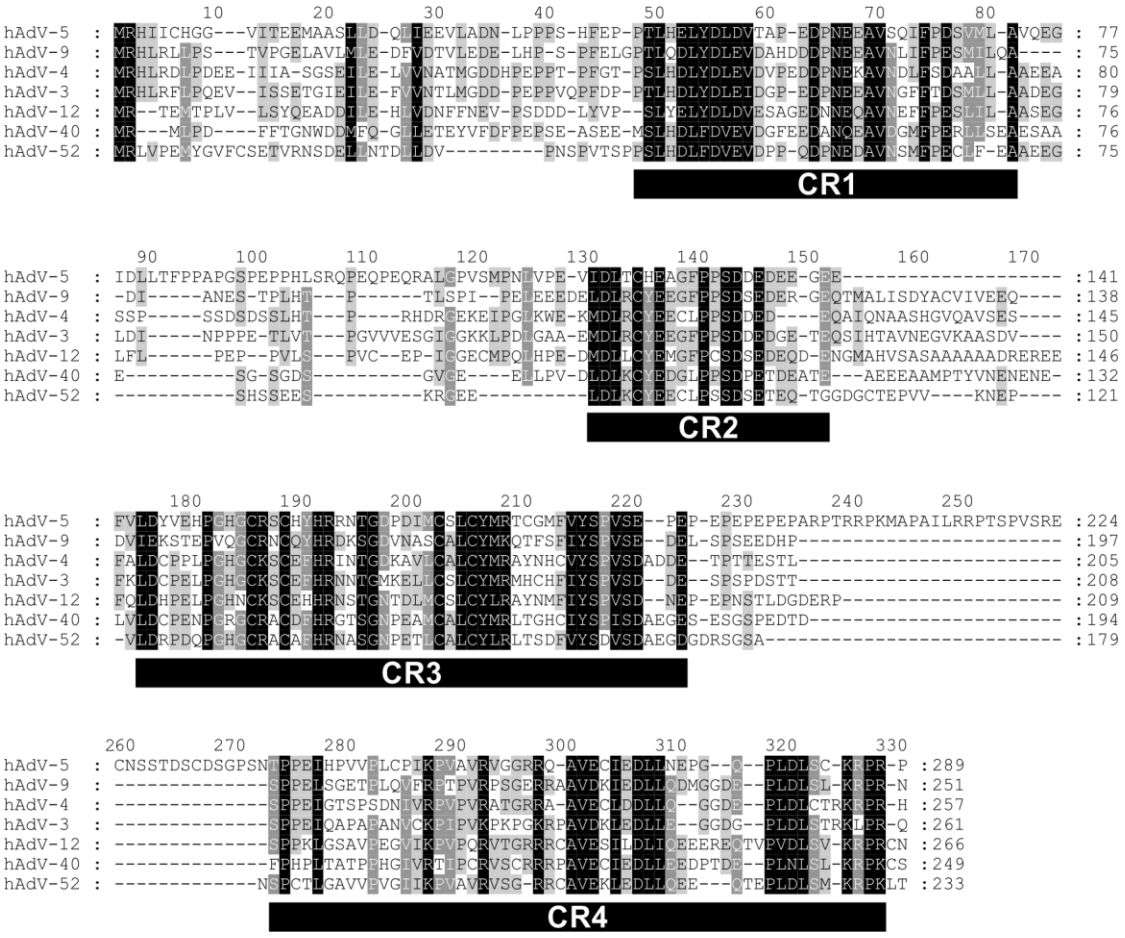


Figure 1.5. Alignment of E1A proteins from representative members of each HAdV species.

The amino acid sequences of the largest E1A isoforms from HAdV-3, -4, -5, -9, -12, -40, and -52 (representing species B, E, C, D, A, F, and G respectively) are shown. Sequence alignment was performed using CLUSTAL W (Thompson et al., 1994). Alignments were edited manually using GeneDoc as previously described (Avvakumov et al., 2004) and shaded to four levels of conservation using the Dayhoff PAM 250 score table. The positions of the conserved regions are indicated as solid bars.

the functions of over 50 distinct cellular targets (Pelka et al., 2008) (Figure 1.6). Indeed, many of these targets are highly-connected hub proteins themselves. By embedding itself so deeply within the cell's protein interaction network, E1A exerts tremendous control over both viral and host gene expression (Berk et al., 1979; Ferrari et al., 2014, 2008; Horwitz et al., 2008). Consequently, E1A is able to modulate the production of other HAdV gene products, as well as suppress the cell's immune response, force the cell into S phase, and block differentiation.

As a relatively small protein, E1A's ability to bind promiscuously to host targets and affect several cellular processes stems from the many features contained within its sequence. Consistent with the constraints of viruses' limited coding potential, E1A serves as a prime example of efficiency; as typifies many hub proteins (both viral and cellular) E1A is largely unstructured (with only a few globular regions) and is packed with short linear interaction motifs (SLiMs) (Davey et al., 2011; Ferreon et al., 2009; Pelka et al., 2008) (Figure 1.7). SLiMs allow E1A to exist in a dynamic and flexible state, enabling it to sample various conformations to carry out its plethora of overlapping protein-protein interactions (Dunker et al., 2005; Kim et al., 2008). While CR1 and CR2 of E1A bear some similarity to parts of human papillomavirus (HPV) E7 and polyomavirus (HPyV) large T antigen, E1A has no known cellular ortholog (Figge et al., 1988; Phelps et al., 1988; Vousden and Jat, 1989). Instead, its protein interaction motifs have likely evolved independently as functional mimics of cellular counterparts, presumably providing selective advantages for HAdV.

1.3.3 E1A can act as either an oncoprotein or tumour suppressor

Radical alterations to cellular protein interaction networks are seen not only during infections by DNA tumour viruses, but also in many types of cancer. Given that the functions of viral oncoproteins like E1A and the cis-acting genome variations found in tumours often converge on the same pathways, E1A is a valuable tool for identifying host proteins affected by the processes of transformation and tumourigenesis (Rozenblatt-Rosen et al., 2012). In fact, trans-acting viral products have been demonstrated to be

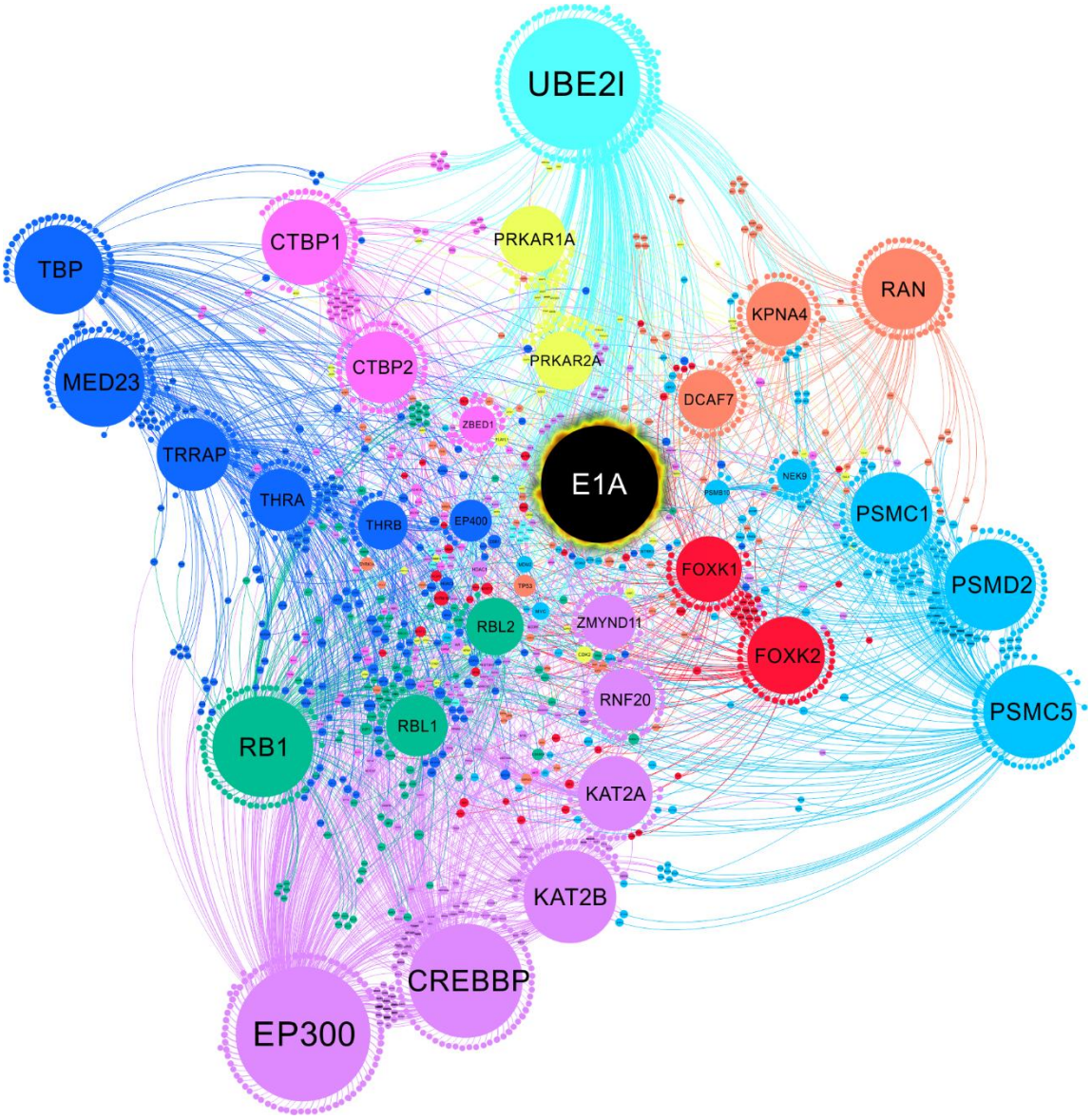


Figure 1.6. The HAdV-5 E1A interactome.

Diagrammatic representation of the E1A protein interaction network. The network was constructed using Gephi 0.9.1 with data from BioGRID build 3.4.144. E1A is placed in the centre of the network with its primary interacting partners depicted as the large circles emanating outward. Each primary interactor is supported by at least two peer-reviewed publications and is sized proportional to the number of its own binding partners. These secondary interactors are depicted as the smaller circles and are coloured and positioned near the primary E1A interactor to which they bind.

capable of successfully predicting cancer-relevant targets at rates on par with functional genomics and large-scale cataloguing of tumour mutations.

As mentioned previously, HAdV does not cause cancer in humans as the lytic infection is typically rapid and self-limiting and the viral genome does not normally integrate into the host genome (Lion, 2014; Mackey et al., 1976; Mende et al., 2004). However, E1A alone is capable of immortalizing cells in culture when introduced by stable transfection (Graham et al., 1977; Houweling et al., 1980; Whittaker et al., 1984). In combination with a cooperating oncogene, such as HAdV E1B or activated Ras, it can fully transform these cells (Gallimore and Turnell, 2001; Subramanian et al., 1991; Van den Elsen et al., 1983). For instance, these properties led to the creation of the widely-used HEK293 cell line which stably express the E1 region of HAdV-5, inserted into chromosome 19 of a cell derived from a primary human embryonic kidney culture (Graham et al., 1977; Louis et al., 1997). E1A is also required for the ability of HAdV to cause tumours in immunocompromised rodents (Trentin et al., 1962). Consequently, E1A can help identify critical regulators of cell growth and gene expression that are likely relevant to cancer.

Paradoxically, exogenous expression of E1A in previously-transformed cell lines can suppress oncogenic phenotypes (Deng et al., 2002, 1998; Frisch and Mymryk, 2002; Mymryk, 1996). This ability to act as a tumour suppressor reduces metastasis, angiogenesis, and tumourigenicity *in vivo*. E1A can also induce apoptosis and convert cells from a mesenchymal-like to an epithelial-like cell type in this context. While the complete mechanisms driving E1A's oncogenic and tumour suppressive abilities are not yet completely understood, E1A can be used as a tool to enhance knowledge of these fundamental processes and shed insight on how both viruses and cancers disrupt them.

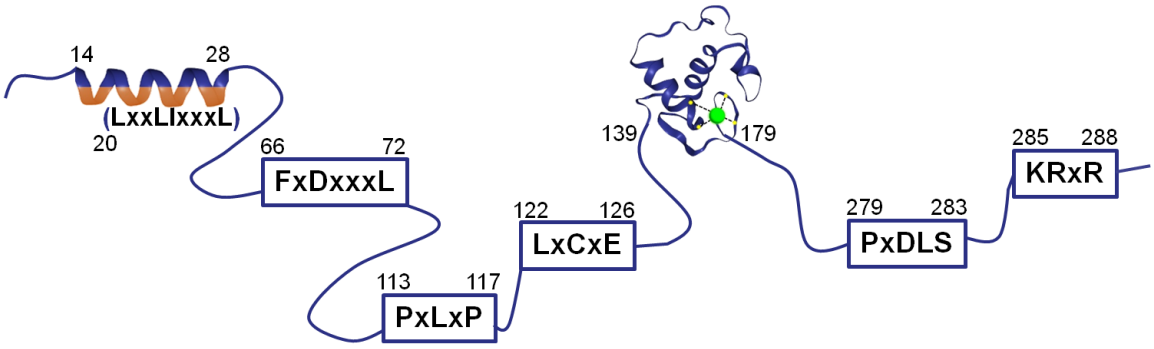
1.3.4 E1A as a tool for identifying eukaryotic regulatory proteins

The E1A protein is extremely modular and multifunctional, as each of its conserved regions (Figure 1.5) can function differently and independently from each other (Bayley and Mymryk, 1994). This highly modular nature has made E1A ideal for mutagenesis

studies, as insertions, deletions, and point mutations typically only affect a subset of functions while leaving others intact. Even small fragments of E1A protein can retain functionality when expressed in isolation. Thus, E1A has a remarkable history for being used to discover and study novel host regulatory proteins (Pelka et al., 2008).

Many of E1A's binding partners are intimately linked with transcription and cell cycle control (Granberg et al., 2005; White, 2001; Zhao et al., 2003) (Figure 1.6). Therefore, the study of these virus-host interactions has revealed deep molecular knowledge of vital cellular processes. One well-known example is the interaction between E1A and retinoblastoma protein (pRb), which was the first example of a viral oncoprotein interacting with a tumour suppressor (Whyte et al., 1988). Here E1A used two distinct binding interfaces to supplant the E2F family of proteins from pRb, benefitting the virus in multiple ways (Fattaey et al., 1993). First, released E2Fs induce activation of HAdV genes, particularly the E2 transcription unit (indeed, E2Fs derive their proper name from this initial observation) (Reichel et al., 1987). Second, E2Fs also activate E2F-responsive cellular genes, which control entry into S-phase of the cell cycle (Ghosh and Harter, 2003; Pagano et al., 1992). In addition, the molecular characterization of the E1A-pRb interaction led to the identification of the LxCxE SLiM (Figure 1.7), which was also subsequently found to be required for pRb-binding by a number of cellular proteins (Avvakumov et al., 2004; Corbeil and Branton, 1994; Dahiya et al., 2000; Ikeda and Nevins, 1993).

Another well-characterized transcriptional repressor, C-terminal binding protein (CtBP1 and 2), was also discovered through its interaction with HAdV E1A (Boyd et al., 1993). As its name suggests, CtBP binds to E1A via a PxDLS SLiM in E1A's C-terminus (Avvakumov et al., 2004; Quinlan et al., 2006; Schaeper et al., 1995). E1A appears to compete with endogenous proteins to bind CtBP1/2 and relieve its repression of host genes involved in cell growth and apoptosis (Bergman and Blaydes, 2006; Chinnadurai, 2009, 2002). E1A may also use CtBP1/2 to bring itself to, and activate, promoters that are normally repressed (effectively converting these cellular repressors into coactivators) (Bruton et al., 2008). These interactions between E1A and various eukaryotic regulatory proteins have been widely exploited in defining the molecular biology of the cell.



Motif	Host target
α-helix	PKA
LxxLIxxxL	TR
FxDxxxL	p300/CBP
PxLxP	BS69
LxCxE	Rb/p107/p130
C4 Zn Finger	TBP, Med23
PxDLS	CtBP1/2
KRxR	Importin α's

Figure 1.7. Diagrammatic representation of E1A's protein interaction motifs.

Experimentally-validated protein interaction motifs are displayed. These include both SLiMs and globular domains. Generic α-helix and zinc finger structures were adapted from the Protein Data Bank (PDB): 2GLH and 1GDC (Andreotti et al., 2006; Baumann et al., 1993). Notable interactions with cellular proteins conferred by these motifs are also listed.

Therefore, by continuing to use genetic and biochemical approaches to discover novel interacting partners of E1A we may identify yet unknown proteins important in various cellular processes.

1.3.5 E1A modulates viral transcription

Many viruses require host enzymes to produce nucleic acid polymers in the form of either viral genomes and/or transcripts. HAdV is no exception and requires both host RNA polymerases and DNA-binding transcription factors in order to transcribe its own genes (Berk, 2013). The HAdV genome has evolved to contain many cis-acting elements capable of engaging with RNAPII and various TFs, but the coordination of these virus-host interactions is multifactorial and complex (Fernandes and Rooney, 1997; Hurst and Jones, 1987; Kovesdi et al., 1986; Parks et al., 1988; Reichel et al., 1987; SivaRaman and Thimmappaya, 1987; Watanabe et al., 1988; Williams et al., 1990; Wu and Berk, 1988). E1A's ability to act as a molecular hub is crucial in this context. It can associate with various eukaryotic transcriptional components and modify their activities to initiate viral gene expression (Figure 1.6). As such, E1A has served as a paradigm for studying viral transcription via the retasking of cellular TFs.

A productive HAdV infection requires E1A-mediated activation of the other viral early genes, as they encode crucial components that perform various tasks during infection including: inhibiting apoptosis, replicating the viral genome, suppressing the immune response, and transporting viral mRNA (discussed in more detail above) (Berk, 2013). Transactivation (a portmanteau of 'transcriptional activation') ability of E1A has been largely mapped to its N-terminus and CR3 regions (both of these can drive reporter gene expression when fused to a heterologous DNA-binding domain), though E1A makes protein-protein interactions along its entire sequence that contribute to modulating transcription, consistent with its modular nature (Bondesson et al., 1994; Lillie and Green, 1989; Martin et al., 1990; Yousef et al., 2009).

Transactivation by E1A has been best-characterized within its CR3 region, one of the few portions of E1A containing a structure (a zinc finger) (Culp et al., 1988; Fahnestock and

Lewis, 1989; Jelsma et al., 1988). Via this region, E1A binds and recruits several components of TFIID complex to nucleate formation of the RNAPII preinitiation complex (PIC) (Geisberg et al., 1994; Webster et al., 1991; Webster and Ricciardi, 1991). These include TATA-binding protein (TBP) and various TBP-associated factors (Geisberg et al., 1995; Mazzarelli et al., 1995). Unsurprisingly, these interactions alone are necessary but not sufficient for wild-type (WT)-levels of E1A transactivation. E1A also binds MED23, a component of the multi-protein Mediator adaptor complex, and its recruitment to promoters serves as a potent activation step (Ablack et al., 2010; Boyer et al., 1999; Cantin et al., 2003; Stevens et al., 2002). While E1A lacks direct DNA-binding ability itself, it does bind to several promoter-targeting transcription factors, including members of the cAMP (cyclic adenosine monophosphate) response element/activating transcription factor (ATF) family, activator protein-1 (AP-1), upstream stimulatory factor (USF), and Sp1 (Liu and Green, 1994, 1990; Ström et al., 1998). Additionally, E1A CR3 binds coactivators (such as pCAF) as well as repressors of transactivation, including GCN5 and BS69 (Ablack et al., 2012; Hateboer et al., 1995; Shuen et al., 2002). Therefore, this portion of E1A appears to coordinate the presence of sequence-specific transcription factors with both activating and repressive complexes, allowing for tight control of viral transcription kinetics.

The N-terminus of E1A also can enhance transcription and it is densely-packed with many protein-protein interaction sites (Figure 1.8). These include motifs that confer interactions with transcription factors/cofactors like p300/CBP, myogenein, AP-2, p400, protein kinase A (PKA), Bre1, TRRAP, thyroid hormone receptor (TR), components of the proteasome, among others (Arany et al., 1995; Deleu et al., 2001; Eckner et al., 1994; Fax et al., 2001; Fonseca et al., 2012; Fuchs et al., 2001; Grand et al., 1999; King et al., 2016a; Meng et al., 2003; Somasundaram et al., 1996; Taylor et al., 1993; Turnell et al., 2000; Wahlström et al., 1999). Many of these interactions remain quite uncharacterized. It is clear, however, that different regions of E1A cooperate to maximize control over specific interaction partners or establish novel connections servicing viral gene expression. Instead of encoding its own RNA polymerase and proteins to act as DNA-binding TFs, HAdV can use a single viral hub (E1A) to coordinate a massive reshuffling of host transcriptional machinery. Excitingly, the roles of many binding partners and

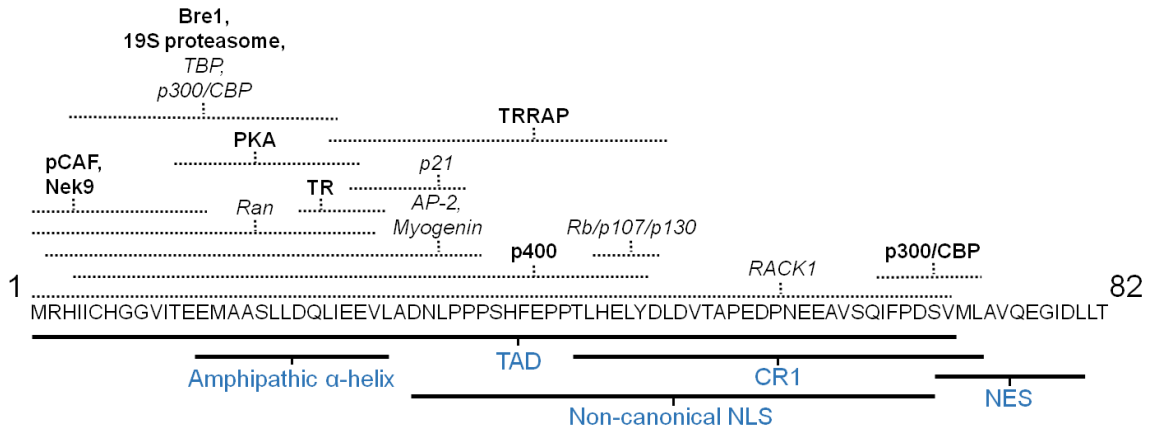


Figure 1.8. Binding partners of the N-terminus of E1A.

Protein sequence of HAdV-5 E1A N-terminus (residues 1-82) is shown. Key features are denoted by blue text and include the N-terminal transactivation domain (TAD), nuclear export signal (NES), and a non-canonical nuclear localization signal (NLS). The mapped binding sites of cellular target proteins are depicted by dashed lines. Less-characterized interactors or those that were experimentally-demonstrated only in yeast are italicized. Note that several interactors have multiple binding sites on full-length E1A, including TBP, p300/CBP, and Rb and family members.

their place within the E1A proteome still require vigorous investigation to delineate their potential roles in transcription in both infected and uninfected conditions.

Research on E1A-mediated viral gene expression has largely focused on the initiation step of transcription. However, transcription is a complex, multi-stage process involving regulation of initiation as well as elongation and termination (Weake and Workman, 2010). More recent studies on E1A have revealed that it also plays a role in enhancing the elongation step of HAdV transcripts produced by RNAPII. The potential for E1A to enhance elongation was originally established from observations that cellular factors recruited to promoters in an E1A-dependent manner were also found associated on downstream areas being actively transcribed (Rasti et al., 2006). These initial findings were expanded upon in studies examining E1A's direct interaction with MED23. MED23 bridged associations between E1A and multiple other components of the Mediator complex, including MED26 and MED26-associated super elongation complex (SEC) (Vijayalingam and Chinnadurai, 2013). These interactions all contributed to E1A-mediated activation of HAdV early gene expression. However, the SEC also contains factors known to regulate the elongation of mRNA transcripts produced by RNAPII. Specifically, the SEC can relieve pausing of RNAPII on nascent transcripts, which serves as a form of checkpoint control (Luo et al., 2012). While a specific role for the SEC on elongation of HAdV transcripts wasn't elucidated, the authors' findings demonstrated that E1A-mediated transactivation was likely more complex than merely triggering the efficient formation of pre-initiation complexes. Subsequent investigation into elongation effects conferred by E1A resulted in discovery of a *bona fide* mechanism driving elongation of HAdV transcripts (Fonseca et al., 2014). E1A associated indirectly with the human Paf1 complex, composed of several proteins that accompany RNAPII from promoter to 3' end of mRNA. In this capacity, Paf1 participates in multiple aspects of transcription including recruitment of histone modifying enzymes, assembly of elongation factors that prevent dissociation of RNAPII from template, and association with factors required for proper termination of newly-synthesized mRNA (Fonseca et al., 2013; Tomson and Arndt, 2013). Loss of an E1A-Paf1 interaction during HAdV infection did not noticeably affect the initiation of HAdV early gene expression;

however, there was a drastic decrease in both E1A and RNAPII occupancy at the 3' ends of early transcription units, implying a severe defect in elongation efficiency of these viral transcripts. This was confirmed by demonstrating a decreased frequency both of full-length transcripts and H3K36 trimethylation (an elongation-specific chromatin mark) in these conditions (Butler and Dent, 2012; Fonseca et al., 2014).

The aforementioned studies have established an enhanced model of E1A-mediated viral transcription that includes both initiation- and elongation-specific functions; however, the understanding of E1A's effects on elongation is in its infancy. If and how E1A interacts with components in other complexes that comprise elongation machinery remains unknown, as does a role for E1A in modulating elongation of host transcripts. Also, while other human pathogens such as influenza A virus (IAV), human immunodefficiency virus (HIV), and herpes simplex virus (HSV) have been previously shown to manipulate elongation in various ways, it remains to be determined whether E1A from all HAdV species behave similarly (Fox et al., 2017; Lu et al., 2013; Pflug et al., 2017).

1.3.6 E1A modulates cellular transcription

The dawn of next-generation sequencing (NGS) technology aided in expanding the scope of E1A-mediated gene expression to include a global view of host cell transcription. During HAdV-5 infection of primary cells, it was discovered that E1A associated with the regulatory regions of more than 17,000 host genes (Ferrari et al., 2008; Horwitz et al., 2008). It was also demonstrated that E1A could either activate or repress many of these genes via the recruitment of cellular transcriptional machinery in a manner similar to how it controls viral gene expression (discussed above). Despite large sets of published data, the means by which E1A individually regulates the overwhelming number of host target genes is still being dissected (Ferrari et al., 2014, 2009; Radko et al., 2015). E1A can recruit transcriptional activators (such as p300/CBP) to activate transcription at previously-repressed sites. Alternatively, it can use these same interactions to recruit itself, along with transcriptional repressors (like pRb and family members p130 and p107), to suppress transcription (Horwitz et al., 2008). E1A-mediated modulation of host

transcription is therefore multifaceted, allowing a mere single viral protein to assert diverse effects on the global kinetics of cellular gene expression.

Additionally, these large-scale studies have revealed how E1A can drastically alter the regulation of cellular chromatin at the epigenetic level (Ferrari et al., 2012, 2008). Histone post-translational modifications (hPTMs) offer an additional level of host genetic regulation by loosening or restricting access of transcriptional machinery to nucleosomes (Chi et al., 2010). Highlighting E1A's ability to invade every aspect of an infected cell's protein network, E1A causes extensive reorganization of these PTMs during HAdV infection. E1A can sterically inhibit histone-modifying enzymes or act as a bridge between enzyme and substrate to enhance and establish novel connections. For instance, E1A can restructure chromatin at cellular Cdc6 and cyclin A genes to stimulate entry into S-phase (Ghosh and Harter, 2003). E1A displaces the repressive E2F4 and p130 proteins associated with histone deacetylase (HDAC1/2) and Suv39H1 histone methylase, which would normally induce repressive heterochromatin. This allows acetylation of H3K9 by the E1A-bound TRRAP-PCAF-p400 acetyltransferase complex, activating transcription from these loci (Fuchs et al., 2001; Sha et al., 2010). Similarly, E1A's concomitant associations with lysine acetyl transferases (KAT), p300 and CBP, and thousands of host gene promoters results in widespread acetylation of H3K18 across the human genome (Ferrari et al., 2008; Horwitz et al., 2008). Consequently, many cellular genes become transcriptionally active from these sites including those involved in cell cycling and macromolecular synthesis. A more recent study from our lab demonstrated E1A's interaction with Bre1 (an E3 ubiquitin ligase involved in modifying histone H2B) could block Bre1's ability to monoubiqutinate H2B during HAdV infection (Fonseca et al., 2012). This resulted in repression of type I interferon-stimulated genes (ISGs) which normally require this hPTM for their full transcription.

The ultimate purpose for E1A's transcriptional reprogramming of host genes is to block innate immunity, induce cell cycle, and reverse the differentiated phenotype in quiescent cells (Berk, 2013, 2005; Pelka et al., 2008). This provides the virus with abundant precursor pools of dNTPs and NTPs for viral DNA and RNA synthesis, and activates pathways required for protein synthesis. It also represses antiviral response genes that

would otherwise inhibit the virus' replication and spread to neighbouring cells. The sum of these effects is the creation of a host environment that is more optimal for a productive HAdV life cycle. Consequently, how E1A repurposes endogenous host factors to alter these downstream pathways remains an intense area of molecular research.

1.4 Protein Kinase A (PKA)

1.4.1 Discovery and structure of PKA

Phosphorylation was first demonstrated to be a means of regulating protein activity in 1958 with the observation that an enzyme, phosphorylase kinase (PHK), was activated by the addition of a phosphate moiety (Krebs et al., 1959; Krebs and Fischer, 1956). Over the past sixty years, the widespread importance of phosphorylation in cell biology has been conclusively demonstrated and extensively studied (Vlastaridis et al., 2017). Entire protein networks in cells can be regulated by the additions or subtractions of phosphate molecules, which can act as reversible switches for protein binding and localization, or modulate the assembly of complex tertiary and quaternary structures (Taylor et al., 2013, 2012; Welch et al., 2010; Westheimer, 1987). Remarkably, approximately 2 percent of the entire human genome encodes for protein kinases (Manning et al., 2002).

Protein kinase A (PKA) was the second kinase ever discovered (Walsh et al., 1968), was the first to have its sequence analyzed (Shoji et al., 1981), and the first to have its structure crystallized (Knighton et al., 1991). Consequently, much of what is known about phosphorylation, intracellular signaling, and kinase structure stems from using PKA as a model protein. PKA genes are conserved down to fungi or even single-celled organisms such as *Plasmodium falciparum* and Trypanosomes (Haste et al., 2012; Kannan et al., 2007). PKA can therefore be considered an ancient evolutionary mechanism contributing to the conversion of extracellular signals into biological responses.

PKA primarily exists as an inactive, tetrameric holoenzyme (Taylor et al., 2013, 2012) (Figure 1.9). Two catalytic subunits (C) are bound by two regulatory subunits (R) that exist as a constitutive homodimer. In mammalian cells, there are three isoforms of the C-subunit (Cα, Cβ, and Cγ) (Skalhegg and Tasken, 2000; Søberg et al., 2013; Turnham and

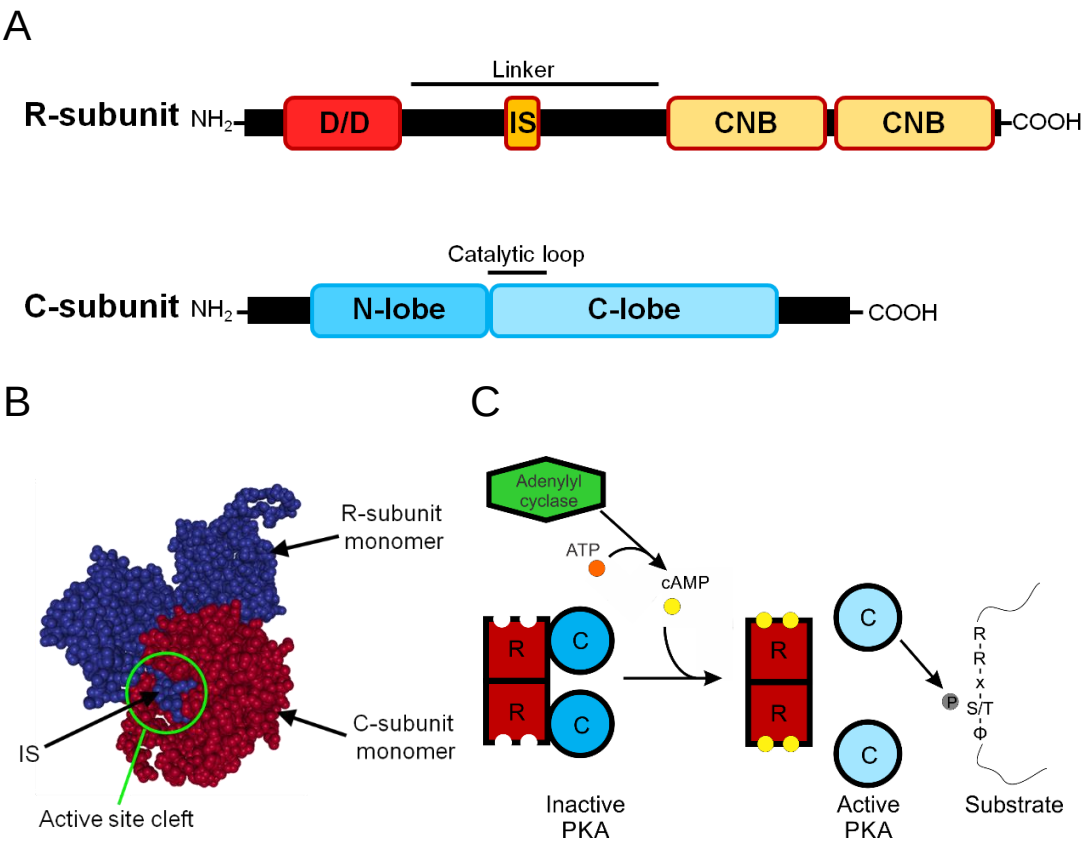


Figure 1.9. Organization and function of PKA.

(A) R-subunits contain a docking/dimerization (D/D) domain in their N-terminus and two cyclic nucleotide binding (CNB) domains in their C-terminus. These structural regions are separated by a flexible linker sequence containing an inhibitory site (IS) that binds the catalytic subunit to maintain its inactive state. C-subunits are comprised of two structural lobes that when folded, surround the active site cleft. The N-terminal lobe binds ATP while the C-terminal lobe forms the framework for catalytic machinery and binding of substrates. (B) Illustration of PKA R- and C-subunit interactions. Adapted from PDB: 2QVS (Wu et al., 2007). (C) General PKA mechanism and downstream function. R-subunit homodimers bind two C-subunits, completing an inactive tetrameric holoenzyme. When local levels of cAMP rise, the binding of this 2nd messenger to the R-subunit CNB domains causes conformational changes that free the C-subunits. Nearby protein substrates with the consensus sequence: R-R-x-S/T-Φ can be phosphorylated on Ser or Thr residues in an ATP-dependent manner. PKA functions in both the cytoplasm and nucleus in a variety of biological pathways.

Scott, 2016). These share about 93% amino acid identity and are functionally-similar. However, Cγ is expressed only in the testis and Cβ is most highly expressed in neural tissue. Cα is ubiquitously-expressed in all tissues, is a binding partner of HAdV E1A and is therefore a focus of this thesis. Additional specificity for PKA is provided by the four unique, non-redundant isoforms of R-subunits (RIα, RIIα, RIβ, and RIIβ) (Canaves and Taylor, 2002; Skalhegg and Tasken, 2000). These share only 40% amino acid identity (at most), and based on their cell type-specific expression and additional protein binding partners, add layers of regulation to PKA catalytic activity. RIα and RIIα are both direct binding targets of E1A and like Cα will be the main focus of this thesis.

As the earliest solved kinase structure, the study of PKA subunits provided crucial insights into the structural nature of all eukaryotic kinases (Hanks et al., 1988; Knighton et al., 1991; Kornev et al., 2006). The catalytic core of the Cα subunit is composed of two 'lobes,' an N-terminal β-sheet and an α-helical C-terminus (a general organization that is common across the human kinome). Highly conserved residues cluster around the active site cleft that lies between the two lobes (Taylor et al., 2013, 2012). Binding of adenosine triphosphate (ATP) commits Cα for catalysis, where it adds a phosphate moiety to serine (Ser) or threonine (Thr) residues on substrates containing the minimal consensus sequence Arg-Arg-x-Ser/Thr-Φ (Kennelly and Krebs, 1991; Masterson et al., 2010; Shabb, 2001).

Both RIα and RIIα subunits form homodimers mediated by an N-terminal 4-helix bundle termed a 'docking/dimerization (D/D) domain' (Taylor et al., 2013, 2012). The spatial organization of the R-subunit isoforms are generally similar (Banky et al., 1998; Kim et al., 2005; Wu et al., 2007). At the C-terminus are two cyclic nucleotide binding (CNB) domains. The D/D and CNB domains are separated by a flexible linker sequence containing an inhibitory sequence (IS). The IS resembles a PKA substrate and binds to the Cα active site cleft, keeping it inactive. The intrinsically disordered nature of the linker dictates the disparate quaternary structures of PKA holoenzymes between the different R-isoforms.

PKA auto-phosphorylates itself during initial folding which is essential for full maturation (Keshwani et al., 2012; Steichen et al., 2012). Additional phosphorylation by either another PKA or 3-phosphoinositide-dependent protein kinase 1 (PDK1) after leaving the ribosome is required for full functionality (Cauthron et al., 1998; Cheng et al., 1998). However, turnover activity is not regulated by auto-phosphorylation, but rather by cyclic adenosine monophosphate (cAMP). Around the same time that protein phosphorylation was being initially characterized, cAMP (produced by adenylyl cyclase) was discovered as a 2nd messenger molecule (Northup et al., 1980; Rall and Sutherland, 1958). Binding by cAMP to each of the CNB domains on PKA R-subunits causes them to undergo major conformational changes that release the C-subunits. Free Cα is then able to phosphorylate its cognate substrates.

1.4.2 Cellular functions of PKA

PKA is ubiquitous in every mammalian cell and carries out functions in both the cytoplasm and nucleus to regulate a wide array of biological events (Taylor et al., 2012). Specificity of function is conferred not only by the numerous PKA subunit isoforms and additional splice variants, but also cell-type specific expression and localization of related signaling components (Skalhegg and Tasken, 2000; Taskén and Aandahl, 2004; Welch et al., 2010; Wong and Scott, 2004). These can include dozens of different G protein-coupled receptors (GPCR), adenylyl cyclase that produces cAMP, phosphodiesterases (PDE) that break cAMP down, phosphatases, A-kinase anchoring proteins (discussed later), and the dedicated PKA substrates. In this sense, cells carefully organize machinery to create discrete foci of cAMP signaling.

The list of cellular gene products predicted or validated as phosphorylated by PKA has long been known to number in the hundreds (Diella et al., 2004; Gao et al., 2008; Shabb, 2001). Unsurprisingly, this kinase exerts functional consequences within a plethora of biological pathways, including memory formation, cell differentiation, proliferation, transcription, metabolism, apoptosis, and trafficking, among others (Johnson et al., 2001; Taylor et al., 2012). Additionally, new substrates for PKA are continually being described, aided by the advent of molecular tools such as catalytic inhibitors (H89 or PKI), cAMP stimulants like forskolin (fsk), and anchoring disruptors (RIAD and

sAKAPis) (Alasbahi and Melzig, 2012; Carlson et al., 2006; Chijiwa et al., 1990; Gold et al., 2006).

In the broadest sense, PKA is a master regulator of intracellular molecular signaling, even participating in feedback within its own signaling pathway. When intracellular cAMP levels are high, PKA phosphorylates the β2-adrenergic receptor which causes the downstream inhibitory G_i protein to inhibit adenylyl cyclase, lowering cAMP production (Daaka et al., 1997; Hausdorff et al., 1990). Similarly, PKA also activates multiple PDEs which hydrolyze cAMP to reduce overall levels of this 2nd messenger (Conti, 2000). In addition to cAMP signaling, PKA cross-talks in other discrete pathways by targeting proteins in the cGMP, calcium, Rho, mitogen-activated protein kinase (MAPK), and protein phosphatase 1 (PP1) signaling networks (Shabb, 2001).

At a more focused level, one of PKA's major functions involves modulating cellular transcription. Many PKA substrates include TFs or cofactors localized within the nucleus that function through the cis-acting cAMP response element (CRE) commonly found in promoters. Well-characterized TFs, whose CRE-binding ability is modulated via phosphorylation by PKA, include CRE-binding protein (CREB), CRE modulator (CREM), and members of the activating transcription factor (ATF) family (De Cesare and Sassone-Corsi, 2000; Gonzalez and Montminy, 1989; Rehfuss et al., 1991). Through CREB alone, PKA is putatively capable of affecting transcription of thousands of cellular genes (Daniel et al., 1998; Mayr and Montminy, 2001; Zhang et al., 2005). Additional TF targets of PKA include NF-κB and nuclear factor of activated T cells (NFAT), though their roles in cAMP-mediated transcriptional signaling are less clear (Serfling et al., 2000; Ye, 2000). Given the roles for both PKA and HAdV E1A in regulating gene expression, PKA is a well-connected and attractive target for molecular reprogramming by E1A during a HAdV infection.

Additional miscellaneous functions of PKA phosphorylation are numerous. They include epigenetic modifications of histones - in fact, histone H1 was the first PKA substrate to have its phosphorylation site established *in vivo* (DeManno et al., 1999; Langan, 1969). PKA also signals within cascades controlling cell death, phosphorylating proapoptotic

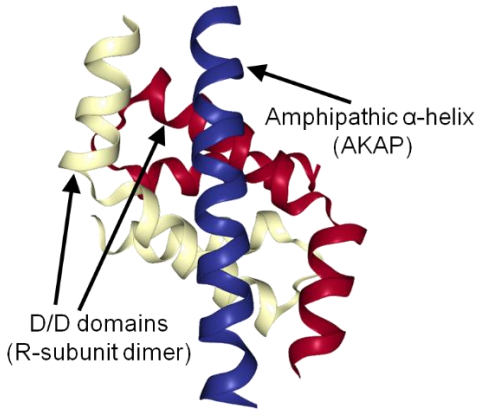
factors such as BAD or glycogen synthase kinase-3 (GSK-3) (Li et al., 2000; Lizcano et al., 2000). Over a third of PKA's known targets are involved in controlling ion channels and water homeostasis, allowing PKA to affect molecular interactions mediating muscle contractions and cell-to-cell signaling in cardiovascular and neuronal tissues (Dagda and Das Banerjee, 2015; Gold et al., 2013; Shabb, 2001). Metabolic targets of PKA include enzymes in the glycolysis and gluconeogenesis pathways, allowing PKA to integrate into the control of blood glucose levels (Okar and Lange, 1999). Lastly, PKA has been well-studied for its capacity to affect cytoskeletal arrangements. PKA activity regulates actin bundling and downstream cell motility and adhesion (Chen et al., 2013; McKenzie et al., 2011). It also participates in the dynein-mediated transport of cargo along microtubule networks, a process actually usurped by HAdV virions during the entry phase of infection (Bremner et al., 2009; Scherer et al., 2014; Suomalainen et al., 2001). To summarize, the diversity of its substrates, along with its flexible recognition properties make PKA a crucial component for molecular control of a cell.

In general, kinases are highly conserved across widely distinct phyla, underlining their biological importance (Kannan et al., 2007). Kinase mutations are often associated with disease, making them ideal targets for drug discovery and PKA is no exception (Taylor et al., 2012). Given the range of functions PKA's reach extends into, it has been studied as a molecular component in several human disease contexts, including cataracts, diabetes, cardiovascular disease, and multiple types of cancer (Gold et al., 2013; McKenzie et al., 2011; Palorini et al., 2016; Pattabiraman et al., 2016). In addition, dysregulation of the cAMP signaling pathway is exploited by a diverse range of viral pathogens such as HSV, HIV, varicella-zoster virus (VZV), HPV, hepatitis C virus (HCV), and hepatitis B virus (HBV) (Barnitz et al., 2010; Benetti and Roizman, 2004; Boon et al., 2015; Cartier et al., 2003; Delury et al., 2013; Desloges et al., 2008; Giroud et al., 2013; Majeau et al., 2007; Okabe et al., 2006). How this occurs in the context of HAdV infection via E1A is the focus of this thesis.

1.4.3 A-kinase anchoring proteins (AKAPs)

Localization of the PKA holoenzyme is a major contributing factor for achieving its functional specificity. This spatiotemporal control of PKA activity is conferred by

A



B

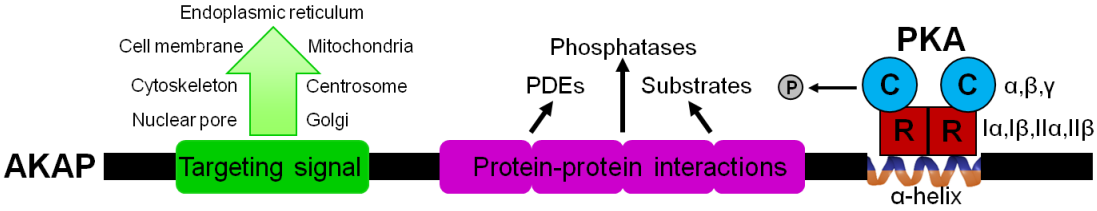


Figure 1.10. Organization and function of AKAPs.

(A) Structural representation of an AKAP amphipathic α-helix motif binding across the D/D domain of PKA R-subunit homodimer. Adapted from PDB: 3IM4 (Sarma et al., 2010). (B) The general organization of functional domains within AKAPs is depicted. AKAPs are variably-sized, polyvalent, and differentially-localized within the cytoplasm of cells. AKAP-PKA interactions can be RI-, RII, or dual-specific. Along with their PKA-binding helix, AKAPs typically contain interaction motifs for other components of cAMP signaling, such as PDEs, phosphatases, and PKA substrates. They also tend to possess a targeting domain that directs AKAPs along with their compartmentalized binding partners to distinct subcellular compartments.

cellular A-kinase anchoring proteins (AKAPs), a set of polyvalent and highly dynamic scaffold proteins (Taylor et al., 2012; Welch et al., 2010; Wong and Scott, 2004). First discovered in the 1980s, there are now over 30 distinct AKAPs in humans, each providing similar but distinct abilities to regulate PKA (Theurkauf and Vallee, 1982). AKAPs all contain a hallmark PKA-binding motif comprised of an amphipathic α-helix (Carr et al., 1991). This directly binds with high-affinity to the hydrophobic groove in the D/D domain of PKA R-subunit dimers (Figure 1.10). AKAP-PKA interactions occur in the cytoplasm and are usually specific for either RIα or RIIα subunits, but dual-specificity AKAPs exist as well (Carlson et al., 2006; Gold et al., 2006).

In addition to binding PKA, AKAPs can contain motifs directing their subcellular localization to specific compartments such as the cytoskeleton, centrosome, golgi, ER, mitochondria, or NPC (Taylor et al., 2012; Welch et al., 2010; Wong and Scott, 2004). Here AKAPs will simultaneously tether interactions between PKA, substrates, and up- or down-stream regulators of the cAMP pathway. By carefully controlling the proximity of this broad-specificity kinase, AKAPs enable tight and efficient control of cAMP signaling mediated through PKA. The interface between AKAPs and PKA therefore offers a target for manipulation, a feature exploited by HAdV E1A.

1.5 Viral Mimicry

How individual viral proteins can radically alter the robust interaction networks found in eukaryotes remains a fascinating topic of study. The highly organized nature of cells requires orchestration of countless protein-protein interactions mediated via structural domains or interaction motifs such as SLiMs (Davey et al., 2015; Dunker et al., 2005; Kim et al., 2008). This feature is also shared by some viral proteins, whose enhanced evolutionary flexibility allows them to quickly adapt to cellular environments, enabling integration into and rewiring of their protein networks (Davey et al., 2011). This includes *ex nihilo* evolution of SLiMs – the ability to rapidly develop a functional domain within a previously non-functional protein sequence. Consequently, viruses always have an advantage in the unceasing arms race between pathogen and host. Viral hubs like HAdV E1A can manipulate target proteins (and secondary interactors) using only small stretches

of amino acid sequence, including motifs that mimic cellular counterparts (King et al., 2016a; Pelka et al., 2008). Instead of encoding entire orthologs of large cellular components or evolving complicated globular structures, this strategy is an efficient means of increasing the functionality of the size-constrained HAdV genome (Avgousti et al., 2016; Chahal et al., 2012; King et al., 2016b). The success of viral mimicry is overwhelmingly demonstrated by how often it has evolved convergently in genetically-distinct viruses (Brito and Pinney, 2017; Chemes et al., 2015; Via et al., 2015). It is also telling that the full complement of cellular processes have been shown as susceptible to viral "corruption" in this manner (Davey et al., 2011). Understanding the mechanisms driving viral hijacking of host components via mimicry will reveal new molecular aspects of cell regulation, viral pathogenesis, and human disease.

1.6 Thesis Overview

The work within this thesis involves the biochemical and functional analysis of the protein-protein interaction between HAdV E1A and cellular PKA. Originally identified in 2001, the E1A-PKA association remained largely uncharacterized and its biological importance unknown (Fax et al., 2001). Here, extensive mutagenesis was utilized to determine the precise molecular mechanism of this virus-host interaction, ultimately revealing a novel example of viral mimicry. Subsequent findings validated the functional importance of this role for E1A in enhancing the HAdV replication cycle. The conservation of this function across the seven HAdV species was also determined, demonstrating its evolutionary importance. Finally, a detailed model was established explaining E1A's ability to engage in mimicry for the purpose of rewiring cellular cAMP signaling to benefit HAdV infection.

1.6.1 Chapter 2: Functional and structural mimicry of cellular protein kinase A anchoring proteins by a viral oncoprotein

In this study, we performed extensive mutational analysis on both HAdV-5 E1A and cellular PKA subunits to characterize the molecular determinants of their protein-protein interaction. We demonstrated that the N-terminus of E1A has evolved to structurally mimic the PKA-binding domain of cellular AKAPs. We also showed this mimicry to be

functional, as E1A could emulate AKAP activity *in vivo*. E1A relocalizes PKA to the nucleus of infected cells via an interaction with the D/D domain in PKA's regulatory subunits. This required an AKAP-like amphipathic α-helix motif in E1A's N-terminus. Importantly, we showed that during HAdV infection, E1A competes with endogenous AKAPs for PKA binding. We also showed that incorporation of E1A's AKAP-like motif into a cellular AKAP mutant unable to bind PKA restored the interaction. Functionally, E1A's ability to act like a 'viral AKAP' (vAKAP) contributed to efficient viral transcription, protein synthesis, and progeny production.

1.6.2 Chapter 3: Mimicry of cellular A kinase anchoring proteins is a conserved and critical function of E1A across various human adenovirus species

Following the previous chapter, we examined the ability of E1A proteins from all seven species of HAdV to act as 'viral AKAPs.' We showed that E1A from representative viruses from five of seven HAdV species make protein-protein interactions with PKA subunits. The conserved mechanism of this binding included each of these distinct E1As using small, AKAP-like motifs in their N-terminal regions to bind the D/D domain of PKA R-subunits. Like with HAdV-5, this caused relocalization of PKA into the nucleus. Interestingly, different HAdV species had a preference for one particular PKA isoform (RIα or RIIα) over another. Notably, we showed these newly characterized vAKAPs could integrate into cAMP signaling to activate transcription from a cAMP-responsive promoter. Additionally, we demonstrated the vAKAP function of E1A contributes to viral genome replication and infectious progeny production for several, unique HAdV species. These data established that mimicry and subversion of this important cellular regulatory pathway is a conserved feature for multiple viral pathogens and enhanced the knowledge of how vAKAPs function.

1.6.3 Chapter 4: General Discussion

To conclude the thesis, I examine the overall significance of my work and address lingering questions related to the projects presented. Additionally, I present potential follow-up experiments including some preliminary analysis of genome-wide changes to cellular transcription possibly linked to the E1A's role as a vAKAP.

1.7 References

Abken, H., Butzler, C., Willecke, K., 1987. Adenovirus type 5 persisting in human lymphocytes is unlikely to be involved in immortalization of lymphoid cells by fusion with cytoplasts or by transfection with DNA of mouse L cells. Anticancer Res. 7, 553–558.

Ablack, J.N.G., Cohen, M., Thillainadesan, G., Fonseca, G.J., Pelka, P., Torchia, J., Mymryk, J.S., 2012. Cellular GCN5 Is a Novel Regulator of Human Adenovirus E1A-Conserved Region 3 Transactivation. J. Virol. 86, 8198–8209.

Ablack, J.N.G., Pelka, P., Yousef, A.F., Turnell, A.S., Grand, R.J.A., Mymryk, J.S., 2010. Comparison of E1A CR3-Dependent Transcriptional Activation across Six Different Human Adenovirus Subgroups. J. Virol. 84, 12771–12781.

Adams, M.J., Lefkowitz, E.J., King, A.M.Q., Harrach, B., Harrison, R.L., Knowles, N.J., Kropinski, A.M., Krupovic, M., Kuhn, J.H., Mushegian, A.R., Nibert, M., Sabanadzovic, S., Sanfaçon, H., Siddell, S.G., Simmonds, P., Varsani, A., Zerbini, F.M., Gorbalenya, A.E., Davison, A.J., 2017. Changes to taxonomy and the International Code of Virus Classification and Nomenclature ratified by the International Committee on Taxonomy of Viruses (2017). Arch. Virol. 162, 2505–2538.

Ahi, Y.S., Mittal, S.K., 2016. Components of Adenovirus Genome Packaging. Front. Microbiol. 7, 1503.

Alasbahi, R.H., Melzig, M.F., 2012. Forskolin and derivatives as tools for studying the role of cAMP. Pharmazie 67, 5–13.

Albinsson, B., Kidd, A.H., 1999. Adenovirus type 41 lacks an RGD alpha(v)-integrin binding motif on the penton base and undergoes delayed uptake in A549 cells. Virus Res. 64, 125–36.

Andersson, M.G., Haasnoot, P.C.J., Xu, N., Berenjian, S., Berkhout, B., Akusjarvi, G., 2005. Suppression of RNA Interference by Adenovirus Virus-Associated RNA. J. Virol. 79, 9556–9565.

Andreotti, G., Méndez, B.L., Amodeo, P., Morelli, M.A.C., Nakamuta, H., Motta, A., 2006. Structural determinants of salmon calcitonin bioactivity: the role of the Leu-based amphipathic alpha-helix. J. Biol. Chem. 281, 24193–203.

Aoki, K., Benkö, M., Davison, A.J., Echavarria, M., Erdman, D.D., Harrach, B., Kajon, A.E., Schnurr, D., Wadell, G., Members of the Adenovirus Research Community, 2011. Toward an integrated human adenovirus designation system that utilizes molecular and serological data and serves both clinical and fundamental virology. J. Virol. 85, 5703–4.

Arany, Z., Newsome, D., Oldread, E., Livingston, D.M., Eckner, R., 1995. A family of transcriptional adaptor proteins targeted by the E1A oncoprotein. Nature 374, 81–4.

Araujo, F.D., Stracker, T.H., Carson, C.T., Lee, D. V, Weitzman, M.D., 2005. Adenovirus type 5 E4orf3 protein targets the Mre11 complex to cytoplasmic aggresomes. J. Virol. 79, 11382–91.

Avgousti, D.C., Herrmann, C., Kulej, K., Pancholi, N.J., Sekulic, N., Petrescu, J., Molden, R.C., Blumenthal, D., Paris, A.J., Reyes, E.D., Ostapchuk, P., Hearing, P., Seeholzer, S.H., Worthen, G.S., Black, B.E., Garcia, B.A., Weitzman, M.D., 2016. A core viral protein binds host nucleosomes to sequester immune danger signals. Nature 535, 173–7.

Avvakumov, N., Kajon, A.E., Hoeben, R.C., Mymryk, J.S., 2004. Comprehensive sequence analysis of the E1A proteins of human and simian adenoviruses. Virology 329, 477–92.

Avvakumov, N., Sahbegovic, M., Zhang, Z., Shuen, M., Mymryk, J.S., 2002a. Analysis of DNA binding by the adenovirus type 5 E1A oncoprotein. J. Gen. Virol. 83, 517–24.

Avvakumov, N., Wheeler, R., D'Halluin, J.C., Mymryk, J.S., 2002b. Comparative sequence analysis of the largest E1A proteins of human and simian adenoviruses. J. Virol. 76, 7968–75.

Banky, P., Huang, L.J., Taylor, S.S., 1998. Dimerization/docking domain of the type Ialpha regulatory subunit of cAMP-dependent protein kinase. Requirements for dimerization and docking are distinct but overlapping. J. Biol. Chem. 273, 35048–55.

Barnitz, R.A., Wan, F., Tripuraneni, V., Bolton, D.L., Lenardo, M.J., 2010. Protein kinase A phosphorylation activates Vpr-induced cell cycle arrest during human immunodeficiency virus type 1 infection. J. Virol. 84, 6410–24.

Baumann, H., Paulsen, K., Kovács, H., Berglund, H., Wright, A.P., Gustafsson, J.A., Härd, T., 1993. Refined solution structure of the glucocorticoid receptor DNA-binding domain. Biochemistry 32, 13463–71.

Bayley, S.T., Mymryk, J.S., 1994. Adenovirus e1a proteins and transformation (review). Int. J. Oncol. 5, 425–44.

Benedict, C.A., Norris, P.S., Prigozy, T.I., Bodmer, J.-L., Mahr, J.A., Garnett, C.T., Martinon, F., Tschopp, J., Gooding, L.R., Ware, C.F., 2001. Three Adenovirus E3 Proteins Cooperate to Evade Apoptosis by Tumor Necrosis Factor-related Apoptosis-inducing Ligand Receptor-1 and -2. J. Biol. Chem. 276, 3270–3278.

Benetti, L., Roizman, B., 2004. Herpes simplex virus protein kinase US3 activates and functionally overlaps protein kinase A to block apoptosis. Proc. Natl. Acad. Sci. U.

S. A. 101, 9411–6.

Bennett, E.M., Bennink, J.R., Yewdell, J.W., Brodsky, F.M., 1999. Cutting edge: adenovirus E19 has two mechanisms for affecting class I MHC expression. J. Immunol. 162, 5049–52.

Bergelson, J.M., Cunningham, J.A., Droguett, G., Kurt-Jones, E.A., Krithivas, A., Hong, J.S., Horwitz, M.S., Crowell, R.L., Finberg, R.W., 1997. Isolation of a common receptor for Coxsackie B viruses and adenoviruses 2 and 5. Science 275, 1320–3.

Berget, S.M., Moore, C., Sharp, P.A., 1977. Spliced segments at the 5' terminus of adenovirus 2 late mRNA. Proc. Natl. Acad. Sci. U. S. A. 74, 3171–5.

Bergman, L.M., Blaydes, J.P., 2006. C-terminal binding proteins: Emerging roles in cell survival and tumorigenesis. Apoptosis 11, 879–888.

Berk, A., 2013. Adenoviridae. In: Fields Virology. pp. 1704–1731.

Berk, A.J., 2005. Recent lessons in gene expression, cell cycle control, and cell biology from adenovirus. Oncogene 24, 7673–85.

Berk, A.J., Lee, F., Harrison, T., Williams, J., Sharp, P.A., 1979. Pre-early adenovirus 5 gene product regulates synthesis of early viral messenger RNAs. Cell 17, 935–44.

Binder, A.M., Biggs, H.M., Haynes, A.K., Chommanard, C., Lu, X., Erdman, D.D., Watson, J.T., Gerber, S.I., 2017. Human Adenovirus Surveillance — United States, 2003–2016. MMWR. Morb. Mortal. Wkly. Rep. 66, 1039–1042.

Blanchette, P., Cheng, C.Y., Yan, Q., Ketner, G., Ornelles, D.A., Dobner, T., Conaway, R.C., Conaway, J.W., Branton, P.E., 2004. Both BC-Box Motifs of Adenovirus Protein E4orf6 Are Required To Efficiently Assemble an E3 Ligase Complex That Degrades p53. Mol. Cell. Biol. 24, 9619–9629.

Bondesson, M., Mannervik, M., Akusjärvi, G., Svensson, C., 1994. An adenovirus E1A transcriptional repressor domain functions as an activator when tethered to a promoter. Nucleic Acids Res. 22, 3053–60.

Boon, S.S., Tomaić, V., Thomas, M., Roberts, S., Banks, L., 2015. Cancer-causing human papillomavirus E6 proteins display major differences in the phospho-regulation of their PDZ interactions. J. Virol. 89, 1579–86.

Boyd, J.M., Subramanian, T., Schaeper, U., La Regina, M., Bayley, S., Chinnadurai, G., 1993. A region in the C-terminus of adenovirus 2/5 E1a protein is required for association with a cellular phosphoprotein and important for the negative modulation of T24-ras mediated transformation, tumorigenesis and metastasis. EMBO J. 12, 469–78.

Boyer, G.S., Denny, F.W., Ginsberg, H.S., 1959. Sequential cellular changes produced by

types 5 and 7 adenoviruses in HeLa cells and in human amniotic cells; cytological studies aided by fluorescein-labelled antibody. J. Exp. Med. 110, 827–44.

Boyer, T.G., Martin, M.E., Lees, E., Ricciardi, R.P., Berk, A.J., 1999. Mammalian Srb/Mediator complex is targeted by adenovirus E1A protein. Nature 399, 276–9.

Bremner, K.H., Scherer, J., Yi, J., Vershinin, M., Gross, S.P., Vallee, R.B., 2009. Adenovirus transport via direct interaction of cytoplasmic dynein with the viral capsid hexon subunit. Cell Host Microbe 6, 523–35.

Bridge, E., Ketner, G., 1990. Interaction of adenoviral E4 and E1b products in late gene expression. Virology 174, 345–53.

Brito, A.F., Pinney, J.W., 2017. Protein-Protein Interactions in Virus-Host Systems. Front. Microbiol. 8, 1557.

Bruton, R.K., Pelka, P., Mapp, K.L., Fonseca, G.J., Torchia, J., Turnell, A.S., Mymryk, J.S., Grand, R.J.A., 2008. Identification of a Second CtBP Binding Site in Adenovirus Type 5 E1A Conserved Region 3. J. Virol. 82, 8476–8486.

Burgert, H.G., Kvist, S., 1985. An adenovirus type 2 glycoprotein blocks cell surface expression of human histocompatibility class I antigens. Cell 41, 987–97.

Burgert, H.G., Maryanski, J.L., Kvist, S., 1987. "E3/19K" protein of adenovirus type 2 inhibits lysis of cytolytic T lymphocytes by blocking cell-surface expression of histocompatibility class I antigens. Proc. Natl. Acad. Sci. U. S. A. 84, 1356–60.

Butler, J.S., Dent, S.Y.R., 2012. Chromatin “resetting” during transcription elongation: a central role for methylated H3K36. Nat. Struct. Mol. Biol. 19, 863–4.

Canaves, J.M., Taylor, S.S., 2002. Classification and phylogenetic analysis of the cAMP-dependent protein kinase regulatory subunit family. J. Mol. Evol. 54, 17–29.

Cantin, G.T., Stevens, J.L., Berk, A.J., 2003. Activation domain-mediator interactions promote transcription preinitiation complex assembly on promoter DNA. Proc. Natl. Acad. Sci. U. S. A. 100, 12003–8.

Carlson, C.R., Lygren, B., Berge, T., Hoshi, N., Wong, W., Taskén, K., Scott, J.D., 2006. Delineation of type I protein kinase A-selective signaling events using an RI anchoring disruptor. J. Biol. Chem. 281, 21535–45.

Carmody, R.J., Maguschak, K., Chen, Y.H., 2006. A novel mechanism of nuclear factor-kappaB regulation by adenoviral protein 14.7K. Immunology 117, 188–95.

Carr, D.W., Stofko-Hahn, R.E., Fraser, I.D., Bishop, S.M., Acott, T.S., Brennan, R.G., Scott, J.D., 1991. Interaction of the regulatory subunit (RII) of cAMP-dependent protein kinase with RII-anchoring proteins occurs through an amphipathic helix

binding motif. J. Biol. Chem. 266, 14188–92.

Cartier, C., Hemonnot, B., Gay, B., Bardy, M., Sanchiz, C., Devaux, C., Briant, L., 2003. Active cAMP-dependent protein kinase incorporated within highly purified HIV-1 particles is required for viral infectivity and interacts with viral capsid protein. J. Biol. Chem. 278, 35211–9.

Cauthron, R.D., Carter, K.B., Liauw, S., Steinberg, R.A., 1998. Physiological phosphorylation of protein kinase A at Thr-197 is by a protein kinase A kinase. Mol. Cell. Biol. 18, 1416–23.

Chahal, J.S., Qi, J., Flint, S.J., 2012. The human adenovirus type 5 E1B 55 kDa protein obstructs inhibition of viral replication by type I interferon in normal human cells. PLoS Pathog. 8, e1002853.

Challberg, M.D., Desiderio, S. V, Kelly, T.J., 1980. Adenovirus DNA replication in vitro: characterization of a protein covalently linked to nascent DNA strands. Proc. Natl. Acad. Sci. U. S. A. 77, 5105–9.

Challberg, M.D., Kelly, T.J., 1979. Adenovirus DNA replication in vitro: origin and direction of daughter strand synthesis. J. Mol. Biol. 135, 999–1012.

Challberg, M.D., Kelly, T.J., 1981. Processing of the adenovirus terminal protein. J. Virol. 38, 272–7.

Chatterjee, P.K., Bruner, M., Flint, S.J., Harter, M.L., 1988. DNA-binding properties of an adenovirus 289R E1A protein. EMBO J. 7, 835–41.

Chemes, L.B., de Prat-Gay, G., Sánchez, I.E., 2015. Convergent evolution and mimicry of protein linear motifs in host-pathogen interactions. Curr. Opin. Struct. Biol. 32, 91–101.

Chen, E.C., Yagi, S., Kelly, K.R., Mendoza, S.P., Tarara, R.P., Canfield, D.R., Maninger, N., Rosenthal, A., Spinner, A., Bales, K.L., Schnurr, D.P., Lerche, N.W., Chiu, C.Y., 2011. Cross-species transmission of a novel adenovirus associated with a fulminant pneumonia outbreak in a new world monkey colony. PLoS Pathog. 7, e1002155.

Chen, J., Morral, N., Engel, D.A., 2007. Transcription releases protein VII from adenovirus chromatin. Virology 369, 411–422.

Chen, L., Brown, J.W., Mok, Y.-F., Hatters, D.M., McKnight, C.J., 2013. The Allosteric Mechanism Induced by Protein Kinase A (PKA) Phosphorylation of Dematin (Band 4.9). J. Biol. Chem. 288, 8313–8320.

Cheng, X., Ma, Y., Moore, M., Hemmings, B.A., Taylor, S.S., 1998. Phosphorylation and activation of cAMP-dependent protein kinase by phosphoinositide-dependent protein kinase. Proc. Natl. Acad. Sci. U. S. A. 95, 9849–54.

Chi, P., Allis, C.D., Wang, G.G., 2010. Covalent histone modifications — miswritten, misinterpreted and mis-erased in human cancers. Nat. Rev. Cancer 10, 457–469.

Chijiwa, T., Mishima, A., Hagiwara, M., Sano, M., Hayashi, K., Inoue, T., Naito, K., Toshioka, T., Hidaka, H., 1990. Inhibition of forskolin-induced neurite outgrowth and protein phosphorylation by a newly synthesized selective inhibitor of cyclic AMP-dependent protein kinase, N-[2-(p-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide (H-89), of PC12D pheochromocytoma cells. J. Biol. Chem. 265, 5267–72.

Chinnadurai, G., 2002. CtBP, an unconventional transcriptional corepressor in development and oncogenesis. Mol. Cell 9, 213–24.

Chinnadurai, G., 2009. The Transcriptional Corepressor CtBP: A Foe of Multiple Tumor Suppressors. Cancer Res. 69, 731–734.

Chiou, S.K., Rao, L., White, E., 1994. Bcl-2 blocks p53-dependent apoptosis. Mol. Cell. Biol. 14, 2556–63.

Chow, L.T., Gelinas, R.E., Broker, T.R., Roberts, R.J., 1977. An amazing sequence arrangement at the 5’ ends of adenovirus 2 messenger RNA. Cell 12, 1–8.

Cohen, M.J., Yousef, A.F., Massimi, P., Fonseca, G.J., Todorovic, B., Pelka, P., Turnell, A.S., Banks, L., Mymryk, J.S., 2013. Dissection of the C-Terminal Region of E1A Redefines the Roles of CtBP and Other Cellular Targets in Oncogenic Transformation. J. Virol. 87, 10348–10355.

Conti, M., 2000. Phosphodiesterases and cyclic nucleotide signaling in endocrine cells. Mol. Endocrinol. 14, 1317–27.

Corbeil, H.B., Branton, P.E., 1994. Functional importance of complex formation between the retinoblastoma tumor suppressor family and adenovirus E1A proteins as determined by mutational analysis of E1A conserved region 2. J. Virol. 68, 6697–709.

Couch, R.B., Cate, T.R., Douglas, R.G., Gerone, P.J., Knight, V., 1966. Effect of route of inoculation on experimental respiratory viral disease in volunteers and evidence for airborne transmission. Bacteriol. Rev. 30, 517–29.

Cuconati, A., White, E., 2002. Viral homologs of BCL-2: role of apoptosis in the regulation of virus infection. Genes Dev. 16, 2465–2478.

Culp, J.S., Webster, L.C., Friedman, D.J., Smith, C.L., Huang, W.J., Wu, F.Y., Rosenberg, M., Ricciardi, R.P., 1988. The 289-amino acid E1A protein of adenovirus binds zinc in a region that is important for trans-activation. Proc. Natl. Acad. Sci. U. S. A. 85, 6450–4.

Daaka, Y., Luttrell, L.M., Lefkowitz, R.J., 1997. Switching of the coupling of the beta2-

adrenergic receptor to different G proteins by protein kinase A. Nature 390, 88–91.

Dagda, R.K., Das Banerjee, T., 2015. Role of protein kinase A in regulating mitochondrial function and neuronal development: implications to neurodegenerative diseases. Rev. Neurosci. 26, 359–70.

Dahiya, A., Gavin, M.R., Luo, R.X., Dean, D.C., 2000. Role of the LXCXE binding site in Rb function. Mol. Cell. Biol. 20, 6799–805.

Dales, S., Chardonnet, Y., 1973. Early events in the interaction of adenoviruses with HeLa cells. IV. Association with microtubules and the nuclear pore complex during vectorial movement of the inoculum. Virology 56, 465–83.

Daniel, P.B., Walker, W.H., Habener, J.F., 1998. Cyclic AMP signaling and gene regulation. Annu. Rev. Nutr. 18, 353–83.

Daniell, E., 1976. Genome structure of incomplete particles of adenovirus. J. Virol. 19, 685–708.

Davey, N.E., Cyert, M.S., Moses, A.M., 2015. Short linear motifs – ex nihilo evolution of protein regulation. Cell Commun. Signal. 13, 43.

Davey, N.E., Travé, G., Gibson, T.J., 2011. How viruses hijack cell regulation. Trends Biochem. Sci. 36, 159–169.

Davison, A.J., Benko, M., Harrach, B., 2003. Genetic content and evolution of adenoviruses. J. Gen. Virol. 84, 2895–2908.

De Cesare, D., Sassone-Corsi, P., 2000. Transcriptional regulation by cyclic AMP-responsive factors. Prog. Nucleic Acid Res. Mol. Biol. 64, 343–69.

Dehghan, S., Seto, J., Liu, E.B., Walsh, M.P., Dyer, D.W., Chodosh, J., Seto, D., 2013. Computational analysis of four human adenovirus type 4 genomes reveals molecular evolution through two interspecies recombination events. Virology 443, 197–207.

Deleu, L., Shellard, S., Alevizopoulos, K., Amati, B., Land, H., 2001. Recruitment of TRRAP required for oncogenic transformation by E1A. Oncogene 20, 8270–5.

Delury, C.P., Marsh, E.K., James, C.D., Boon, S.S., Banks, L., Knight, G.L., Roberts, S., 2013. The role of protein kinase A regulation of the E6 PDZ-binding domain during the differentiation-dependent life cycle of human papillomavirus type 18. J. Virol. 87, 9463–72.

DeManno, D.A., Cottom, J.E., Kline, M.P., Peters, C.A., Maizels, E.T., Hunzicker-Dunn, M., 1999. Follicle-Stimulating Hormone Promotes Histone H3 Phosphorylation on Serine-10. Mol. Endocrinol. 13, 91–105.

Deng, J., Kloosterbooer, F., Xia, W., Hung, M.-C., 2002. The NH(2)-terminal and

conserved region 2 domains of adenovirus E1A mediate two distinct mechanisms of tumor suppression. Cancer Res. 62, 346–50.

Deng, J., Xia, W., Hung, M.-C., 1998. Adenovirus 5 E1A-mediated tumor suppression associated with E1A-mediated apoptosis in vivo. Oncogene 17, 2167–2175.

Desloges, N., Rahaus, M., Wolff, M.H., 2008. The phosphorylation profile of protein kinase A substrates is modulated during Varicella-zoster virus infection. Med. Microbiol. Immunol. 197, 353–60.

Devaux, C., Caillet-Boudin, M.L., Jacrot, B., Boulanger, P., 1987. Crystallization, enzymatic cleavage, and the polarity of the adenovirus type 2 fiber. Virology 161, 121–8.

Diella, F., Cameron, S., Gemünd, C., Linding, R., Via, A., Kuster, B., Sicheritz-Pontén, T., Blom, N., Gibson, T.J., 2004. Phospho.ELM: a database of experimentally verified phosphorylation sites in eukaryotic proteins. BMC Bioinformatics 5, 79.

DiMaio, D., Fan, H., 2013. Viruses, Cell Transformation, and Cancer. In: Fields Virology. pp. 153–188.

Dobner, T., Horikoshi, N., Rubenwolf, S., Shenk, T., 1996. Blockage by adenovirus E4orf6 of transcriptional activation by the p53 tumor suppressor. Science 272, 1470–3.

Dunker, A.K., Cortese, M.S., Romero, P., Iakoucheva, L.M., Uversky, V.N., 2005. Flexible nets. The roles of intrinsic disorder in protein interaction networks. FEBS J. 272, 5129–5148.

Eckner, R., Ewen, M.E., Newsome, D., Gerdes, M., DeCaprio, J.A., Lawrence, J.B., Livingston, D.M., 1994. Molecular cloning and functional analysis of the adenovirus E1A-associated 300-kD protein (p300) reveals a protein with properties of a transcriptional adaptor. Genes Dev. 8, 869–84.

Ersching, J., Hernandez, M.I.M., Cezarotto, F.S., Ferreira, J.D.S., Martins, A.B., Switzer, W.M., Xiang, Z., Ertl, H.C.J., Zanetti, C.R., Pinto, A.R., 2010. Neutralizing antibodies to human and simian adenoviruses in humans and New-World monkeys. Virology 407, 1–6.

Evans, J.D., Hearing, P., 2005. Relocalization of the Mre11-Rad50-Nbs1 Complex by the Adenovirus E4 ORF3 Protein Is Required for Viral Replication. J. Virol. 79, 6207–6215.

Fahnestock, M.L., Lewis, J.B., 1989. Genetic dissection of the transactivating domain of the E1a 289R protein of adenovirus type 2. J. Virol. 63, 1495–504.

Farrow, S.N., White, J.H.M., Martinou, I., Raven, T., Pun, K.-T., Grinham, C.J., Martinou, J.-C., Brown, R., 1995. Cloning of a bcl-2 homologue by interaction with

adenovirus E1B 19K. Nature 374, 731–733.

Fattaey, A.R., Harlow, E., Helin, K., 1993. Independent regions of adenovirus E1A are required for binding to and dissociation of E2F-protein complexes. Mol. Cell. Biol. 13, 7267–77.

Fax, P., Carlson, C.R., Collas, P., Taskén, K., Esche, H., Brockmann, D., 2001. Binding of PKA-RIIalpha to the Adenovirus E1A12S oncoprotein correlates with its nuclear translocation and an increase in PKA-dependent promoter activity. Virology 285, 30–41.

Fernandes, E.R., Rooney, R.J., 1997. The adenovirus E1A-regulated transcription factor E4F is generated from the human homolog of nuclear factor phiAP3. Mol. Cell. Biol. 17, 1890–903.

Ferrari, R., Berk, A.J., Kurdistani, S.K., 2009. Viral manipulation of the host epigenome for oncogenic transformation. Nat. Rev. Genet. 10, 290–4.

Ferrari, R., Gou, D., Jawdekar, G., Johnson, S.A., Nava, M., Su, T., Yousef, A.F., Zemke, N.R., Pellegrini, M., Kurdistani, S.K., Berk, A.J., 2014. Adenovirus small E1A employs the lysine acetylases p300/CBP and tumor suppressor Rb to repress select host genes and promote productive virus infection. Cell Host Microbe 16, 663–76.

Ferrari, R., Pellegrini, M., Horwitz, G.A., Xie, W., Berk, A.J., Kurdistani, S.K., 2008. Epigenetic reprogramming by adenovirus e1a. Science 321, 1086–8.

Ferrari, R., Su, T., Li, B., Bonora, G., Oberai, A., Chan, Y., Sasidharan, R., Berk, A.J., Pellegrini, M., Kurdistani, S.K., 2012. Reorganization of the host epigenome by a viral oncogene. Genome Res. 22, 1212–21.

Ferreon, J.C., Martinez-Yamout, M.A., Dyson, H.J., Wright, P.E., 2009. Structural basis for subversion of cellular control mechanisms by the adenoviral E1A oncoprotein. Proc. Natl. Acad. Sci. U. S. A. 106, 13260–5.

Fessler, S.P., Young, C.S., 1998. Control of adenovirus early gene expression during the late phase of infection. J. Virol. 72, 4049–56.

Field, J., Gronostajski, R.M., Hurwitz, J., 1984. Properties of the adenovirus DNA polymerase. J. Biol. Chem. 259, 9487–95.

Figge, J., Webster, T., Smith, T.F., Paucha, E., 1988. Prediction of similar transforming regions in simian virus 40 large T, adenovirus E1A, and myc oncoproteins. J. Virol. 62, 1814–8.

Flint, J., Shenk, T., 1997. Viral transactivating proteins. Annu. Rev. Genet. 31, 177–212.

Fonseca, G.J., Cohen, M.J., Mymryk, J.S., 2014. Adenovirus E1A Recruits the Human Paf1 Complex To Enhance Transcriptional Elongation. J. Virol. 88, 5630–5637.

Fonseca, G.J., Cohen, M.J., Nichols, A.C., Barrett, J.W., Mymryk, J.S., 2013. Viral Retasking of hBre1/RNF20 to Recruit hPaf1 for Transcriptional Activation. PLoS Pathog. 9, e1003411.

Fonseca, G.J., Thillainadesan, G., Yousef, A.F., Ablack, J.N., Mossman, K.L., Torchia, J., Mymryk, J.S., 2012. Adenovirus evasion of interferon-mediated innate immunity by direct antagonism of a cellular histone posttranslational modification. Cell Host Microbe 11, 597–606.

Fox, H.L., Dembowski, J.A., DeLuca, N.A., 2017. A Herpesviral Immediate Early Protein Promotes Transcription Elongation of Viral Transcripts. MBio 8, e00745-17.

Fox, J.P., Hall, C.E., Cooney, M.K., 1977. The Seattle Virus Watch. VII. Observations of adenovirus infections. Am. J. Epidemiol. 105, 362–86.

Freeman, A.E., Black, P.H., Vanderpool, E.A., Henry, P.H., Austin, J.B., Huebner, R.J., 1967. Transformation of primary rat embryo cells by adenovirus type 2. Proc. Natl. Acad. Sci. U. S. A. 58, 1205–12.

Frese, K.K., Lee, S.S., Thomas, D.L., Latorre, I.J., Weiss, R.S., Glaunsinger, B.A., Javier, R.T., 2003. Selective PDZ protein-dependent stimulation of phosphatidylinositol 3-kinase by the adenovirus E4-ORF1 oncoprotein. Oncogene 22, 710–21.

Friedman, J.M., Horwitz, M.S., 2002. Inhibition of tumor necrosis factor alpha-induced NF-kappa B activation by the adenovirus E3-10.4/14.5K complex. J. Virol. 76, 5515–21.

Frisch, S.M., Mymryk, J.S., 2002. Adenovirus-5 E1A: paradox and paradigm. Nat. Rev. Mol. Cell Biol. 3, 441–52.

Fuchs, M., Gerber, J., Drapkin, R., Sif, S., Ikura, T., Ogryzko, V., Lane, W.S., Nakatani, Y., Livingston, D.M., 2001. The p400 complex is an essential E1A transformation target. Cell 106, 297–307.

Gaggar, A., Shayakhmetov, D.M., Lieber, A., 2003. CD46 is a cellular receptor for group B adenoviruses. Nat. Med. 9, 1408–12.

Gallimore, P.H., 1972. Tumour Production in Immunosuppressed Rats with Cells Transformed in vitro by Adenovirus Type 2. J. Gen. Virol. 16, 99–102.

Gallimore, P.H., Turnell, A.S., 2001. Adenovirus E1A: remodelling the host cell, a life or death experience. Oncogene 20, 7824–35.

Gao, X., Jin, C., Ren, J., Yao, X., Xue, Y., 2008. Proteome-wide prediction of PKA phosphorylation sites in eukaryotic kingdom. Genomics 92, 457–63.

Garnett, C.T., Talekar, G., Mahr, J.A., Huang, W., Zhang, Y., Ornelles, D.A., Gooding,

L.R., 2009. Latent species C adenoviruses in human tonsil tissues. J. Virol. 83, 2417–28.

Geisberg, J. V, Chen, J.L., Ricciardi, R.P., 1995. Subregions of the adenovirus E1A transactivation domain target multiple components of the TFIID complex. Mol. Cell. Biol. 15, 6283–90.

Geisberg, J. V, Lee, W.S., Berk, A.J., Ricciardi, R.P., 1994. The zinc finger region of the adenovirus E1A transactivating domain complexes with the TATA box binding protein. Proc. Natl. Acad. Sci. U. S. A. 91, 2488–92.

Ghebremedhin, B., 2014. Human adenovirus: Viral pathogen with increasing importance. Eur. J. Microbiol. Immunol. 4, 26–33.

Ghosh, M.K., Harter, M.L., 2003. A viral mechanism for remodeling chromatin structure in G0 cells. Mol. Cell 12, 255–60.

Ginsberg, H.S., Pereira, H.G., Valentine, R.C., Wilcox, W.C., 1966. A proposed terminology for the adenovirus antigens and virion morphological subunits. Virology 28, 782–3.

Giroud, C., Chazal, N., Gay, B., Eldin, P., Brun, S., Briant, L., 2013. HIV-1-associated PKA acts as a cofactor for genome reverse transcription. Retrovirology 10, 157.

Gold, M.G., Gonen, T., Scott, J.D., 2013. Local cAMP signaling in disease at a glance. J. Cell Sci. 126, 4537–4543.

Gold, M.G., Lygren, B., Dokurno, P., Hoshi, N., McConnachie, G., Taskén, K., Carlson, C.R., Scott, J.D., Barford, D., 2006. Molecular basis of AKAP specificity for PKA regulatory subunits. Mol. Cell 24, 383–95.

Gonzalez, G.A., Montminy, M.R., 1989. Cyclic AMP stimulates somatostatin gene transcription by phosphorylation of CREB at serine 133. Cell 59, 675–80.

Gonzalez, R.A., Flint, S.J., 2002. Effects of mutations in the adenoviral E1B 55-kilodalton protein coding sequence on viral late mRNA metabolism. J. Virol. 76, 4507–19.

Gonzalez, R., Huang, W., Finnen, R., Bragg, C., Flint, S.J., 2006. Adenovirus E1B 55-Kilodalton Protein Is Required for both Regulation of mRNA Export and Efficient Entry into the Late Phase of Infection in Normal Human Fibroblasts. J. Virol. 80, 964–974.

Gooding, L.R., Elmore, L.W., Tollefson, A.E., Brady, H.A., Wold, W.S., 1988. A 14,700 MW protein from the E3 region of adenovirus inhibits cytolysis by tumor necrosis factor. Cell 53, 341–6.

Gooding, L.R., Sofola, I.O., Tollefson, A.E., Duerksen-Hughes, P., Wold, W.S., 1990.

The adenovirus E3-14.7K protein is a general inhibitor of tumor necrosis factor-mediated cytolysis. J. Immunol. 145, 3080–6.

Gräble, M., Hearing, P., 1992. cis and trans requirements for the selective packaging of adenovirus type 5 DNA. J. Virol. 66, 723–31.

Graham, F.L., Smiley, J., Russell, W.C., Nairn, R., 1977. Characteristics of a Human Cell Line Transformed by DNA from Human Adenovirus Type 5. J. Gen. Virol. 36, 59–72.

Granberg, F., Svensson, C., Pettersson, U., Zhao, H., 2005. Modulation of host cell gene expression during onset of the late phase of an adenovirus infection is focused on growth inhibition and cell architecture. Virology 343, 236–45.

Grand, R.J., Turnell, A.S., Mason, G.G., Wang, W., Milner, A.E., Mymryk, J.S., Rookes, S.M., Rivett, A.J., Gallimore, P.H., 1999. Adenovirus early region 1A protein binds to mammalian SUG1-a regulatory component of the proteasome. Oncogene 18, 449–458.

Greber, U.F., Suomalainen, M., Stidwill, R.P., Boucke, K., Ebersold, M.W., Helenius, A., 1997. The role of the nuclear pore complex in adenovirus DNA entry. EMBO J. 16, 5998–6007.

Greber, U.F., Webster, P., Weber, J., Helenius, A., 1996. The role of the adenovirus protease on virus entry into cells. EMBO J. 15, 1766–77.

Greber, U.F., Willetts, M., Webster, P., Helenius, A., 1993. Stepwise dismantling of adenovirus 2 during entry into cells. Cell 75, 477–86.

Green, M., Daesch, G.E., 1961. Biochemical studies on adenovirus multiplication. II. Kinetics of nucleic acid and protein synthesis in suspension cultures. Virology 13, 169–76.

Halbert, D.N., Cutt, J.R., Shenk, T., 1985. Adenovirus early region 4 encodes functions required for efficient DNA replication, late gene expression, and host cell shutoff. J. Virol. 56, 250–7.

Hanks, S.K., Quinn, A.M., Hunter, T., 1988. The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. Science 241, 42–52.

Harada, J.N., Shevchenko, A., Shevchenko, A., Pallas, D.C., Berk, A.J., 2002. Analysis of the adenovirus E1B-55K-anchored proteome reveals its link to ubiquitination machinery. J. Virol. 76, 9194–206.

Hardy, S., Engel, D.A., Shenk, T., 1989. An adenovirus early region 4 gene product is required for induction of the infection-specific form of cellular E2F activity. Genes Dev. 3, 1062–74.

Haste, N.M., Talabani, H., Doo, A., Merckx, A., Langsley, G., Taylor, S.S., 2012. Exploring the Plasmodium falciparum cyclic-adenosine monophosphate (cAMP)-dependent protein kinase (PfPKA) as a therapeutic target. Microbes Infect. 14, 838–50.

Hateboer, G., Gennissen, A., Ramos, Y.F., Kerkhoven, R.M., Sonntag-Buck, V., Stunnenberg, H.G., Bernards, R., 1995. BS69, a novel adenovirus E1A-associated protein that inhibits E1A transactivation. EMBO J. 14, 3159–69.

Hausdorff, W.P., Caron, M.G., Lefkowitz, R.J., 1990. Turning off the signal: desensitization of beta-adrenergic receptor function. FASEB J. 4, 2881–9.

Hawkins, L.K., Wold, W.S., 1992. A 12,500 MW protein is coded by region E3 of adenovirus. Virology 188, 486–94.

Hearing, P., Samulski, R.J., Wishart, W.L., Shenk, T., 1987. Identification of a repeated sequence element required for efficient encapsidation of the adenovirus type 5 chromosome. J. Virol. 61, 2555–8.

Hearing, P., Shenk, T., 1983. The adenovirus type 5 E1A transcriptional control region contains a duplicated enhancer element. Cell 33, 695–703.

Helin, K., Harlow, E., 1994. Heterodimerization of the transcription factors E2F-1 and DP-1 is required for binding to the adenovirus E4 (ORF6/7) protein. J. Virol. 68, 5027–35.

Hilleman, M.R., Werner, J.H., 1954. Recovery of new agent from patients with acute respiratory illness. Proc. Soc. Exp. Biol. Med. 85, 183–8.

Hoppe, E., Pauly, M., Gillespie, T.R., Akoua-Koffi, C., Hohmann, G., Fruth, B., Karhemere, S., Madinda, N.F., Mugisha, L., Muyembe, J.-J., Todd, A., Petrzelkova, K.J., Gray, M., Robbins, M., Bergl, R.A., Wittig, R.M., Zuberbühler, K., Boesch, C., Schubert, G., Leendertz, F.H., Ehlers, B., Calvignac-Spencer, S., 2015. Multiple Cross-Species Transmission Events of Human Adenoviruses (HAdV) during Hominine Evolution. Mol. Biol. Evol. 32, 2072–2084.

Horwitz, G.A., Zhang, K., McBrian, M.A., Grunstein, M., Kurdistani, S.K., Berk, A.J., 2008. Adenovirus Small e1a Alters Global Patterns of Histone Modification. Science (80-.). 321, 1084–1085.

Horwitz, M.S., Scharff, M.D., Maizel, J. V, 1969. Synthesis and assembly of adenovirus 2. I. Polypeptide synthesis, assembly of capsomeres, and morphogenesis of the virion. Virology 39, 682–94.

Houweling, A., van den Elsen, P.J., van der Eb, A.J., 1980. Partial transformation of primary rat cells by the leftmost 4.5% fragment of adenovirus 5 DNA. Virology 105, 537–50.

Howley, P.M., Livingston, D.M., 2009. Small DNA tumor viruses: Large contributors to biomedical sciences. Virology 384, 256–259.

Huang, M.M., Hearing, P., 1989. The adenovirus early region 4 open reading frame 6/7 protein regulates the DNA binding activity of the cellular transcription factor, E2F, through a direct complex. Genes Dev. 3, 1699–710.

Hurst, H.C., Jones, N.C., 1987. Identification of factors that interact with the E1A-inducible adenovirus E3 promoter. Genes Dev. 1, 1132–46.

Ikeda, M.A., Nevins, J.R., 1993. Identification of distinct roles for separate E1A domains in disruption of E2F complexes. Mol. Cell. Biol. 13, 7029–35.

Jelsma, T.N., Howe, J.A., Evelegh, C.M., Cunniff, N.F., Skiadopoulos, M.H., Floroff, M.R., Denman, J.E., Bayley, S.T., 1988. Use of deletion and point mutants spanning the coding region of the adenovirus 5 E1A gene to define a domain that is essential for transcriptional activation. Virology 163, 494–502.

Jogler, C., Hoffmann, D., Theegarten, D., Grunwald, T., Uberla, K., Wildner, O., 2006. Replication properties of human adenovirus in vivo and in cultures of primary cells from different animal species. J. Virol. 80, 3549–58.

Johnson, D.A., Akamine, P., Radzio-Andzelm, E., Madhusudan, M., Taylor, S.S., 2001. Dynamics of cAMP-dependent protein kinase. Chem. Rev. 101, 2243–70.

Jones, M.S., Harrach, B., Ganac, R.D., Gozum, M.M.A., Dela Cruz, W.P., Riedel, B., Pan, C., Delwart, E.L., Schnurr, D.P., 2007. New adenovirus species found in a patient presenting with gastroenteritis. J. Virol. 81, 5978–84.

Jones, N., Shenk, T., 1979. An adenovirus type 5 early gene function regulates expression of other early viral genes. Proc. Natl. Acad. Sci. U. S. A. 76, 3665–9.

Kajon, A.E., Lu, X., Erdman, D.D., Louie, J., Schnurr, D., George, K.S., Koopmans, M.P., Allibhai, T., Metzgar, D., 2010. Molecular epidemiology and brief history of emerging adenovirus 14-associated respiratory disease in the United States. J. Infect. Dis. 202, 93–103.

Kannan, N., Wu, J., Anand, G.S., Yooseph, S., Neuwald, A.F., Venter, J.C., Taylor, S.S., 2007. Evolution of allostery in the cyclic nucleotide binding module. Genome Biol. 8, R264.

Karen, K.A., Hearing, P., 2011. Adenovirus core protein VII protects the viral genome from a DNA damage response at early times after infection. J. Virol. 85, 4135–42.

Karen, K.A., Hoey, P.J., Young, C.S.H., Hearing, P., 2009. Temporal regulation of the Mre11-Rad50-Nbs1 complex during adenovirus infection. J. Virol. 83, 4565–73.

Kasel, J., 1979. Adenoviruses. In: Diagnostic Procedures for Viral, Rickettsial and

Chlamydial Infections. pp. 229–256.

Kelly, T.J., Lechner, R.L., 1979. The structure of replicating adenovirus DNA molecules: characterization of DNA-protein complexes from infected cells. Cold Spring Harb. Symp. Quant. Biol. 43 Pt 2, 721–8.

Kennelly, P.J., Krebs, E.G., 1991. Consensus sequences as substrate specificity determinants for protein kinases and protein phosphatases. J. Biol. Chem. 266, 15555–8.

Keshwani, M.M., Klammt, C., von Daake, S., Ma, Y., Kornev, A.P., Choe, S., Insel, P.A., Taylor, S.S., 2012. Cotranslational cis-phosphorylation of the COOH-terminal tail is a key priming step in the maturation of cAMP-dependent protein kinase. Proc. Natl. Acad. Sci. U. S. A. 109, E1221-9.

Kidd, A.H., Chroboczek, J., Cusack, S., Ruigrok, R.W.H., 1993. Adenovirus Type 40 Virions Contain Two Distinct Fibers. Virology 192, 73–84.

Kim, C., Xuong, N.-H., Taylor, S.S., 2005. Crystal Structure of a Complex Between the Catalytic and Regulatory (RI) Subunits of PKA. Science (80-.). 307, 690–696.

Kim, H.-J., Foster, M.P., 2002. Characterization of Ad5 E3-14.7K, an adenoviral inhibitor of apoptosis: structure, oligomeric state, and metal binding. Protein Sci. 11, 1117–28.

Kim, P.M., Sboner, A., Xia, Y., Gerstein, M., 2008. The role of disorder in interaction networks: a structural analysis. Mol. Syst. Biol. 4, 179.

Kimelman, D., Miller, J.S., Porter, D., Roberts, B.E., 1985. E1a regions of the human adenoviruses and of the highly oncogenic simian adenovirus 7 are closely related. J. Virol. 53, 399–409.

King, A.J., Teertstra, W.R., Blanco, L., Salas, M., van der Vliet, P.C., 1997. Processive proofreading by the adenovirus DNA polymerase. Association with the priming protein reduces exonucleolytic degradation. Nucleic Acids Res. 25, 1745–52.

King, C.R., Cohen, M.J., Fonseca, G.J., Dirk, B.S., Dikeakos, J.D., Mymryk, J.S., 2016a. Functional and Structural Mimicry of Cellular Protein Kinase A Anchoring Proteins by a Viral Oncoprotein. PLoS Pathog. 12, e1005621.

King, C.R., Tessier, T.M., Mymryk, J.S., 2016b. Color Me Infected: Painting Cellular Chromatin with a Viral Histone Mimic. Trends Microbiol. 24, 774–776.

King, C.R., Zhang, A., Mymryk, J.S., 2016c. The Persistent Mystery of Adenovirus Persistence. Trends Microbiol. 24, 323–4.

Knighton, D.R., Zheng, J.H., Ten Eyck, L.F., Ashford, V.A., Xuong, N.H., Taylor, S.S., Sowadski, J.M., 1991. Crystal structure of the catalytic subunit of cyclic adenosine

monophosphate-dependent protein kinase. Science 253, 407–14.

Komatsu, T., Haruki, H., Nagata, K., 2011. Cellular and viral chromatin proteins are positive factors in the regulation of adenovirus gene expression. Nucleic Acids Res. 39, 889–901.

Kornev, A.P., Haste, N.M., Taylor, S.S., Eyck, L.F. Ten, 2006. Surface comparison of active and inactive protein kinases identifies a conserved activation mechanism. Proc. Natl. Acad. Sci. U. S. A. 103, 17783–8.

Kovesdi, I., Reichel, R., Nevins, J.R., 1986. Identification of a cellular transcription factor involved in E1A trans-activation. Cell 45, 219–28.

Krebs, E.G., Fischer, E.H., 1956. The phosphorylase b to a converting enzyme of rabbit skeletal muscle. Biochim. Biophys. Acta 20, 150–7.

Krebs, E.G., Graves, D.J., Fischer, E.H., 1959. Factors affecting the activity of muscle phosphorylase b kinase. J. Biol. Chem. 234, 2867–73.

Krilov, L.R., 2005. Adenovirus infections in the immunocompromised host. Pediatr. Infect. Dis. J. 24, 555–6.

Langan, T.A., 1969. Phosphorylation of liver histone following the administration of glucagon and insulin. Proc. Natl. Acad. Sci. U. S. A. 64, 1276–83.

Law, L.K., Davidson, B.L., 2005. What Does It Take to Bind CAR? Mol. Ther. 12, 599–609.

Lechner, R.L., Kelly, T.J., 1977. The structure of replicating adenovirus 2 DNA molecules. Cell 12, 1007–20.

Lenman, A., Liaci, A.M., Liu, Y., Årdahl, C., Rajan, A., Nilsson, E., Bradford, W., Kaeshammer, L., Jones, M.S., Frängsmyr, L., Feizi, T., Stehle, T., Arnberg, N., 2015. Human Adenovirus 52 Uses Sialic Acid-containing Glycoproteins and the Coxsackie and Adenovirus Receptor for Binding to Target Cells. PLOS Pathog. 11, e1004657.

Leopold, P.L., Kreitzer, G., Miyazawa, N., Rempel, S., Pfister, K.K., Rodriguez-Boulan, E., Crystal, R.G., 2000. Dynein- and microtubule-mediated translocation of adenovirus serotype 5 occurs after endosomal lysis. Hum. Gene Ther. 11, 151–65.

Levine, A.J., van der Vliet, P.C., Rosenwirth, B., Anderson, C., Rabek, J., Levinson, A., Anderson, S., 1976. Characterization of an adenovirus early protein required for viral DNA replication: a single strand specific DNA binding proteins. Mol. Cell. Biochem. 11, 79–95.

Lewis, P.F., Schmidt, M.A., Lu, X., Erdman, D.D., Campbell, M., Thomas, A., Cieslak, P.R., Grenz, L.D., Tsaknardis, L., Gleaves, C., Kendall, B., Gilbert, D., 2009. A

community-based outbreak of severe respiratory illness caused by human adenovirus serotype 14. J. Infect. Dis. 199, 1427–34.

Li, M., Wang, X., Meintzer, M.K., Laessig, T., Birnbaum, M.J., Heidenreich, K.A., 2000. Cyclic AMP promotes neuronal survival by phosphorylation of glycogen synthase kinase 3beta. Mol. Cell. Biol. 20, 9356–63.

Lichtenstein, D.L., Toth, K., Doronin, K., Tollefson, A.E., Wold, W.S.M., 2004. Functions and mechanisms of action of the adenovirus E3 proteins. Int. Rviews Immunol. 23, 75–111.

Lillie, J.W., Green, M.R., 1989. Transcription activation by the adenovirus E1a protein. Nature 338, 39–44.

Lion, T., 2014. Adenovirus infections in immunocompetent and immunocompromised patients. Clin. Microbiol. Rev. 27, 441–62.

Liu, F., Green, M.R., 1990. A specific member of the ATF transcription factor family can mediate transcription activation by the adenovirus E1a protein. Cell 61, 1217–24.

Liu, F., Green, M.R., 1994. Promoter targeting by adenovirus E1a through interaction with different cellular DNA-binding domains. Nature 368, 520–525.

Lizcano, J.M., Morrice, N., Cohen, P., 2000. Regulation of BAD by cAMP-dependent protein kinase is mediated via phosphorylation of a novel site, Ser155. Biochem. J. 349, 547–57.

Louis, N., Evelegh, C., Graham, F.L., 1997. Cloning and sequencing of the cellular-viral junctions from the human adenovirus type 5 transformed 293 cell line. Virology 233, 423–9.

Lu, H., Li, Z., Xue, Y., Zhou, Q., 2013. Viral-host interactions that control HIV-1 transcriptional elongation. Chem. Rev. 113, 8567–82.

Luo, Z., Lin, C., Shilatifard, A., 2012. The super elongation complex (SEC) family in transcriptional control. Nat. Rev. Mol. Cell Biol. 13, 543–547.

Mabit, H., Nakano, M.Y., Prank, U., Saam, B., Döhner, K., Sodeik, B., Greber, U.F., 2002. Intact microtubules support adenovirus and herpes simplex virus infections. J. Virol. 76, 9962–71.

Mackey, J.K., Rigden, P.M., Green, M., 1976. Do highly oncogenic group A human adenoviruses cause human cancer? Analysis of human tumors for adenovirus 12 transforming DNA sequences. Proc. Natl. Acad. Sci. U. S. A. 73, 4657–61.

Madisch, I., Harste, G., Pommer, H., Heim, A., 2005. Phylogenetic Analysis of the Main Neutralization and Hemagglutination Determinants of All Human Adenovirus Prototypes as a Basis for Molecular Classification and Taxonomy. J. Virol. 79,

15265–15276.

Majeau, N., Bolduc, M., Duvignaud, J.-B., Fromentin, R., Leclerc, D., 2007. Effect of cAMP-dependent protein kinase A (PKA) on HCV nucleocapsid assembly and degradation. Biochem. Cell Biol. 85, 78–87.

Manning, G., Whyte, D.B., Martinez, R., Hunter, T., Sudarsanam, S., 2002. The Protein Kinase Complement of the Human Genome. Science (80-.). 298, 1912–1934.

Martin-Fernandez, M., Longshaw, S. V, Kirby, I., Santis, G., Tobin, M.J., Clarke, D.T., Jones, G.R., 2004. Adenovirus type-5 entry and disassembly followed in living cells by FRET, fluorescence anisotropy, and FLIM. Biophys. J. 87, 1316–27.

Martin, K.J., Lillie, J.W., Green, M.R., 1990. Evidence for interaction of different eukaryotic transcriptional activators with distinct cellular targets. Nature 346, 147–152.

Masterson, L.R., Cheng, C., Yu, T., Tonelli, M., Kornev, A., Taylor, S.S., Veglia, G., 2010. Dynamics connect substrate recognition to catalysis in protein kinase A. Nat. Chem. Biol. 6, 821–8.

Mathews, M.B., Shenk, T., 1991. Adenovirus virus-associated RNA and translation control. J. Virol. 65, 5657–62.

Mayr, B., Montminy, M., 2001. Transcriptional regulation by the phosphorylation-dependent factor CREB. Nat. Rev. Mol. Cell Biol. 2, 599–609.

Mazzarelli, J.M., Atkins, G.B., Geisberg, J. V, Ricciardi, R.P., 1995. The viral oncoproteins Ad5 E1A, HPV16 E7 and SV40 TAg bind a common region of the TBP-associated factor-110. Oncogene 11, 1859–64.

McBride, W.D., Wiener, A., 1964. In vitro transformation of hamster kidney cells by human adenovirus type 12. Proc. Soc. Exp. Biol. Med. 115, 870–4.

McKenzie, A.J., Campbell, S.L., Howe, A.K., 2011. Protein kinase A activity and anchoring are required for ovarian cancer cell migration and invasion. PLoS One 6, e26552.

Meier, O., Greber, U.F., 2003. Adenovirus endocytosis. J. Gene Med. 5, 451–462.

Mellman, I., 1992. The importance of being acid: the role of acidification in intracellular membrane traffic. J. Exp. Biol. 172, 39–45.

Mende, Y., Schneider, P.M., Baldus, S.E., Doerfler, W., 2004. PCR-screening of human esophageal and bronchial cancers reveals absence of adenoviral DNA sequences. Virus Res. 104, 81–5.

Meng, X., Yang, Y.-F., Cao, X., Govindan, M. V, Shuen, M., Hollenberg, A.N.,

Mymryk, J.S., Walfish, P.G., 2003. Cellular context of coregulator and adaptor proteins regulates human adenovirus 5 early region 1A-dependent gene activation by the thyroid hormone receptor. Mol. Endocrinol. 17, 1095–105.

Metzgar, D., Osuna, M., Kajon, A.E., Hawksworth, A.W., Irvine, M., Russell, K.L., 2007. Abrupt emergence of diverse species B adenoviruses at US military recruit training centers. J. Infect. Dis. 196, 1465–73.

Miller, M.S., Pelka, P., Fonseca, G.J., Cohen, M.J., Kelly, J.N., Barr, S.D., Grand, R.J.A., Turnell, A.S., Whyte, P., Mymryk, J.S., 2012. Characterization of the 55-Residue Protein Encoded by the 9S E1A mRNA of Species C Adenovirus. J. Virol. 86, 4222–4233.

Miyake-Stoner, S.J., O'Shea, C.C., 2014. Metabolism goes viral. Cell Metab. 19, 549–50.

Moise, A.R., Grant, J.R., Vitalis, T.Z., Jefferies, W.A., 2002. Adenovirus E3-6.7K maintains calcium homeostasis and prevents apoptosis and arachidonic acid release. J. Virol. 76, 1578–87.

Montell, C., Courtois, G., Eng, C., Berk, A., 1984. Complete transformation by adenovirus 2 requires both E1A proteins. Cell 36, 951–61.

Montell, C., Fisher, E.F., Caruthers, M.H., Berk, A.J., 1982. Resolving the functions of overlapping viral genes by site-specific mutagenesis at a mRNA splice site. Nature 295, 380–4.

Muller, S., Dobner, T., 2008. The adenovirus E1B-55K oncoprotein induces SUMO modification of p53. Cell Cycle 7, 754–8.

Mymryk, J.S., 1996. Tumour suppressive properties of the adenovirus 5 E1A oncogene. Oncogene 13, 1581–9.

Nevins, J.R., Ginsberg, H.S., Blanchard, J.M., Wilson, M.C., Darnell, J.E., 1979. Regulation of the primary expression of the early adenovirus transcription units. J. Virol. 32, 727–33.

Nicklin, S.A., Wu, E., Nemerow, G.R., Baker, A.H., 2005. The influence of adenovirus fiber structure and function on vector development for gene therapy. Mol. Ther. 12, 384–93.

Norrby, E., 1966. The relationship between the soluble antigens and the virion of adenovirus type 3. II. Identification and characterization of an incomplete hemagglutinin. Virology 30, 608–17.

Norrby, E., Skaaret, P., 1967. The relationship between soluble antigens and the virion of adenovirus type 3. 3. Immunological identification of fiber antigen and isolated vertex capsomer antigen. Virology 32, 489–502.

Northup, J.K., Sternweis, P.C., Smigel, M.D., Schleifer, L.S., Ross, E.M., Gilman, A.G., 1980. Purification of the regulatory component of adenylate cyclase. Proc. Natl. Acad. Sci. U. S. A. 77, 6516–20.

O'Shea, C., Klupsch, K., Choi, S., Bagus, B., Soria, C., Shen, J., McCormick, F., Stokoe, D., 2005. Adenoviral proteins mimic nutrient/growth signals to activate the mTOR pathway for viral replication. EMBO J. 24, 1211–1221.

Okabe, M., Enomoto, M., Maeda, H., Kuroki, K., Ohtsuki, K., 2006. Biochemical characterization of suramin as a selective inhibitor for the PKA-mediated phosphorylation of HBV core protein in vitro. Biol. Pharm. Bull. 29, 1810–4.

Okar, D.A., Lange, A.J., 1999. Fructose-2,6-bisphosphate and control of carbohydrate metabolism in eukaryotes. Biofactors 10, 1–14.

Ostapchuk, P., Hearing, P., 2003. Regulation of adenovirus packaging. Curr. Top. Microbiol. Immunol. 272, 165–85.

Ostapchuk, P., Hearing, P., 2005. Control of adenovirus packaging. J. Cell. Biochem. 96, 25–35.

Pagano, M., Draetta, G., Jansen-Dürr, P., 1992. Association of cdk2 kinase with the transcription factor E2F during S phase. Science 255, 1144–7.

Page, R.D., 1996. TreeView: an application to display phylogenetic trees on personal computers. Comput. Appl. Biosci. 12, 357–8.

Palorini, R., Votta, G., Pirola, Y., De Vitto, H., De Palma, S., Airoldi, C., Vasso, M., Ricciardiello, F., Lombardi, P.P., Cirulli, C., Rizzi, R., Nicotra, F., Hiller, K., Gelfi, C., Alberghina, L., Chiaradonna, F., 2016. Protein Kinase A Activation Promotes Cancer Cell Resistance to Glucose Starvation and Anoikis. PLoS Genet. 12, e1005931.

Parks, C.L., Banerjee, S., Spector, D.J., 1988. Organization of the transcriptional control region of the E1b gene of adenovirus type 5. J. Virol. 62, 54–67.

Pattabiraman, D.R., Bierie, B., Kober, K.I., Thiru, P., Krall, J.A., Zill, C., Reinhardt, F., Tam, W.L., Weinberg, R.A., 2016. Activation of PKA leads to mesenchymal-to-epithelial transition and loss of tumor-initiating ability. Science (80-.). 351, aad3680-aad3680.

Pelka, P., Ablack, J.N.G., Fonseca, G.J., Yousef, A.F., Mymryk, J.S., 2008. Intrinsic structural disorder in adenovirus E1A: a viral molecular hub linking multiple diverse processes. J. Virol. 82, 7252–63.

Pennella, M.A., Liu, Y., Woo, J.L., Kim, C.A., Berk, A.J., 2010. Adenovirus E1B 55-kilodalton protein is a p53-SUMO1 E3 ligase that represses p53 and stimulates its nuclear export through interactions with promyelocytic leukemia nuclear bodies. J.

Virol. 84, 12210–25.

Perricaudet, M., Akusjärvi, G., Virtanen, A., Pettersson, U., 1979. Structure of two spliced mRNAs from the transforming region of human subgroup C adenoviruses. Nature 281, 694–6.

Pflug, A., Lukarska, M., Resa-Infante, P., Reich, S., Cusack, S., 2017. Structural insights into RNA synthesis by the influenza virus transcription-replication machine. Virus Res. 234, 103–117.

Phelps, W.C., Yee, C.L., Münger, K., Howley, P.M., 1988. The human papillomavirus type 16 E7 gene encodes transactivation and transformation functions similar to those of adenovirus E1A. Cell 53, 539–47.

Querido, E., Blanchette, P., Yan, Q., Kamura, T., Morrison, M., Boivin, D., Kaelin, W.G., Conaway, R.C., Conaway, J.W., Branton, P.E., 2001. Degradation of p53 by adenovirus E4orf6 and E1B55K proteins occurs via a novel mechanism involving a Cullin-containing complex. Genes Dev. 15, 3104–3117.

Quinlan, K.G.R., Verger, A., Kwok, A., Lee, S.H.Y., Perdomo, J., Nardini, M., Bolognesi, M., Crossley, M., 2006. Role of the C-terminal binding protein PXDLS motif binding cleft in protein interactions and transcriptional repression. Mol. Cell. Biol. 26, 8202–13.

Radko, S., Jung, R., Olanubi, O., Pelka, P., 2015. Effects of Adenovirus Type 5 E1A Isoforms on Viral Replication in Arrested Human Cells. PLoS One 10, e0140124.

Rall, T.W., Sutherland, E.W., 1958. Formation of a cyclic adenine ribonucleotide by tissue particles. J. Biol. Chem. 232, 1065–76.

Rao, L., Debbas, M., Sabbatini, P., Hockenbery, D., Korsmeyer, S., White, E., 1992. The adenovirus E1A proteins induce apoptosis, which is inhibited by the E1B 19-kDa and Bcl-2 proteins. Proc. Natl. Acad. Sci. U. S. A. 89, 7742–6.

Rasti, M., Grand, R.J.A., Yousef, A.F., Shuen, M., Mymryk, J.S., Gallimore, P.H., Turnell, A.S., 2006. Roles for APIS and the 20S proteasome in adenovirus E1A-dependent transcription. EMBO J. 25, 2710–22.

Rehfuss, R.P., Walton, K.M., Loriaux, M.M., Goodman, R.H., 1991. The cAMP-regulated enhancer-binding protein ATF-1 activates transcription in response to cAMP-dependent protein kinase A. J. Biol. Chem. 266, 18431–4.

Reich, P.R., Forget, B.G., Weissman, S.M., 1966. RNA of low molecular weight in KB cells infected with adenovirus type 2. J. Mol. Biol. 17, 428–39.

Reichel, P.A., Merrick, W.C., Siekierka, J., Mathews, M.B., 1985. Regulation of a protein synthesis initiation factor by adenovirus virus-associated RNA. Nature 313, 196–200.

Reichel, R., Kovesdi, I., Nevins, J.R., 1987. Developmental control of a promoter-specific factor that is also regulated by the E1A gene product. Cell 48, 501–6.

Rekosh, D.M., Russell, W.C., Bellet, A.J., Robinson, A.J., 1977. Identification of a protein linked to the ends of adenovirus DNA. Cell 11, 283–95.

Roberts, R.J., O'Neill, K.E., Yen, C.T., 1984. DNA sequences from the adenovirus 2 genome. J. Biol. Chem. 259, 13968–75.

Robinson, C.M., Singh, G., Lee, J.Y., Dehghan, S., Rajaiya, J., Liu, E.B., Yousuf, M.A., Betensky, R.A., Jones, M.S., Dyer, D.W., Seto, D., Chodosh, J., 2013. Molecular evolution of human adenoviruses. Sci. Rep. 3, 1812.

Roelvink, P.W., Lizonova, A., Lee, J.G., Li, Y., Bergelson, J.M., Finberg, R.W., Brough, D.E., Kovesdi, I., Wickham, T.J., 1998. The coxsackievirus-adenovirus receptor protein can function as a cellular attachment protein for adenovirus serotypes from subgroups A, C, D, E, and F. J. Virol. 72, 7909–15.

Rowe, D.T., Graham, F.L., Branton, P.E., 1983. Intracellular localization of adenovirus type 5 tumor antigens in productively infected cells. Virology 129, 456–68.

Rowe, W.P., Huebner, R.J., Gilmore, L.K., Parrott, R.H., Ward, T.G., 1953. Isolation of a cytopathogenic agent from human adenoids undergoing spontaneous degeneration in tissue culture. Proc. Soc. Exp. Biol. Med. 84, 570–3.

Roy, S., Vandenberghe, L.H., Kryazhimskiy, S., Grant, R., Calcedo, R., Yuan, X., Keough, M., Sandhu, A., Wang, Q., Medina-Jaszek, C.A., Plotkin, J.B., Wilson, J.M., 2009. Isolation and characterization of adenoviruses persistently shed from the gastrointestinal tract of non-human primates. PLoS Pathog. 5, e1000503.

Rozenblatt-Rosen, O., Deo, R.C., Padi, M., Adelmant, G., Calderwood, M.A., Rolland, T., Grace, M., Dricot, A., Askenazi, M., Tavares, M., Pevzner, S.J., Abderazzaq, F., Byrdsong, D., Carvunis, A.-R., Chen, A.A., Cheng, J., Correll, M., Duarte, M., Fan, C., Feltkamp, M.C., Ficarro, S.B., Franchi, R., Garg, B.K., Gulbahce, N., Hao, T., Holthaus, A.M., James, R., Korkhin, A., Litovchick, L., Mar, J.C., Pak, T.R., Rabello, S., Rubio, R., Shen, Y., Singh, S., Spangle, J.M., Tasan, M., Wanamaker, S., Webber, J.T., Roecklein-Canfield, J., Johannsen, E., Barabási, A.-L., Beroukhim, R., Kieff, E., Cusick, M.E., Hill, D.E., Münger, K., Marto, J.A., Quackenbush, J., Roth, F.P., DeCaprio, J.A., Vidal, M., 2012. Interpreting cancer genomes using systematic host network perturbations by tumour virus proteins. Nature 487, 491–5.

Russell, K.L., Broderick, M.P., Franklin, S.E., Blyn, L.B., Freed, N.E., Moradi, E., Ecker, D.J., Kammerer, P.E., Osuna, M.A., Kajon, A.E., Morn, C.B., Ryan, M.A.K., 2006. Transmission dynamics and prospective environmental sampling of adenovirus in a military recruit setting. J. Infect. Dis. 194, 877–85.

Russell, W.C., 2009. Adenoviruses: update on structure and function. J. Gen. Virol. 90, 1–20.

San Martín, C., 2012. Latest insights on adenovirus structure and assembly. Viruses 4, 847–77.

Sarma, G.N., Kinderman, F.S., Kim, C., von Daake, S., Chen, L., Wang, B.-C., Taylor, S.S., 2010. Structure of D-AKAP2:PKA RI complex: insights into AKAP specificity and selectivity. Structure 18, 155–66.

Schaack, J., Bennett, M.L., Colbert, J.D., Torres, A. V., Clayton, G.H., Ornelles, D., Moorhead, J., 2004. E1A and E1B proteins inhibit inflammation induced by adenovirus. Proc. Natl. Acad. Sci. 101, 3124–3129.

Schaeper, U., Boyd, J.M., Verma, S., Uhlmann, E., Subramanian, T., Chinnadurai, G., 1995. Molecular cloning and characterization of a cellular phosphoprotein that interacts with a conserved C-terminal domain of adenovirus E1A involved in negative modulation of oncogenic transformation. Proc. Natl. Acad. Sci. U. S. A. 92, 10467–71.

Scherer, J., Yi, J., Vallee, R.B., 2014. PKA-dependent dynein switching from lysosomes to adenovirus: a novel form of host-virus competition. J. Cell Biol. 205, 163–77.

Schneider, R.J., Weinberger, C., Shenk, T., 1984. Adenovirus VAI RNA facilitates the initiation of translation in virus-infected cells. Cell 37, 291–8.

Segerman, A., Atkinson, J.P., Marttila, M., Dennerquist, V., Wadell, G., Arnberg, N., 2003. Adenovirus type 11 uses CD46 as a cellular receptor. J. Virol. 77, 9183–91.

Serfling, E., Berberich-Siebelt, F., Chuvpilo, S., Jankevics, E., Klein-Hessling, S., Twardzik, T., Avots, A., 2000. The role of NF-AT transcription factors in T cell activation and differentiation. Biochim. Biophys. Acta 1498, 1–18.

Seto, J., Walsh, M.P., Mahadevan, P., Purkayastha, A., Clark, J.M., Tibbetts, C., Seto, D., 2009. Genomic and bioinformatics analyses of HAdV-14p, reference strain of a re-emerging respiratory pathogen and analysis of B1/B2. Virus Res. 143, 94–105.

Sha, J., Ghosh, M.K., Zhang, K., Harter, M.L., 2010. E1A interacts with two opposing transcriptional pathways to induce quiescent cells into S phase. J. Virol. 84, 4050–9.

Shabb, J.B., 2001. Physiological substrates of cAMP-dependent protein kinase. Chem. Rev. 101, 2381–411.

Shah, G.A., O'Shea, C.C., 2015. Viral and Cellular Genomes Activate Distinct DNA Damage Responses. Cell 162, 987–1002.

Shaw, A.R., Ziff, E.B., 1980. Transcripts from the adenovirus-2 major late promoter yield a single early family of 3' coterminal mRNAs and five late families. Cell 22, 905–16.

Shisler, J., Yang, C., Walter, B., Ware, C.F., Gooding, L.R., 1997. The adenovirus E3-

10.4K/14.5K complex mediates loss of cell surface Fas (CD95) and resistance to Fas-induced apoptosis. J. Virol. 71, 8299–306.

Shoji, S., Parmelee, D.C., Wade, R.D., Kumar, S., Ericsson, L.H., Walsh, K.A., Neurath, H., Long, G.L., Demaille, J.G., Fischer, E.H., Titani, K., 1981. Complete amino acid sequence of the catalytic subunit of bovine cardiac muscle cyclic AMP-dependent protein kinase. Proc. Natl. Acad. Sci. U. S. A. 78, 848–51.

Shuen, M., Avvakumov, N., Walfish, P.G., Brandl, C.J., Mymryk, J.S., 2002. The adenovirus E1A protein targets the SAGA but not the ADA transcriptional regulatory complex through multiple independent domains. J. Biol. Chem. 277, 30844–51.

Sirena, D., Lilienfeld, B., Eisenhut, M., Kälin, S., Boucke, K., Beerli, R.R., Vogt, L., Ruedl, C., Bachmann, M.F., Greber, U.F., Hemmi, S., 2004. The human membrane cofactor CD46 is a receptor for species B adenovirus serotype 3. J. Virol. 78, 4454–62.

SivaRaman, L., Thimmappaya, B., 1987. Two promoter-specific host factors interact with adjacent sequences in an EIA-inducible adenovirus promoter. Proc. Natl. Acad. Sci. U. S. A. 84, 6112–6.

Skalhegg, B.S., Tasken, K., 2000. Specificity in the cAMP/PKA signaling pathway. Differential expression,regulation, and subcellular localization of subunits of PKA. Front. Biosci. 5, D678-93.

Søberg, K., Jahnsen, T., Rognes, T., Skålhegg, B.S., Laerdahl, J.K., 2013. Evolutionary paths of the cAMP-dependent protein kinase (PKA) catalytic subunits. PLoS One 8, e60935.

Sohn, S.-Y., Hearing, P., 2012. Adenovirus Regulates Sumoylation of Mre11-Rad50-Nbs1 Components through a Paralog-Specific Mechanism. J. Virol. 86, 9656–9665.

Somasundaram, K., Jayaraman, G., Williams, T., Moran, E., Frisch, S., Thimmapaya, B., 1996. Repression of a matrix metalloprotease gene by E1A correlates with its ability to bind to cell type-specific transcription factor AP-2. Proc. Natl. Acad. Sci. U. S. A. 93, 3088–93.

Soria, C., Estermann, F.E., Espantman, K.C., O'Shea, C.C., 2010. Heterochromatin silencing of p53 target genes by a small viral protein. Nature 466, 1076–1081.

Spector, D.J., Johnson, J.S., Baird, N.L., Engel, D.A., 2003. Adenovirus type 5 DNA-protein complexes from formaldehyde cross-linked cells early after infection. Virology 312, 204–12.

Spector, D.J., McGrogan, M., Raskas, H.J., 1978. Regulation of the appearance of cytoplasmic RNAs from region 1 of the adenovirus 2 genome. J. Mol. Biol. 126, 395–414.

Steenbergh, P.H., Maat, J., van Ormondt, H., Sussenbach, J.S., 1977. The nucleotide sequence at the termini of adenovirus type 5 DNA. Nucleic Acids Res. 4, 4371–89.

Steichen, J.M., Kuchinskas, M., Keshwani, M.M., Yang, J., Adams, J.A., Taylor, S.S., 2012. Structural Basis for the Regulation of Protein Kinase A by Activation Loop Phosphorylation. J. Biol. Chem. 287, 14672–14680.

Stephens, C., Harlow, E., 1987. Differential splicing yields novel adenovirus 5 E1A mRNAs that encode 30 kd and 35 kd proteins. EMBO J. 6, 2027–35.

Stevens, J.L., Cantin, G.T., Wang, G., Shevchenko, A., Shevchenko, A., Berk, A.J., 2002. Transcription control by E1A and MAP kinase pathway via Sur2 mediator subunit. Science 296, 755–8.

Stracker, T.H., Carson, C.T., Weitzman, M.D., 2002. Adenovirus oncoproteins inactivate the Mre11–Rad50–NBS1 DNA repair complex. Nature 418, 348–352.

Ström, A.C., Ohlsson, P., Akusjärvi, G., 1998. AR1 is an integral part of the adenovirus type 2 E1A-CR3 transactivation domain. J. Virol. 72, 5978–83.

Subramanian, T., Malstrom, S.E., Chinnadurai, G., 1991. Requirement of the C-terminal region of adenovirus E1a for cell transformation in cooperation with E1b. Oncogene 6, 1171–3.

Suomalainen, M., Nakano, M.Y., Boucke, K., Keller, S., Greber, U.F., 2001. Adenovirus-activated PKA and p38/MAPK pathways boost microtubule-mediated nuclear targeting of virus. EMBO J. 20, 1310–9.

Svensson, C., Pettersson, U., Akusjärvi, G., 1983. Splicing of adenovirus 2 early region 1A mRNAs is non-sequential. J. Mol. Biol. 165, 475–95.

Svensson, U., 1985. Role of vesicles during adenovirus 2 internalization into HeLa cells. J. Virol. 55, 442–9.

Taskén, K., Aandahl, E.M., 2004. Localized effects of cAMP mediated by distinct routes of protein kinase A. Physiol. Rev. 84, 137–67.

Täuber, B., Dobner, T., 2001. Molecular regulation and biological function of adenovirus early genes: the E4 ORFs. Gene 278, 1–23.

Taylor, D.A., Kraus, V.B., Schwarz, J.J., Olson, E.N., Kraus, W.E., 1993. E1A-mediated inhibition of myogenesis correlates with a direct physical interaction of E1A12S and basic helix-loop-helix proteins. Mol. Cell. Biol. 13, 4714–27.

Taylor, S.S., Ilouz, R., Zhang, P., Kornev, A.P., 2012. Assembly of allosteric macromolecular switches: lessons from PKA. Nat. Rev. Mol. Cell Biol. 13, 646–58.

Taylor, S.S., Zhang, P., Steichen, J.M., Keshwani, M.M., Kornev, A.P., 2013. PKA:

Lessons learned after twenty years. Biochim. Biophys. Acta - Proteins Proteomics 1834, 1271–1278.

Ternovoi, V. V, Le, L.P., Belousova, N., Smith, B.F., Siegal, G.P., Curiel, D.T., 2005. Productive replication of human adenovirus type 5 in canine cells. J. Virol. 79, 1308–11.

Thai, M., Graham, N.A., Braas, D., Nehil, M., Komisopoulou, E., Kurdistani, S.K., McCormick, F., Graeber, T.G., Christofk, H.R., 2014. Adenovirus E4ORF1-Induced MYC Activation Promotes Host Cell Anabolic Glucose Metabolism and Virus Replication. Cell Metab. 19, 694–701.

Theurkauf, W.E., Vallee, R.B., 1982. Molecular characterization of the cAMP-dependent protein kinase bound to microtubule-associated protein 2. J. Biol. Chem. 257, 3284–90.

Thomas, M.A., Spencer, J.F., La Regina, M.C., Dhar, D., Tollefson, A.E., Toth, K., Wold, W.S.M., 2006. Syrian hamster as a permissive immunocompetent animal model for the study of oncolytic adenovirus vectors. Cancer Res. 66, 1270–6.

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–80.

Tollefson, A.E., Scaria, A., Hermiston, T.W., Ryerse, J.S., Wold, L.J., Wold, W.S., 1996a. The adenovirus death protein (E3-11.6K) is required at very late stages of infection for efficient cell lysis and release of adenovirus from infected cells. J. Virol. 70, 2296–306.

Tollefson, A.E., Scaria, A., Hermiston, T.W., Ryerse, J.S., Wold, L.J., Wold, W.S., 1996b. The adenovirus death protein (E3-11.6K) is required at very late stages of infection for efficient cell lysis and release of adenovirus from infected cells. J. Virol. 70, 2296–306.

Tollefson, A.E., Scaria, A., Saha, S.K., Wold, W.S., 1992. The 11,600-MW protein encoded by region E3 of adenovirus is expressed early but is greatly amplified at late stages of infection. J. Virol. 66, 3633–42.

Tollefson, A.E., Toth, K., Doronin, K., Kuppuswamy, M., Doronina, O.A., Lichtenstein, D.L., Hermiston, T.W., Smith, C.A., Wold, W.S.M., 2001. Inhibition of TRAIL-Induced Apoptosis and Forced Internalization of TRAIL Receptor 1 by Adenovirus Proteins. J. Virol. 75, 8875–8887.

Tollefson, A.E., Ying, B., Doronin, K., Sidor, P.D., Wold, W.S.M., 2007. Identification of a New Human Adenovirus Protein Encoded by a Novel Late l-Strand Transcription Unit. J. Virol. 81, 12918–12926.

Tomko, R.P., Xu, R., Philipson, L., 1997. HCAR and MCAR: the human and mouse cellular receptors for subgroup C adenoviruses and group B coxsackieviruses. Proc. Natl. Acad. Sci. U. S. A. 94, 3352–6.

Tomson, B.N., Arndt, K.M., 2013. The many roles of the conserved eukaryotic Paf1 complex in regulating transcription, histone modifications, and disease states. Biochim. Biophys. Acta 1829, 116–26.

Toth, K., Spencer, J.F., Tollefson, A.E., Kuppuswamy, M., Doronin, K., Lichtenstein, D.L., Regina, M.C. La, Prince, G.A., Wold, W.S.M., 2005. Cotton Rat Tumor Model for the Evaluation of Oncolytic Adenoviruses. Hum. Gene Ther. 16, 139–146.

Trentin, J.J., Yabe, Y., Taylor, G., 1962. The quest for human cancer viruses. Science 137, 835–41.

Trotman, L.C., Mosberger, N., Fornerod, M., Stidwill, R.P., Greber, U.F., 2001. Import of adenovirus DNA involves the nuclear pore complex receptor CAN/Nup214 and histone H1. Nat. Cell Biol. 3, 1092–100.

Turnell, A.S., Grand, R.J., Gorbea, C., Zhang, X., Wang, W., Mymryk, J.S., Gallimore, P.H., 2000. Regulation of the 26S proteasome by adenovirus E1A. EMBO J. 19, 4759–4773.

Turnham, R.E., Scott, J.D., 2016. Protein kinase A catalytic subunit isoform PRKACA; History, function and physiology. Gene 577, 101–8.

Ulfendahl, P.J., Linder, S., Kreivi, J.P., Nordqvist, K., Sevensson, C., Hultberg, H., Akusjärvi, G., 1987. A novel adenovirus-2 E1A mRNA encoding a protein with transcription activation properties. EMBO J. 6, 2037–44.

Ullman, A.J., Hearing, P., 2008. Cellular proteins PML and Daxx mediate an innate antiviral defense antagonized by the adenovirus E4 ORF3 protein. J. Virol. 82, 7325–35.

van Breukelen, B., Brenkman, A.B., Holthuizen, P.E., van der Vliet, P.C., 2003. Adenovirus type 5 DNA binding protein stimulates binding of DNA polymerase to the replication origin. J. Virol. 77, 915–22.

Van den Elsen, P., Houweling, A., Van der Eb, A., 1983. Expression of region E1b of human adenoviruses in the absence of region E1a is not sufficient for complete transformation. Virology 128, 377–90.

van der Vliet, P.C., Levine, A.J., 1973. DNA-binding proteins specific for cells infected by adenovirus. Nat. New Biol. 246, 170–4.

van Ormondt, H., Hesper, B., 1983. Comparison of the nucleotide sequences of early region E1b DNA of human adenovirus types 12, 7 and 5 (subgroups A, B and C).

Gene 21, 217–26.

Varga, M.J., Weibull, C., Everitt, E., 1991. Infectious entry pathway of adenovirus type 2. J. Virol. 65, 6061–70.

Velicer, L.F., Ginsberg, H.S., 1970. Synthesis, transport, and morphogenesis of type adenovirus capsid proteins. J. Virol. 5, 338–52.

Via, A., Uyar, B., Brun, C., Zanzoni, A., 2015. How pathogens use linear motifs to perturb host cell networks. Trends Biochem. Sci. 40, 36–48.

Vijayalingam, S., Chinnadurai, G., 2013. Adenovirus L-E1A activates transcription through mediator complex-dependent recruitment of the super elongation complex. J. Virol. 87, 3425–34.

Virtanen, A., Pettersson, U., 1983. The molecular structure of the 9S mRNA from early region 1A of adenovirus serotype 2. J. Mol. Biol. 165, 496–9.

Vlastaridis, P., Kyriakidou, P., Chaliotis, A., Van de Peer, Y., Oliver, S.G., Amoutzias, G.D., 2017. Estimating the total number of phosphoproteins and phosphorylation sites in eukaryotic proteomes. Gigascience 6, 1–11.

Vousden, K.H., Jat, P.S., 1989. Functional similarity between HPV16E7, SV40 large T and adenovirus E1a proteins. Oncogene 4, 153–8.

Wahlström, G.M., Vennström, B., Bolin, M.B., 1999. The adenovirus E1A protein is a potent coactivator for thyroid hormone receptors. Mol. Endocrinol. 13, 1119–29.

Walls, T., Shankar, A.G., Shingadia, D., 2003. Adenovirus: an increasingly important pathogen in paediatric bone marrow transplant patients. Lancet. Infect. Dis. 3, 79–86.

Walsh, D.A., Perkins, J.P., Krebs, E.G., 1968. An adenosine 3',5'-monophosphate-dependant protein kinase from rabbit skeletal muscle. J. Biol. Chem. 243, 3763–5.

Walsh, M.P., Seto, J., Liu, E.B., Dehghan, S., Hudson, N.R., Lukashev, A.N., Ivanova, O., Chodosh, J., Dyer, D.W., Jones, M.S., Seto, D., 2011. Computational Analysis of Two Species C Human Adenoviruses Provides Evidence of a Novel Virus. J. Clin. Microbiol. 49, 3482–3490.

Watanabe, H., Imai, T., Sharp, P.A., Handa, H., 1988. Identification of two transcription factors that bind to specific elements in the promoter of the adenovirus early-region 4. Mol. Cell. Biol. 8, 1290–300.

Weake, V.M., Workman, J.L., 2010. Inducible gene expression: diverse regulatory mechanisms. Nat. Rev. Genet. 11, 426–437.

Webster, L.C., Ricciardi, R.P., 1991. trans-dominant mutants of E1A provide genetic

evidence that the zinc finger of the trans-activating domain binds a transcription factor. Mol. Cell. Biol. 11, 4287–96.

Webster, L.C., Zhang, K., Chance, B., Ayene, I., Culp, J.S., Huang, W.J., Wu, F.Y., Ricciardi, R.P., 1991. Conversion of the E1A Cys4 zinc finger to a nonfunctional His2,Cys2 zinc finger by a single point mutation. Proc. Natl. Acad. Sci. U. S. A. 88, 9989–93.

Weinmann, R., Raskas, H.J., Roeder, R.G., 1974. Role of DNA-dependent RNA polymerases II and III in transcription of the adenovirus genome late in productive infection. Proc. Natl. Acad. Sci. U. S. A. 71, 3426–39.

Weitzman, M.D., Ornelles, D.A., 2005. Inactivating intracellular antiviral responses during adenovirus infection. Oncogene 24, 7686–7696.

Welch, E.J., Jones, B.W., Scott, J.D., 2010. Networking with AKAPs: context-dependent regulation of anchored enzymes. Mol. Interv. 10, 86–97.

Westheimer, F.H., 1987. Why nature chose phosphates. Science 235, 1173–8.

Wevers, D., Metzger, S., Babweteera, F., Bieberbach, M., Boesch, C., Cameron, K., Couacy-Hymann, E., Cranfield, M., Gray, M., Harris, L.A., Head, J., Jeffery, K., Knauf, S., Lankester, F., Leendertz, S.A.J., Lonsdorf, E., Mugisha, L., Nitsche, A., Reed, P., Robbins, M., Travis, D.A., Zommers, Z., Leendertz, F.H., Ehlers, B., 2011. Novel adenoviruses in wild primates: a high level of genetic diversity and evidence of zoonotic transmissions. J. Virol. 85, 10774–84.

White, E., 2001. Regulation of the cell cycle and apoptosis by the oncogenes of adenovirus. Oncogene 20, 7836–7846.

Whittaker, J.L., Byrd, P.J., Grand, R.J., Gallimore, P.H., 1984. Isolation and characterization of four adenovirus type 12-transformed human embryo kidney cell lines. Mol. Cell. Biol. 4, 110–6.

Whyte, P., Buchkovich, K.J., Horowitz, J.M., Friend, S.H., Raybuck, M., Weinberg, R.A., Harlow, E., 1988. Association between an oncogene and an anti-oncogene: the adenovirus E1A proteins bind to the retinoblastoma gene product. Nature 334, 124–9.

Wickham, T.J., Carrion, M.E., Kovesdi, I., 1995. Targeting of adenovirus penton base to new receptors through replacement of its RGD motif with other receptor-specific peptide motifs. Gene Ther. 2, 750–6.

Wickham, T.J., Mathias, P., Cheresh, D.A., Nemerow, G.R., 1993. Integrins alpha v beta 3 and alpha v beta 5 promote adenovirus internalization but not virus attachment. Cell 73, 309–19.

Williams, J.L., Garcia, J., Harrich, D., Pearson, L., Wu, F., Gaynor, R., 1990. Lymphoid

specific gene expression of the adenovirus early region 3 promoter is mediated by NF-kappa B binding motifs. EMBO J. 9, 4435–42.

Winberg, G., Shenk, T., 1984. Dissection of overlapping functions within the adenovirus type 5 E1A gene. EMBO J. 3, 1907–12.

Wold, W.S., Cladaras, C., Deutscher, S.L., Kapoor, Q.S., 1985. The 19-kDa glycoprotein coded by region E3 of adenovirus. Purification, characterization, and structural analysis. J. Biol. Chem. 260, 2424–31.

Wold, W.S.M., Ison, M.G., 2013. Adenoviruses. In: Fields Virology. pp. 1732–1767.

Wong, W., Scott, J.D., 2004. AKAP signalling complexes: focal points in space and time. Nat. Rev. Mol. Cell Biol. 5, 959–70.

Wu, E., Trauger, S.A., Pache, L., Mullen, T.-M., von Seggern, D.J., Siuzdak, G., Nemerow, G.R., 2004. Membrane cofactor protein is a receptor for adenoviruses associated with epidemic keratoconjunctivitis. J. Virol. 78, 3897–905.

Wu, J., Brown, S.H.J., von Daake, S., Taylor, S.S., 2007. PKA type IIalpha holoenzyme reveals a combinatorial strategy for isoform diversity. Science 318, 274–9.

Wu, L., Berk, A., 1988. Constraints on spacing between transcription factor binding sites in a simple adenovirus promoter. Genes Dev. 2, 403–11.

Yaniv, M., 2009. Small DNA tumour viruses and their contributions to our understanding of transcription control. Virology 384, 369–374.

Yatherajam, G., Huang, W., Flint, S.J., 2011. Export of adenoviral late mRNA from the nucleus requires the Nxf1/Tap export receptor. J. Virol. 85, 1429–38.

Ye, R.D., 2000. beta-Adrenergic agonists regulate NF-kappaB activation through multiple mechanisms. Am. J. Physiol. Lung Cell. Mol. Physiol. 279, L615-7.

Yew, P.R., Berk, A.J., 1992. Inhibition of p53 transactivation required for transformation by adenovirus early 1B protein. Nature 357, 82–85.

Yousef, A.F., Brandl, C.J., Mymryk, J.S., 2009. Requirements for E1A dependent transcription in the yeast Saccharomyces cerevisiae. BMC Mol. Biol. 10, 32.

Zhang, X., Odom, D.T., Koo, S.-H., Conkright, M.D., Canettieri, G., Best, J., Chen, H., Jenner, R., Herbolsheimer, E., Jacobsen, E., Kadam, S., Ecker, J.R., Emerson, B., Hogenesch, J.B., Unterman, T., Young, R.A., Montminy, M., 2005. Genome-wide analysis of cAMP-response element binding protein occupancy, phosphorylation, and target gene activation in human tissues. Proc. Natl. Acad. Sci. U. S. A. 102, 4459–64.

Zhang, Y., Bergelson, J.M., 2005. Adenovirus Receptors. J. Virol. 79, 12125–12131.

Zhang, Y., Huang, W., Ornelles, D.A., Gooding, L.R., 2010. Modeling adenovirus latency in human lymphocyte cell lines. J. Virol. 84, 8799–810.

Zhang, Z., Mui, M.Z., Chan, F., Roopchand, D.E., Marcellus, R.C., Blanchette, P., Li, S., Berghuis, A.M., Branton, P.E., 2011. Genetic analysis of B55alpha/Cdc55 protein phosphatase 2A subunits: association with the adenovirus E4orf4 protein. J. Virol. 85, 286–95.

Zhao, H., Granberg, F., Elfineh, L., Pettersson, U., Svensson, C., 2003. Strategic attack on host cell gene expression during adenovirus infection. J. Virol. 77, 11006–15.

Zheng, Y., Stamminger, T., Hearing, P., 2016. E2F/Rb Family Proteins Mediate Interferon Induced Repression of Adenovirus Immediate Early Transcription to Promote Persistent Viral Infection. PLoS Pathog. 12, e1005415.

Zu, Y.L., Takamatsu, Y., Zhao, M.J., Maekawa, T., Handa, H., Ishii, S., 1992. Transcriptional regulation by a point mutant of adenovirus-2 E1a product lacking DNA binding activity. J. Biol. Chem. 267, 20181–7.

Chapter 2

2 Functional and structural mimicry of cellular protein kinase A anchoring proteins by a viral oncoprotein

"Research is what I'm doing when I don't know what I'm doing."

– *Wernher von Braun*

2.1 Introduction

As obligate intracellular parasites, all viruses are critically dependent upon the host cell. Intensive selective pressure, rapid replicative cycle times and severe restrictions on viral genome size combine to drive virus evolution. As a consequence, viral regulatory proteins have been relentlessly forged into exquisitely sophisticated instruments that functionally reprogram the infected cell (Rozenblatt-Rosen et al., 2012). Studies of human adenovirus (HAdV), a small DNA tumour virus, illustrate the profound impact of viral proteins on multiple host functions to maximize viral propagation (Ferrari et al., 2014, 2008; Fonseca et al., 2012; Miyake-Stoner and O'Shea, 2014; Shah and O'Shea, 2015; Soria et al., 2010).

The multifunctional E1A proteins of HAdV are particularly adept at targeting key cellular regulators. Through these interactions, E1A creates a cellular milieu more conducive for replication by altering or inhibiting the normal function of host targets and establishing new connections in the host protein interaction network. Indeed, E1A enhances cell cycle entry, subverts innate immunity and intensively reprograms the cellular gene expression program (Ferrari et al., 2014, 2008; Pelka et al., 2008). The modular E1A proteins are dense with short linear sequence motifs that bind to and alter the activity of dozens of critical cellular proteins (Ferrari et al., 2009; Ferreon et al., 2013). Many of the interaction motifs in E1A are functional mimics of highly similar sequences present in cellular regulatory proteins. Thus, viral evolution has converged to generate specific high affinity protein interaction surfaces that perturb cell regulation by competing with endogenous targets.

Cellular compartmentalization of proteins is a widespread cellular mechanism that ensures the interaction of signalling molecules with a localized subset of appropriate effector proteins. As one well studied example, the activation of protein kinase A (PKA) signalling by the second messenger cyclic AMP (cAMP) is precisely restricted to discrete subcellular regions (Taylor et al., 2012). This is primarily achieved by a diverse set of cytoplasmic scaffolds collectively known as A-kinase anchoring proteins (AKAPs). AKAPs bind to PKA regulatory subunits via a well characterized amphipathic α-helix, localizing them to distinct cellular loci near PKA's substrates (Wong and Scott, 2004). Compartmentalization of PKA allows its enzymatic activity to be directed in a spatially defined and temporally specified manner and dysregulation of this compartmentalization has pathophysiological consequences (Gold et al., 2013).

Although the E1A proteins from multiple HAdVs can synergize with cAMP to alter viral and cellular gene expression (Engel et al., 1991, 1988; Hardy and Shenk, 1988; B H Lee and Mathews, 1997; Müller et al., 1989) the exact mechanism remains unclear. Interestingly, HAdV-12 E1A binds directly to the regulatory subunits of PKA, resulting in the relocalization of one isoform from the cytoplasm to the nucleus (Brockmann and Esche, 2003; Fax et al., 2001). These results suggest that E1A may function as a 'viral AKAP' by redirecting the subcellular localization of PKA to alter transcription.

Here we show that HAdV E1A mimics cellular AKAPs in both structure and function. We found that the PKA RIα and RIIα subunits are conserved targets of most HAdV E1A species. Structural modeling and a docking analysis predict a remarkable similarity between the binding of E1A and cellular AKAPs to PKA, which was confirmed experimentally. In addition, we observed E1A-mediated relocalization of PKA subunits and competition between E1A and cellular AKAPs during infection that contribute to HAdV gene expression and overall viral replication. Together, our studies identify E1A as the first known viral AKAP, and reveal a unique example of viral subversion of the PKA pathway via structural mimicry.

2.2 Materials and Methods

2.2.1 Cell lines and transfections

Human A549 (provided by Russ Wheeler, Molecular Pathology/Genetics London Health Sciences Centre), HT1080 (purchased from the American Type Culture Collection), and HEK293 cells (Graham et al., 1977) were grown at 37°C with 5% CO_2 in DMEM (Multicell) supplemented with 10% fetal bovine serum (FBS) (Gibco). Plasmids were transfected into A549 and HT1080 cells using XtremegeneHP (Roche) following the manufacturer's recommendation. After 24 hours of incubation, transfected cells were used for downstream experiments.

2.2.2 Virus infection of cells

All viruses were derived from the HAdV-5 *dl*309 background and express the 289R and 243R E1A proteins (Egan et al., 1988; Jones and Shenk, 1979). A549 cells were infected with WT (*dl*309) or HAdV containing the indicated E1A mutant: ΔE1A (*dl*312), Δ4-25 (*dl*1101). Cells were infected at a multiplicity of infection (MOI) of 5 pfu (plaque-forming units)/cell. Cell cultures were infected at 50% confluence and subconfluent cells were collected at indicated time points for downstream experiments.

2.2.3 RNAi knockdown

Downregulation of PKA subunits RIα, RIIα, and Cα was performed using Silencer Select siRNA (Thermo). The following PKA oligos were utilized: s286 (PRKAR1A), s11086 (PRKAR2A), and s11065 (PRKACA). Four hours after seeding, siRNA was delivered to A549 cells via transfection with Silentfect (BioRad) according to the manufacturer's instructions. A scrambled siRNA was used as a negative control. Treated cells were used for experiments 48 hours post-transfection. Downregulation of E1A in HEK293 cells was performed using a cocktail of E1A-specific siRNAs generated by Thermo Fisher's design platform: (GAUUUUUCCCGAGUCUGUAtt, CGGAGGUGUUAUUACCGAAtt, and CUGUAUGAUUUAGACGUGAtt).

Table 2.1. List of plasmids used in this chapter

Clone	Backbone(s)	Notes
13S Ad3 E1A	pEGFP-C2	Species B
13S Ad4 E1A	pEGFP-C2	Species E
13S Ad5 E1A	pEGFP-C2, pcDNA3	Species C
13S Ad9 E1A	pEGFP-C2	Species D
13S Ad12 E1A	pEGFP-C2	Species A
13S Ad40 E1A	pEGFP-C2	Species F
Ad5 E1A 1-82	pEGFP-C2	N-terminus and CR1
Ad5 E1A 93-139	pEGFP-C2	CR2
Ad5 E1A 139-204	pEGFP-C2	CR3
Ad5 E1A 187-289	pEGFP-C2	Exon 2 including CR4
*Ad5 E1A Δ1-82	pcDNA3	
*Ad5 E1A Δ1-29	pcDNA3	
*Ad5 E1A Δ1-14	pcDNA3	
Ad5 E1A Δ4-25	pcDNA3	*dl*1101
*Ad5 E1A Δ16-28	pcDNA3	
Ad5 E1A Δ26-35	pcDNA3	
Ad5 E1A Δ30-49	pcDNA3	
Ad5 E1A Δ48-60	pcDNA3	
Ad5 E1A Δ61-69	pcDNA3	
Ad5 E1A Δ70-81	pcDNA3	
*Ad5 E1A 1-29	pEGFP-C2	
*Ad5 E1A 1-14	pEGFP-C2	
*Ad5 E1A 14-28	pEGFP-C2	
*Ad5 E1A 16-28	pEGFP-C2	
*Ad5 E1A 29-49	pEGFP-C2	
*Ad5 E1A D21K	pcDNA3	
*Ad5 E1A E26K	pcDNA3	
*Ad5 E1A V27K	pcDNA3	
*Ad5 E1A E26A V27A	pcDNA3	
*Ad5 E1A E25K	pcDNA3	
*PKA RIα	pcDNA-HA, pcDNA-MYC	From Addgene 23741
*PKA RIIα	pcDNA-HA, pcDNA-MYC	From Addgene 23789
*PKA Cα	pcDNA-HA	From Addgene 23495
*PKA RIα Q28E	pcDNA-HA	
*PKA RIα L31A K32A	pcDNA-HA	
*PKA RIα I35A V36A	pcDNA-HA	
*PKA RIα Δ1-63	pcDNA-MYC	ΔD/D
*PKA RIIα Δ1-45	pcDNA-MYC	ΔD/D
*PKA RIα 1-63	pEGFP-N1	D/D only
*PKA RIIα 1-45	pEGFP-N1	D/D only
RIAD	pEGFP-C2	LEQYANQLADQIIKEAT, from Alan Howe
sAKAPis	pEGFP-C2	QIEYLAKQIVDNAIQQAK, from Alan Howe
D-AKAP1	pEGFP-C2	From Thomas Kuntzinger
*D-AKAP1 Δ344-357	pEGFP-C2	
*AKAP-E1A	pEGFP-C2	AKAP-E1A chimera

*Novel clone generated for this study

2.2.4 Plasmids

All constructs were expressed in vectors under control of the CMV promoter. WT RIα, RIIα, and Cα were PCR amplified (from Addgene 23741, 23789 and 23495) and cloned into pcDNA4-HA and pCANmyc. RIα Δ1-63 and RIIα Δ1-45 were similarly derived and expressed in pCANmyc. D/D fragments of RIα and RIIα were both expressed as EGFP fusions from pEGFP-N1. E1A fragments were expressed as fusions to EGFP and either described previously (1-82, 93-139, 139-204, 187-289) (Pelka et al., 2011) or derived via PCR and cloned into pEGFP-C2 (1-29, 1-14, 14-28, 16-28, 29-49). WT HAdV-5 E1A and its associated deletion mutants were all expressed in pcDNA3. These constructs were previously described (Δ4-25, Δ26-35, Δ30-49, Δ48-60, Δ61-69, Δ70-81) (Pelka et al., 2011) or generated via PCR (Δ1-82, Δ1-14, Δ1-29, Δ16-28). Point of mutants of E1A (D21K, E26K, V27K, E26A V27A, E25K) and RIα (Q28E, L31A K32A, I35A V36A) were generated by PCR and expressed in pcDNA3 and pcDNA-HA respectively. The largest E1A isoform from six HAdV species were cloned as EGFP fusions. D-AKAP1 mutants were generated via PCR of a construct kindly provided by Thomas Kuntziger (Oslo) and expressed in pEGFP-C2. RIAD-EGFP and sAKAPis-EGFP were provided by Alan Howe (Vermont). A list of plasmids used in this chapter can be found in table 2.1.

2.2.5 Western blotting and co-immunoprecipitation

Cells were lysed in NP-40 lysis buffer (150mM NaCl, 50mM Tris-HCL pH 7.5, 0.1% NP-40) with protease inhibitor cocktail. Protein concentrations were determined using BioRad protein assay reagent using BSA as a standard. Immunoprecipitations were carried out at 4°C for 4 hours, or overnight for endogenous interactions. 2% of sample was kept as input control. After washing with NP-40 buffer, complexes were boiled in 25 µL of LDS sample buffer for 5 minutes. Samples were separated on NuPage 4-12% Bis-Tris gradient gels (Life Technologies) and transferred onto a PVDF membrane (Amersham). Membranes were blocked in 5% skim milk constituted in TBS with 0.1% Tween-20. Horseradish peroxidise-conjugated secondary antibody was detected using Luminata Forte or Crescendo substrate (Millipore). For biochemical fractionation of

Table 2.2. List of primary antibodies used in this chapter

Reactivity	Purpose	Description	Company
RIα	Western	Mouse monoclonal	BD
RIα	IF, ChIP	Rabbit polyclonal	Thermo
RIIα	Western	Mouse monoclonal	BD
RIIα	IF, ChIP	Rabbit polyclonal	Santa Cruz
Cα	Western	Mouse monoclonal	BD
Cα	IF, ChIP	Rabbit polyclonal	Santa Cruz
M73 (E1A)	Western, IP, IF	Mouse monoclonal	In-house
M58 (E1A)	Western	Mouse monoclonal	In-house
Actin	Western	Rabbit polyclonal	Sigma
EGFP	Western, IP	Rabbit polyclonal	Clontech
9E10 (MYC)	Western	Mouse monoclonal	In-house
3F10 (HA)	Western	Rat monoclonal	Roche
AKAP7	Western, IP	Rabbit polyclonal	Santa Cruz
2A6 (E1B-55K)	Western	Mouse monoclonal	In-house
B68 (DBP)	Western, IF	Mouse monoclonal	In-house
E3-19K	Western	Rabbit serum	In-house
1807-4 (E4orf6)	Western	Rabbit polyclonal	In-house
Ad5 capsid	Western	Rabbit polyclonal	Abcam
Histone H3	Western	Rabbit polyclonal	Abcam

infected A549 cells, nuclear and cytoplasmic extracts were acquired using an NE-PER kit from Thermo-Fisher. Primary antibodies used in this chapter can be found in table 2.2.

2.2.6 Immunofluorescence microscopy and image analysis

Cells were fixed in 3.7% paraformaldehyde, permeabilized on ice using 0.2% Triton X-100, and blocked using 3% BSA in phosphate-buffered saline (PBS). Samples were incubated in the indicated primary antibody for 1 hour at room temperature or 4°C overnight and another hour at room temperature with secondary antibodies (Alexa Fluor 594 α-rabbit, Alexa Fluor 488 α-mouse) (Life Technologies). Samples were mounted with Prolong Gold reagent containing DAPI (Life Technologies). Confocal images were acquired using a Fluoview 1000 laser scanning confocal microscope (Olympus Corp). Non-confocal images were acquired using an Eclipse Ti-U inverted laser microscope (Nikon). Quantification of total cellular signal and nuclear signal was conducted using ImageJ. Cells were normalized for both cytoplasmic and nuclear size and %nuclear signal was determined as previously described (Magico and Bell, 2011).

2.2.7 Quantitative RT-PCR

Total RNA was prepared with Trizol extraction (Life Technologies). A total of 1 µg of RNA was reverse transcribed into cDNA by random priming using the qScript cDNA supermix (Quanta Biosciences) following the manufacturer's instructions. Quantification of cDNA was done using Power SYBR-Green mastermix (Applied Biosystems) with oligonucleotide sequences that specifically recognize the indicated target. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a control for total cDNA along with a no-RT negative control. Results were normalized to the GAPDH and uninfected samples and calculated using the ΔΔCt method (Schmittgen and Livak, 2008). A list of all primers used in this chapter can be found in tables 2.3 and 2.4.

2.2.8 Chromatin immunoprecipitation

Approximately 10^7 cells per sample were cross-linked in 2mM ethylene glycol bis(succinimidyl succinate) (EGS) for 1 hour followed by 1% formaldehyde for 15 minutes at room temperature. Reactions were quenched with 0.125M glycine and

Table 2.3. List of qPCR primers used in this chapter

Target	Region	Location	Forward	Reverse	Size
GAPDH	Transcript	566-644	ACTGCTTAGCACCCCTGGCCAA	ATGGCATGGACTGTGGTCATGAGTC	79
E1A	Transcript	715-834	ACACCTCCTGAGATACACCC	TTATTGCCCAGGCTCGTTAAGC	120
E1B	Transcript	578-697	GACAATTACAGAGGATGGGC	CACTCAGGACGGTGTCTGG	120
E2	Transcript	17-155	GGGGGTGGTTTCGCGCTGCTCC	GCGGATGAGGCGGCGTATCGAG	138
E3	Transcript	13-162	GAGGCAGAGCAACTGCGCC	GCTCTCCCTGGGCGGTAAGCCGG	150
E4	Transcript	3-129	GCCCCCATAGGAGGTATAAC	GGCTGCCGCTGTGGAAGCGC	127
GAPDH	Promoter	231 bp upstream of TSS	TTCGCCCCAGGCTGGATGG	AGGCGGAGGACAGGATGGC	126
E1B	Promoter	1546-1708	GGTGTAAACCTGTGATTGCG	CAGATGTAACCAAGATTAGCCC	162
E3	Promoter	27501-27633	GGCGGCTTTCGTCACAGGG	TCTGAAATGTCCCGTCCGG	132
E4	Promoter	35585-35694	CAGCTCAATCAGTCACAGTGTAAAAAAGGGCC	TGCGGTTTTCTGGGTGTTTT	110

Table 2.4. List of cloning primers used in this chapter

Clone	Forward	Reverse	Notes
Ad5 E1A Δ1-82	ATCCGAATTCATGTTTCCGCCGGCG CCCGG	CGGCTCGAGCTCCACACACGCAA TCACAGG	
Ad5 E1A Δ1-29	CCACGAATTCGATAATCTTCCACCT CCTAGC	CGGCTCGAGCTCCACACACGCAA TCACAGG	
Ad5 E1A Δ1-14	TTCGAATTCATGGCCGCCAGTCTTT TG	ACTGTCGACTTATGGCCTGGGAC GTTTACAGCTC	
Ad5 E1A Δ16-28	ATTACCGAAGAAATGGCTGATAAT CTTCCACC	GGTGGAAGATTATCAGCCATTTC TTCGGTAAT	Internal
	CGACGAATTCATGAGACATATTATC TGC	CGGCTCGAGCTCCACACACGCAA TCACAGG	Flanking
Ad5 E1A 1-29	CGACGAATTCATGAGACATATTATC TGC	GTTGTCGACTTAAGCCAGTACCT CTTCG	
Ad5 E1A 1-14	CGACGAATTCATGAGACATATTATC TGC	CATCCTCGAGCTATTCTTCGGTA ATAAC	
Ad5 E1A 14-28	GATGGAATTCGAAATGGCCGCCAG TC	CATCCTCGAGCTACAGTACCTCT TCGATC	
Ad5 E1A 16-28	GATGGAATTCGCCGCCAGTCTTTTG	CATCCTCGAGCTACAGTACCTCT TCGATC	
Ad5 E1A 29-49	GATGGAATTCGCTGATAATCTTCCA C	CATCCTCGAGCTATAAATCATAC AGTTCG	
Ad5 E1A D21K	CCGCCAGTCTTTTGAAACAGCTGAT CGAAG	CTTCGATCAGCTGTTTCAAAAGA CTGGCGG	Internal
	CGACGAATTCATGAGACATATTATC TGC	CGGCTCGAGCTCCACACACGCAA TCACAGG	Flanking
Ad5 E1A E26K	GACCAGCTGATCGAAAAAGTACTG GCTGATAATC	GATTATCAGCCAGTACTTTTTCG ATCAGCTGGTC	Internal
	CGACGAATTCATGAGACATATTATC TGC	CGGCTCGAGCTCCACACACGCAA TCACAGG	Flanking
Ad5 E1A V27K	CAGCTGATCGAAGAGAAACTGGCT GATAATCTTC	GAAGATTATCAGCCAGTTTCTCT TCGATCAGCTG	Internal
	CGACGAATTCATGAGACATATTATC TGC	CGGCTCGAGCTCCACACACGCAA TCACAGG	Flanking
Ad5 E1A E26A V27A	GACCAGCTGATCGAAGCCGCCCTG GCTGATAATC	GATTATCAGCCAGGGCGGCTTCG ATCAGCTGGTC	Internal
	CGACGAATTCATGAGACATATTATC TGC	CGGCTCGAGCTCCACACACGCAA TCACAGG	Flanking
Ad5 E1A E25K	TTGGACCAGCTGATCAAAGAGGTA CTGGCTGAT	ATCAGCCAGTACCTCTTTGATCA GCTGGTCCAA	Internal
	CGACGAATTCATGAGACATATTATC TGC	CGGCTCGAGCTCCACACACGCAA TCACAGG	Flanking
PKA RIα	ATCCGGATCCATGGAGTCTGGCAGT ACC	GATGCTCGAGTCAGACAGACAGT GACACAAAAC	
PKA RIIα	AGTCGGATCCATGAGCCACATCCA GATC	TATTCTCGAGCTACTGCCCGAGG TTGCCCAG	
PKA Cα	ATACGGATCCATGGGCAACGCCGC CGCC	GCCGGTCGACCTAAAACTCAGAA AACTCCTT	
PKA RIα Q28E	CAGAAGCATAACATTGAAGCGCTG CTCAAAGAT	ATCTTTGAGCAGCGCTTCAATGT TATGCTTCTG	Internal
	ATCCGGATCCATGGAGTCTGGCAGT ACC	GATGCTCGAGTCAGACAGACAGT GACACAAAAC	Flanking
PKA RIα L31A K32A	AACATTCAAGCGCTGGCCGCCGAT TCTATTGTGC	GCACAATAGAATCGGCGGCCAG CGCTTGAATGTT	Internal
	ATCCGGATCCATGGAGTCTGGCAGT ACC	GATGCTCGAGTCAGACAGACAGT GACACAAAAC	Flanking

PKA RIα I35A V36A	CTGCTCAAAGATTCTGCCGCCCAGT TGTGCACTGC	GCAGTGCACAACTGGGCGGCAG AATCTTTGAGCAG	Internal
	ATCCGGATCCATGGAGTCTGGCAGT ACC	GATGCTCGAGTCAGACAGACAGT GACACAAAAC	Flanking
PKA RIα Δ1-63	ATCCGGATCCCAGATTCAGAATCTG CAG	GATGCTCGAGTCAGACAGACAGT	
PKA RIIα Δ1-45	AGCCGGATCCATGCCAGCCTCAGT CCTGCCCGC	TATTCTCGAGCTACTGCCCGAGG TTGCCCAG	
PKA RIα 1-63	ATCCCTCGAGATGGAGTCTGGCAGT ACC	CATCGGATCCTTTGCCTCCTCCTT CTC	
PKA RIIα 1-45	AGCCCTCGAGATGAGCCACATCCA GATC	CATAGGATCCGCGCGGGCCTCGC GCAG	
D-AKAP1 Δ344-357	GATAGAAATGAGGAGGAAGCAACC GAACAGG	CCTGTTCGGTTGCTTCCTCCTCAT TTCTATC	Internal
	GATGGAATTCATGGCAATCCAGTTC CG	CATCGCGGCCGCTCAAAGGCTTG TGTAGTAG	Flanking
AKAP1-E1A	GAGGTACTGGAAGCAACCGAACAG GTG	CGGCCATTTCCTCCTCATTTCTAT CCAAG	Internal (AKAP template)
	GAAATGAGGAGGAAATGGCCGCCA GTC	CGGTTGCTTCCAGTACCTCTTCG ATCAG	Internal (E1A template)
	GATGGAATTCATGGCAATCCAGTTC CG	CATCGCGGCCGCTCAAAGGCTTG TGTAGTAG	Flanking (AKAP-E1A)

washed twice with cold PBS. Cell pellets were processed in ChIP buffer 1 (10mM HEPES [pH 6.5], 10mM EDTA, 0.5mM EGTA, 0.25% Triton X-100), ChIP buffer 2 (10mM HEPES [pH 6.5], 1mM EDTA, 0.5 mM EGTA, 200mM NaCl), and ChIP buffer 3 (50mM Tris-HCl [pH 8], 10mM EDTA, 0.5% Triton X-100, 1% SDS, and protease inhibitors). Lysates were sonicated in an ultrasonic bioruptor bath (Diogenode) to yield DNA fragments between 200-500 basepairs. Eighty µg of chromatin supernatant was used for ChIP, 1% of this was kept for input controls. Samples were diluted 10-fold in ChIP dilution buffer (50mM Tric-HCl [pH 8], 10mM EDTA, 150mM NaCl, 0.1% Triton X-100, protease inhibitors) and precleared with 30µL of Protein G Dynabeads (Invitrogen) for 1 hour at 4°C. Immunoprecipitations were performed overnight at 4°C using 5µg of the indicated antibody. The next morning, 30µL of Dynabeads were incubated with each sample for 2 hours. Beads were then washed with twice each with wash buffer 1 (20mM Tris-HCl [pH 8], 2mM EDTA, 150mM NaCl, 1% Triton X-100, 0.1% SDS), wash buffer 2 (20mM Tris-HCl [pH 8], 2mM EDTA, 500mM NaCl, 1% Triton X-100, 0.1% SDS), and wash buffer 3 (10mM Tris-HCl [pH 8], 1mM EDTA). Immunocomplexes were extracted twice with 150µL of elution buffer (0.1M NaHCO3, 1% SDS). Twenty five µL of 2.5M NaCl was added to the 300µL pooled elutions and incubated overnight at 65°C to de-crosslink the complexes. DNA was purified using a PCR purification kit (Thermo). qPCR using SYBR-Green was performed as described previously using 80nM oligos and 0.5µL of ChIP DNA per 15µL reaction.

2.2.9 Statistical analysis

All experiments were carried out with three biological replicates performed in duplicate. Graphs represent mean and standard error of the mean (S.E.M.) of all biological replicates. For western blots a representative image was selected. Statistical significance of numerical differences was calculated using one-way ANOVA and Holm-Sidak post-hoc comparison between experimental conditions.

2.2.10 Docking methods

To model the interaction between PKA and E1A, we first performed a structural prediction of the amino terminus of E1A by submitting the primary sequence to Phyre 2

(Kelley et al., 2015). The predicted structure of E1A was subsequently docked onto PKA (PDB ID: 3IM4) using the standard settings profile of ClusPro2.0 (Kozakov et al., 2013). Residues forming an E1A-PKA binding interface within 4 Angstroms were selected for further experimental analysis. All images were generated in the PyMOL Molecular Graphics System, Version 1.8 Schrödinger, LLC. Additional *in silico* comparisons of HAdV-5 and HAdV-4 E1A were conducted using Clustal Omega (Goujon et al., 2010) and the UCL Department of Computer Science's PSI-PRED protein sequence analysis workbench (Buchan et al., 2013).

2.3 Results

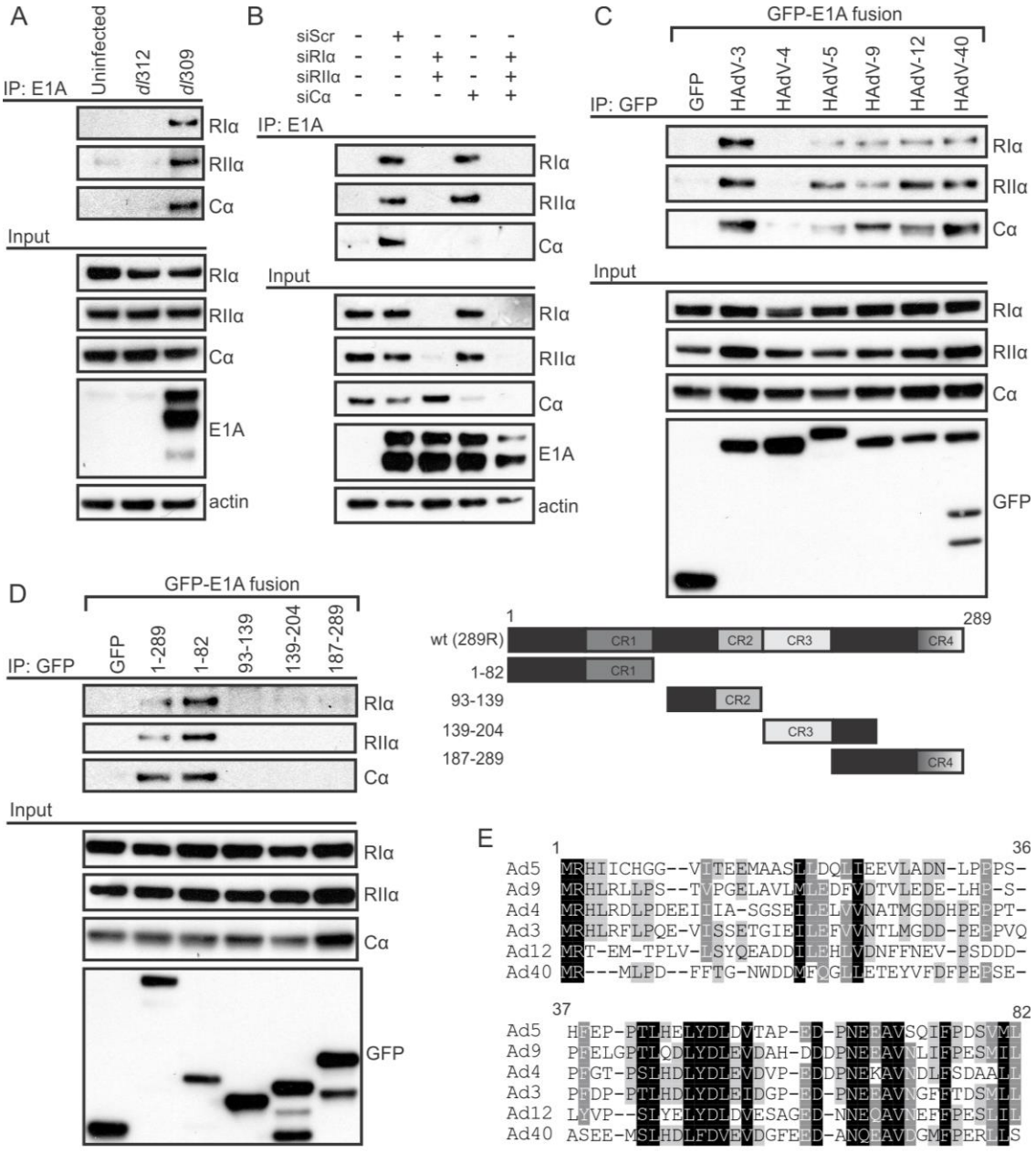
2.3.1 Multiple PKA subunits are conserved targets of HAdV E1A

The E1A proteins from multiple HAdVs synergize with cAMP to alter viral and cellular gene expression. A direct interaction between HAdV-12 E1A and the type I and type II regulatory subunits of PKA (RIα and RIIα) was previously reported, but has not been investigated further (Fax et al., 2001). It was also not known if this interaction was specific to HAdV-12 E1A. To further explore the E1A-PKA interaction, A549 lung adenocarcinoma cells were infected with wildtype (WT) HAdV-5 or a ΔE1A virus and co-immunoprecipitations were performed (Figure 2.1A). Similarly to HAdV-12 E1A, HAdV-5 E1A interacted with endogenous PKA regulatory subunits RIα and RIIα. Interestingly, we also found a previously unknown interaction between HAdV-5 E1A and the endogenous PKA catalytic subunit Cα. siRNA-mediated downregulation of specific PKA subunits demonstrated that E1A's association with Cα required expression of RIα and RIIα (Figure 2.1B). This suggests that the interaction with the Cα subunit may be indirect and that E1A binds the entire PKA holoenzyme.

To determine if the interactions between E1A and the PKA subunits are evolutionarily conserved across the different HAdV species, HT1080 fibrosarcoma cells were transfected with vectors expressing the PKA subunits and the largest E1A isoform from six different HAdV species. Co-immunoprecipitation analysis revealed that RIα, RIIα, and Cα all interacted with each of the E1A proteins tested, with the exception of HAdV-4

Figure 2.1. Multiple subunits of PKA are conserved targets of HAdV-5 E1A during infection.

(A, B) A549 cells were infected with either WT (*dl*309) or ΔE1A HAdV-5 (*dl*312) at an MOI of 5 pfu/cell and cell lysates were harvested for co-immunoprecipitation. (A) Associations between E1A and endogenous PKA subunits RIα, RIIα, and Cα are shown. (B) Cells were treated with the indicated siRNAs (shown in the inset panel) prior to infection. Associations between E1A and endogenous PKA subunits RIα, RIIα, and Cα are shown. The interaction between E1A and the PKA catalytic subunit Cα required the presence of PKA regulatory subunits, indicating an indirect association. E1A's interaction with the regulatory subunits was unaffected by the Cα knockdown. **(**C, D) HT1080 cells were co-transfected with PKA subunits and various E1A constructs expressed as fusions to EGFP and cell lysates were harvested for co-immunoprecipitation. (C) Full-length E1A proteins from 6 different HAdV species all interacted with PKA subunits RIα, RIIα, and Cα to varying degrees, with the exception of HAdV-4. (D) Of the E1A fragments tested (shown in the inset panel) only the N-terminus was sufficient for interaction with PKA. This region of E1A has several regions of high amino acid sequence conservation between various HAdV species (E).


A
Uninfected
d/312
d/309
IP: E1A
RIα
RIIα
Cα
Input
RIα
RIIα
Cα
E1A
actin
B
siScr - + - - -
siRIα - - + - +
siRIIα - - + - +
siCα - - - + +
IP: E1A
RIα
RIIα
Cα
Input
RIα
RIIα
Cα
E1A
actin
C
GFP-E1A fusion
IP: GFP
GFP
HAdV-3
HAdV-4
HAdV-5
HAdV-9
HAdV-12
HAdV-40
RIα
RIIα
Cα
Input
RIα
RIIα
Cα
GFP
D
GFP-E1A fusion
IP: GFP
GFP
1-289
1-82
93-139
139-204
187-289
RIα
RIIα
Cα
Input
RIα
RIIα
Cα
GFP
1
289
wt (289R)
CR1
CR2
CR3
CR4
1-82
93-139
139-204
187-289
E
1
36
Ad5 MRHIICHGG--VITEEMAASLLDQLIEEVLADN-LPPPS-
Ad9 MRHLRLLPS--TVPGELAVLMLEDFVDTVLEDE-LHP-S-
Ad4 MRHLRDLPDEEIIIA-SGSEILELVVNATMGDDHPEPPT-
Ad3 MRHLRFLPQE-VISSETGIEILEFVVNTLMGDD-PEPPVQ
Ad12 MRT-EM-TPLV-LSYQEADDILEHLVDNFFNEV-PSDDD-
Ad40 MR---MLPD--FFTG-NWDDVFQGLLETEYVFDFPEPSE-
37
82
Ad5 HGEP-PTLHELYDLDVTAP-EDD-PNEEAVSQIFPDSVML
Ad9 PGELGPTLQDLYDLEVDAH-DDDPNEEAVNLIFPESMIL
Ad4 PGGT-PSLHDLYDLEVDVP-EDDPNEKAVNDLFSDAALL
Ad3 PGDP-PTLHDLYDLEIDGP-EDD-PNEEAVNGFFTDSVLL
Ad12 LVVP--SLYELYDLDVESAGEDD-NNEQAVNEFFPESLIL
Ad40 ASEE-MSLHDLFDVEVDGFEEDD-ANQEAVDGMFPERLLS

(Figure 2.1C). The conservation of the E1A-PKA interaction across most HAdV species suggests that targeting of PKA is an important evolutionarily conserved function of E1A.

E1A is comprised of a series of protein interaction modules that often can function independently (Pelka et al., 2008). To grossly define which portion of E1A is required for PKA interaction, lysates from HT1080 cells expressing the PKA subunits and the indicated large fragments of HAdV-5 E1A expressed as EGFP-fusions, were subjected to Co-IP. The N-terminal 82 residues of HAdV-5 E1A were sufficient for association with PKA (Figure 2.1D). Interestingly, this region of E1A has been previously shown to be involved in alterations in cAMP signaling (Gedrich et al., 1992). In addition, the interaction of HAdV-12 E1A with PKA similarly mapped to residues 1-79 in a yeast interaction assay (Fax et al., 2001). As can be seen from the amino acid sequence alignment, there are several areas of high sequence similarity in this region in the E1A proteins from various HAdV species (Figure 2.1E).

2.3.2 The sequence of E1A that binds PKA resembles a cellular AKAP

To determine the minimal region of HAdV-5 E1A necessary and sufficient for PKA interaction, we carried out a detailed mutational analysis of the N-terminus of E1A. Cells were co-transfected with vectors expressing PKA subunits and the indicated E1A mutants, each expressed in the context of full-length HAdV-5 E1A and containing a small in-frame deletion in the N-terminus. As expected, deletion of residues 1-82 abrogated interaction with PKA, confirming that the E1A N-terminus as necessary and sufficient for binding PKA (Figure 2.2A). Several smaller, overlapping deletions also had similar defects for PKA-binding, specifically Δ1-29, Δ4-25, Δ16-28, and Δ26-35. However, adjacent deletion mutants Δ1-14 and Δ30-49, or more distant deletions retained interaction. This suggests that a region spanning residues 14-29 of HAdV-5 E1A is necessary for PKA binding.

We next co-transfected cells with PKA and small E1A fragments expressed as EGFP fusions. Co-immunoprecipitation on lysates of these cells demonstrates that the 14-28 region of E1A was sufficient to confer an interaction with PKA (Figure 2.2B). This

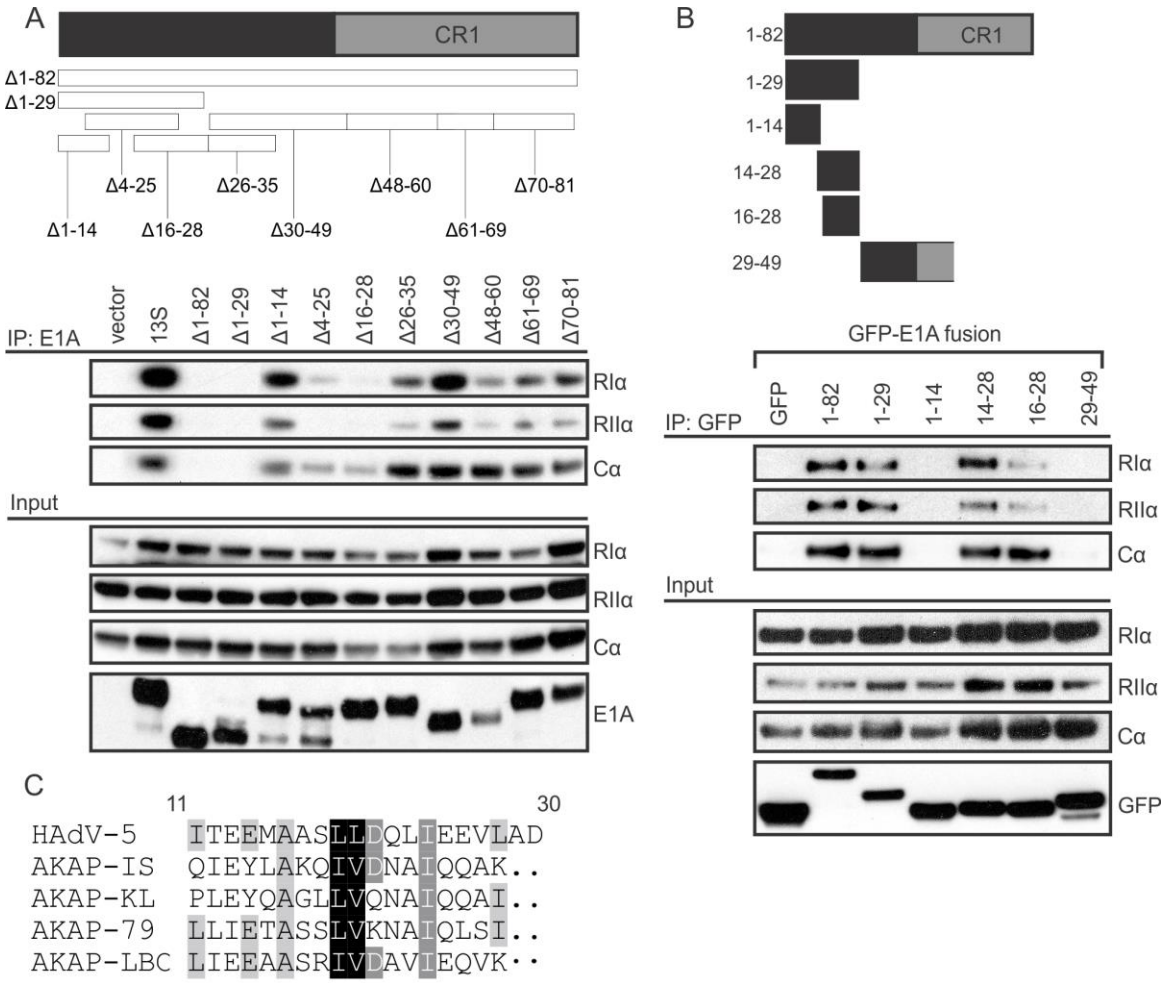


Figure 2.2. E1A contains an AKAP-like domain that is necessary and sufficient for binding PKA.

HT1080 cells were co-transfected with PKA subunits and various E1A constructs and cell lysates were harvested for co-immunoprecipitation. (A) Mutational analysis using N-terminal deletion mutants of full-length E1A (shown in the inset panel) revealed that amino acids 14-28 are necessary for binding PKA subunits. (B) The N-terminal region of E1A, when expressed as a fragment fused to EGFP (shown in the inset panel), was also sufficient for binding PKA subunits. This region of E1A bears amino acid similarity to a variety of known AKAPs and is predicted to contain an amphipathic α-helix (C).

region is similar in the E1A proteins from most HAdV species (Figure 2.1E) and also has noticeable sequence similarity to the PKA-binding regions of a number of cellular AKAPs (Figure 2.2C). Interestingly, AKAPs bind PKA regulatory subunits via an amphipathic α-helix secondary structure motif (Newlon et al., 1999; Sarma et al., 2010), and modeling of the N-terminus of HAdV-5 E1A predicts it also forms an amphipathic α-helix (Figure 2.3). Furthermore, the E1A proteins from all HAdV species are strongly predicted to form an α-helix in this region (Gedrich et al., 1992; Pelka et al., 2008), with the exception of HAdV-4 E1A, which is predicted to form a lower-confidence helix (Figure 2.4A) and does not bind PKA efficiently (Figure 2.1C). Taken together, this suggests that E1A binds PKA by structurally mimicking the AKAPs' amphipathic α-helix motif.

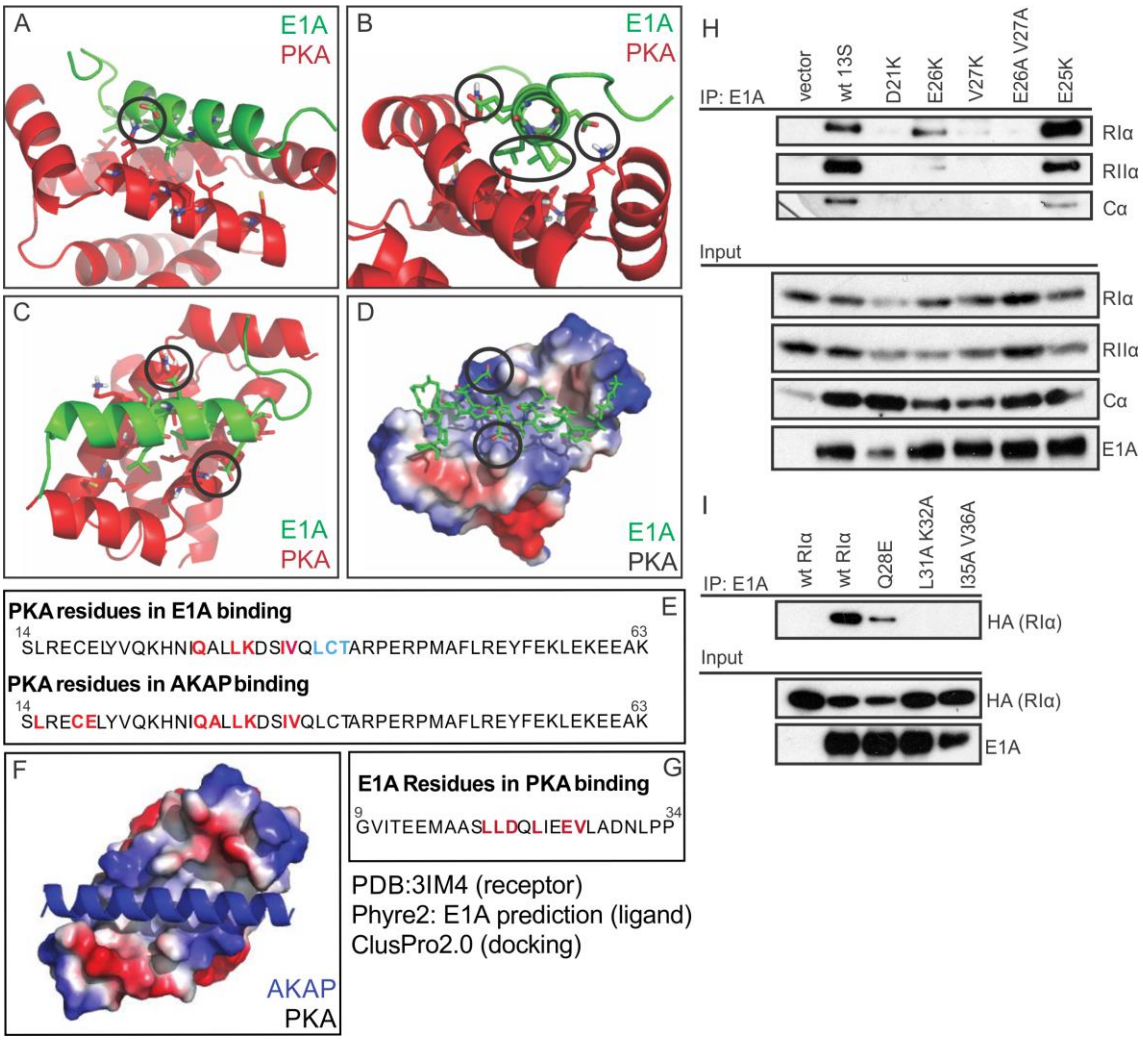
2.3.3 E1A binds the same surface of PKA targeted by cellular AKAPs

We performed *in silico* molecular modeling to predict the docking of the N-terminus of E1A with PKA. Docking simulations performed using the crystal structure of a dual-specificity cellular AKAP in complex with the RIα homodimer of PKA suggest that the interaction of E1A with RIα is virtually equivalent to that of the cellular AKAP (Figure 2.3A-D, F). This model predicts a number of distinct interactions between E1A and RIα (Figure 2.3E, G), which were experimentally tested (Figure 2.3H, I). E1A mutants D21K, E26K, V27K and E26A/V27A, reduced the interaction with RIα as predicted, whereas substitution of E25 with K, which is not predicted to alter binding, had no effect (Figure 2.3H). Similarly, RIα mutants Q28E, L31A K32A and I35A/V36A displayed a reduced ability to bind E1A as predicted by the model (Figure 2.3I). These results indicate that the docking model can correctly predict key residues necessary for binding, which further suggests that E1A structurally mimics a cellular AKAP in order to bind PKA.

Cellular AKAPs bind to the docking/dimerization (D/D) domain located at the N-terminus of the PKA regulatory subunits RIα and RIIα (Banky et al., 1998; Newlon et al., 1999). Given the sequence and predicted structural similarity between E1A and cellular AKAPs, we tested if the D/D domain was necessary for the interaction with E1A.

Figure 2.3. E1A is predicted and confirmed to dock to PKA equivalently to a cellular AKAP.

(A,B,C) Cartoon and stick representations of the predicted E1A helix docking to Protein Kinase A regulatory subunit R1α (dimer;red). Residues represented in sticks are within 4A of each other, suggesting potential interactions. Key predicted interactions are circled. (D) Electrostatic surface representation of PKA R1α and alpha carbon tracing of E1A demonstrates limited potential electrostatic interactions, and multiple hydrophobic interactions. (E) Sequence analysis reveals the residues of PKA R1α important for AKAP binding are similar to the residues implicated in the predicted interaction with E1A. (F) Crystal structure of AKAP interacting with PKA regulatory subunit RIα (PDB ID 3IM4). AKAP structure is shown in blue, Surface electrostatics of PKA are depicted, with blue and red representing positive and negative charges respectively. (G) Sequence of the predicted E1A helix and the residues implicated in PKA interaction according to ClusPro2.0 docking. (H) E1A mutants D21K, E26K, V27K and E26A/V27A reduced interaction with RIα, whereas substitution of E25 with K, which is not predicted to alter binding, had no effect. (I) RIα mutants Q28E, L31A K32A and I35A/V36A displayed a reduced ability to bind E1A.


A
E1A
PKA
B
E1A
PKA
C
E1A
PKA
D
E1A
PKA
E
PKA residues in E1A binding
14 SLRECELYVQKHNIQALLKDSIVQLCTARPERPMAFLREYFEKLEKEEAK 63
PKA residues in AKAP binding
14 SLRECELYVQKHNIQALLKDSIVQLCTARPERPMAFLREYFEKLEKEEAK 63
F
AKAP
PKA
G
E1A Residues in PKA binding
9 GVITEEMAASLLDQLIEEVLADNLPP 34
PDB:3IM4 (receptor)
Phyre2: E1A prediction (ligand)
ClusPro2.0 (docking)
H
IP: E1A
vector
wt 13S
D21K
E26K
V27K
E26A V27A
E25K
RIα
RIIα
Cα
Input
RIα
RIIα
Cα
E1A
I
IP: E1A
wt RIα
wt RIα
Q28E
L31A K32A
I35A V36A
HA (RIα)
Input
HA (RIα)
E1A

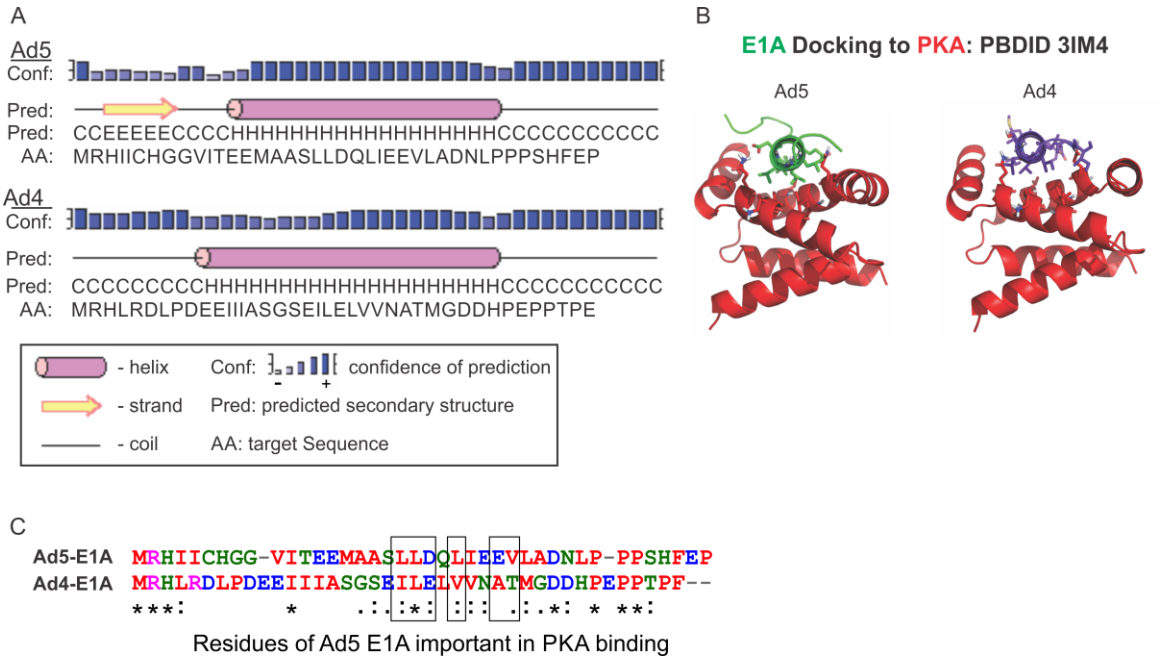


Figure 2.4. HAdV-4 E1A is not predicted to form a helix that is capable of binding PKA in an equivalent manner as the HAdV-5 E1A AKAP-like sequence.

(A) The PSI-PRED protein sequence analysis workbench was used to predict the helical propensity of the N-terminal regions of HAdV-5 and HAdV-4 E1A. Although both sequences are predicted to form helices, HAdV-4 has a lower confidence in forming this secondary structure. (B) When attempting to dock the HAdV-4 E1A sequence to RIα using Clus-Pro, electrostatic interactions at both the amino and carboxy ends of this lower confidence structure are absent that are predicted to contribute to the AKAP like interaction with PKA observed with HAdV-5 E1A. As expected, in the absence of these interactions, even in the most energy minimized states calculated for HAdV-4, HAdV-5 E1A demonstrated a more stable energy minimization. (C) Using Clustal, the sequences of HAdV-5 and HAdV-4 E1A are compared, with the residues demonstrated as crucial for PKA-binding (Figure 2.3) highlighted by boxes here. The corresponding residues in HAdV-4 E1A are quite different and lack the requisite chemical properties to form bonds with PKA. Additionally, several bulky alphatic residues present in HAdV-5 E1A, which appear to stabilize the interaction with PKA via hydrophobic interactions, are also absent in HAdV-4 E1A.

Transfected HAdV-5 E1A was unable to co-immunoprecipitate RIα or RIIα lacking their D/D domain (Δ1-63 and Δ1-45, respectively, Figures 2.5A, B). In addition, when the D/D domains of RIα and RIIα were expressed as fusions to EGFP, they alone were sufficient to co-immunoprecipitate E1A (Figure 2.5C). Thus, the N-terminus of E1A not only resembles an AKAP based on sequence, but also binds to the same site on the PKA regulatory subunits targeted by cellular AKAPs.

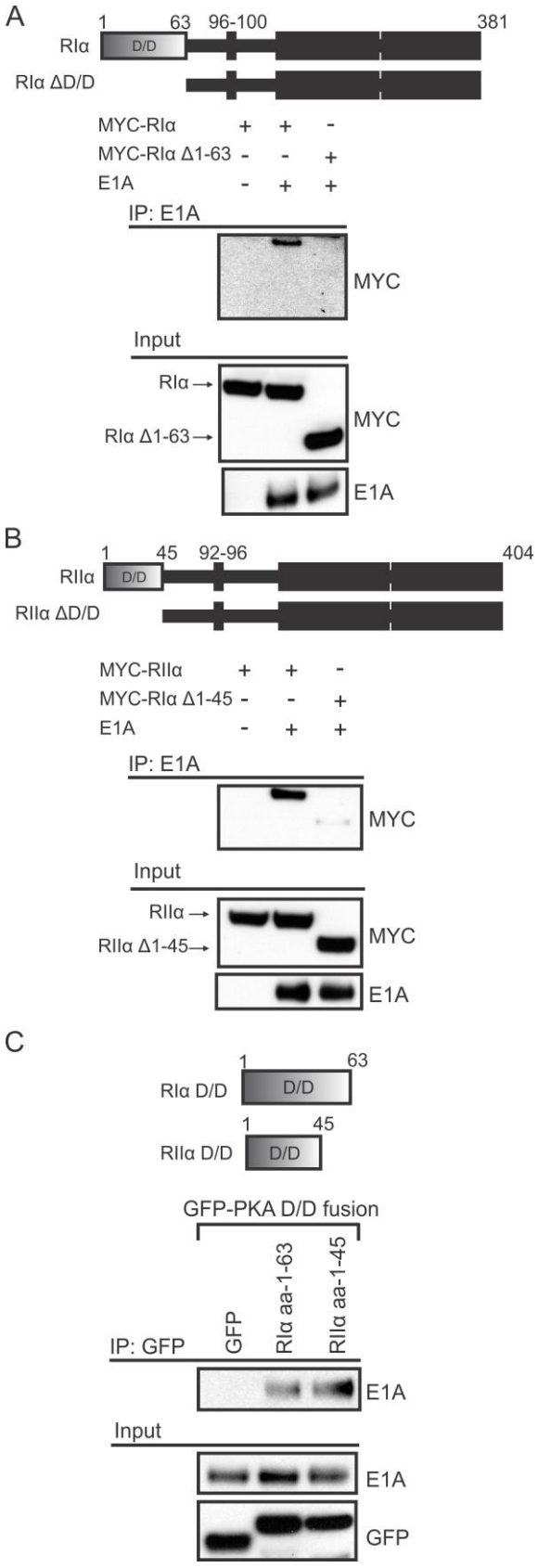
2.3.4 E1A functions as a viral AKAP

We next determined if the structural similarity between E1A and cellular AKAPs extended to functional similarity. We tested whether E1A could compete with endogenous AKAPs for PKA-binding during infection. A549 cells were infected with WT HAdV-5, a ΔE1A virus, or a virus expressing an E1A mutant unable to bind PKA (Δ4-25). Cell lysates were prepared 18 hours post-infection, subjected to immunoprecipitation with an anti-AKAP7 antibody and any co-precipitating PKA subunits were detected via western blot with specific antibodies for each target. AKAP7 is a dual-specificity AKAP (Gold et al., 2006), which binds both RIα and RIIα directly, and indirectly binds Cα. Infection with HAdV-5 did not alter the expression of AKAP7 or the various PKA subunits. However, infection disrupted the endogenous interactions between AKAP7 and PKA. Disruption of the AKAP7-PKA interaction during infection required E1A and was dependent on the AKAP-like domain in E1A (Figure 2.6A). These data establish that the AKAP-like region in E1A competes with endogenous AKAPs for PKA interaction during infection. These results also suggests that E1A can out-compete at least some cellular AKAPs for binding to PKA, which likely contributes to previously observed perturbation of cellular cAMP signalling by HAdV infection (Engel et al., 1991, 1988; Gedrich et al., 1992).

We next tested whether *in silico*-designed peptide inhibitors, which block AKAP-PKA interactions by binding PKA regulatory subunits with sub-nanomolar affinities, could affect E1A's interaction with PKA. These well characterized inhibitors are short peptides expressed as EGFP-fusions which specifically block binding to RIα (RIAD) or RIIα (sAKAPis) (Carlson et al., 2006; Gold et al., 2006). HT1080 cells were co-transfected

Figure 2.5. The D/D domains of PKA regulatory subunits are necessary and sufficient for binding E1A.

HT1080 cells were co-transfected with E1A and a variety of PKA regulatory subunit constructs and cell lysates were harvested for co-immunoprecipitation. Deletion of the D/D domain (shown in the inset panels) in either RIα (A) or RIIα (B) reduced the interaction with WT E1A. (C) When expressed as EGFP fusions (shown in the inset panel) these D/D domains were sufficient for binding E1A. Images are cropped to exclude the IgG heavy chain signal.


A
1
63
96-100
381
RIα
D/D
RIα ΔD/D
MYC-RIα + + -
MYC-RIα Δ1-63 - - +
E1A - + +
IP: E1A
MYC
Input
RIα
RIα Δ1-63
MYC
E1A
B
1
45
92-96
404
RIIα
D/D
RIIα ΔD/D
MYC-RIIα + + -
MYC-RIα Δ1-45 - - +
E1A - + +
IP: E1A
MYC
Input
RIIα
RIIα Δ1-45
MYC
E1A
C
1
63
RIα D/D
D/D
1
45
RIIα D/D
D/D
GFP-PKA D/D fusion
GFP
RIα aa-1-63
RIIα aa-1-45
IP: GFP
E1A
Input
E1A
GFP

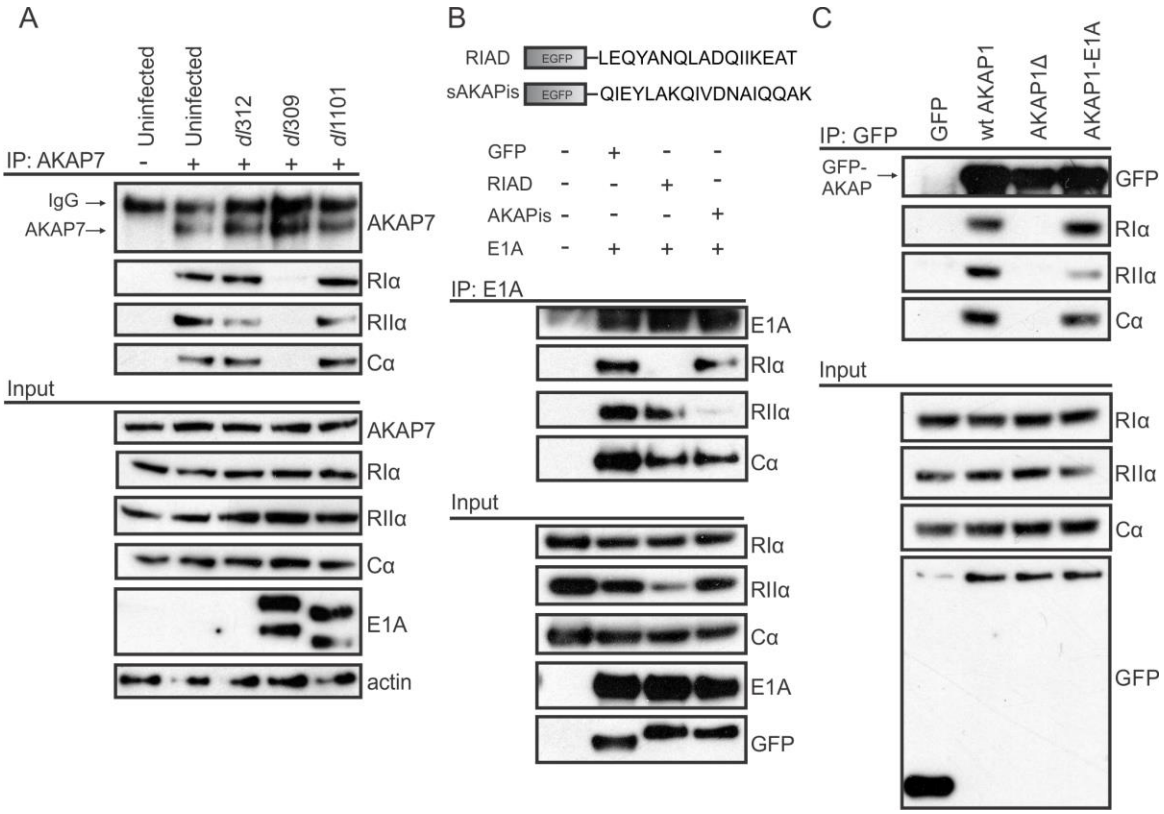


Figure 2.6. E1A can compete with and function like an AKAP.

(A) A549 cells were infected with either WT HAdV-5, ΔE1A virus, or a virus that lacks PKA-binding (Δ4-25[*dl*1101]) and cell lysates were harvested for co-immunoprecipitation. Interactions between the endogenous dual-specificity AKAP7 and PKA subunits was disrupted in the presence of WT E1A, but remained intact in presence of an E1A mutant unable to bind PKA. (B) HT1080 cells were co-transfected with E1A, PKA, and the indicated high affinity AKAP-PKA binding inhibitors (shown in the inset panel). The binding inhibitors disrupted the E1A-PKA interactions in an isoform-specific manner. (C) HT1080 cells were co-transfected with PKA and the indicated D-AKAP1 construct. An AKAP1 mutant lacking its binding for PKA was rescued by cloning in the AKAP-like sequence of HAdV-5 E1A.

with vectors expressing the PKA subunits, WT E1A, and each of the inhibitors. Lysates were subjected to immunoprecipitation with an anti-E1A antibody and interacting PKA subunits were detected by western blot. As expected based on their high affinity, both RIAD and sAKAPis competitively reduced E1A's interaction with PKA in a subunit-specific manner, reinforcing E1A's role as a dual-specificity viral AKAP (Figure 2.6B).

Using an expression construct for a known cellular dual-specificity AKAP (AKAP1) (Gold et al., 2006), we next tested E1A's ability to rescue the PKA-binding function of this AKAP when its PKA-binding domain was deleted. HT1080 cells were co-transfected with PKA subunits and EGFP-fusions of WT AKAP1, an AKAP1 mutant lacking its PKA-binding domain (AKAP1Δ), or an AKAP1 construct with E1A residues 14-28 cloned in lieu of the deletion (AKAP1-E1A). Lysates were subjected to immunoprecipitation with an anti-EGFP antibody and co-precipitating PKA was detected via western blot. As expected, the AKAP1Δ mutant lost the ability to bind PKA. However, incorporation of the E1A AKAP-like sequence into this mutant rescued PKA-binding to WT levels (Figure 2.6C). Together, these results strongly suggest that the AKAP-like motif in E1A is functionally indistinguishable from that found in an authentic cellular AKAP.

2.3.5 E1A alters PKA subcellular localization

Transfection of cells with HAdV-12 E1A induces a relocalization of the RIIα subunit of PKA from the cytoplasm to the nucleus (Fax et al., 2001). We tested E1A's ability to alter PKA's subcellular localization *in vivo* during a HAdV-5 infection (Figures 2.7, 2.8A). A549 cells were infected with WT virus (*dl*309), a ΔE1A virus (*dl*312), or the Δ4-25 E1A deletion mutant virus (*dl*1101) that does not bind PKA. At 18 hours post-infection, cells were subjected to immunofluorescence staining and biochemical fractionation to determine the subcellular localization of PKA. In WT-infected cells, endogenous RIα was rerouted from the cytoplasm into the nucleus. Additionally, in infected cells, RIα appeared to overlap with the HAdV-5 encoded DNA-binding protein (DBP), suggesting possible co-localization with viral replication centres during infection (Figure 2.9). In contrast, the distribution of PKA subunits in cells infected with either the

Figure 2.7. E1A alters PKA R1α subcellular localization during infection.

A549 cells were infected with either WT HAdV-5, ΔE1A virus or a virus mutant unable to bind PKA (Δ4-25). Cells were fixed, permeabilized and stained for confocal immunofluorescence. In the presence of WT E1A, RIα exhibited a drastic shift from exclusively cytoplasmic localization into the cell nucleus (A). This redistribution did not occur during ΔE1A or Δ4-25 infections. RIIα and Cα were not re-localized in the same manner as RIα (Figure 2.8A) Scale bars represent 200μm. (B) Quantification of nuclear signal relative to total cellular signal. Statistically significant differences are denoted (*$p<0.001$) n=50. (C) Nuclear and cytoplasmic extracts from infected cells were prepared by biochemical fractionation and the presence of E1A and PKA subunits in each fraction were detected by western blot. The presence of nuclear RIα in WT-infected cells confirms the results observed by immunofluorescence.

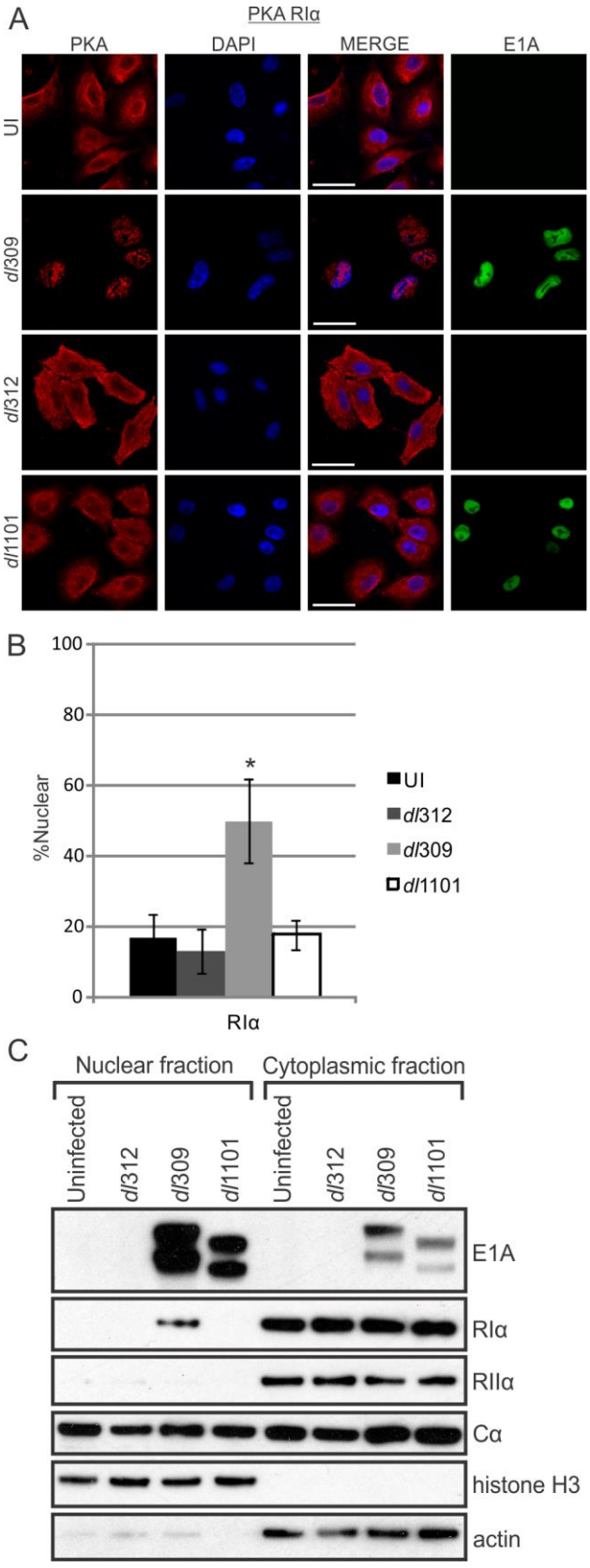

A
PKA RIα
PKA
DAPI
MERGE
E1A
UI
dl309
dl312
dl1101
B
100
80
60
40
20
0
%Nuclear
*
UI
dl312
dl309
dl1101
RIα
C
Nuclear fraction
Cytoplasmic fraction
Uninfected
dl312
dl309
dl1101
Uninfected
dl312
dl309
dl1101
E1A
RIα
RIIα
Cα
histone H3
actin

Figure 2.8. Subcellular localization of RIIα and Cα during HAdV-5 infection and localization of all PKA subunits in HEK293 cells.

(A) A549 cells were infected with either WT HAdV-5 (*dl*309), ΔE1A virus (*dl*312) or a virus lacking PKA-binding (*dl*1101; Δ4-25). Cells were fixed, permeabilized and stained for confocal immunofluorescence. RIIα appears cytoplasmic in all experimental conditions. Cα appears nuclear-cytoplasmic in all experimental conditions, although it may be enriched for nuclear localization in the presence of WT E1A. (B) HEK293 cells (which are stably transformed due to expression of HAdV-5 E1A and E1B) were stained and individual PKA subunits and were demonstrated to have a similar localization phenotypes as in HAdV-infected cells. The nuclear relocalization of RIα appears more diffuse and less punctate in these cells, possibly due to lack of recruitment to viral replication centres as there is no infection occurring in these virally transformed cells. In a separate experiment, endogenous E1A was successfully knocked down to undetectable levels via siRNA-transfection (the knockdown efficiency of various E1A-specific siRNAs generated for this experiment is shown in the inset panel). The amount of RIα detected in the nucleus is greatly reduced when E1A expression is knocked down. Scale bars represent 200μm.

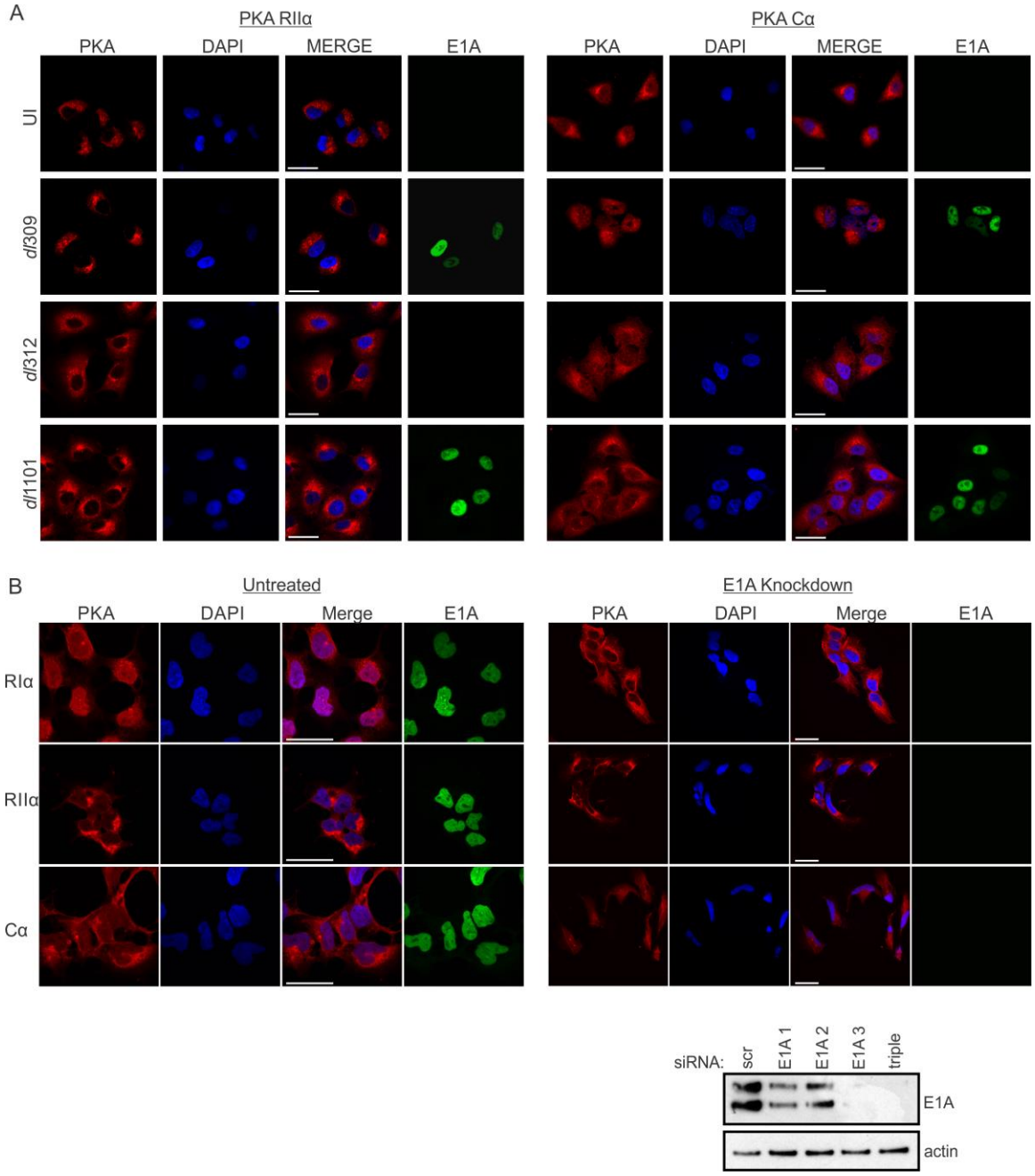
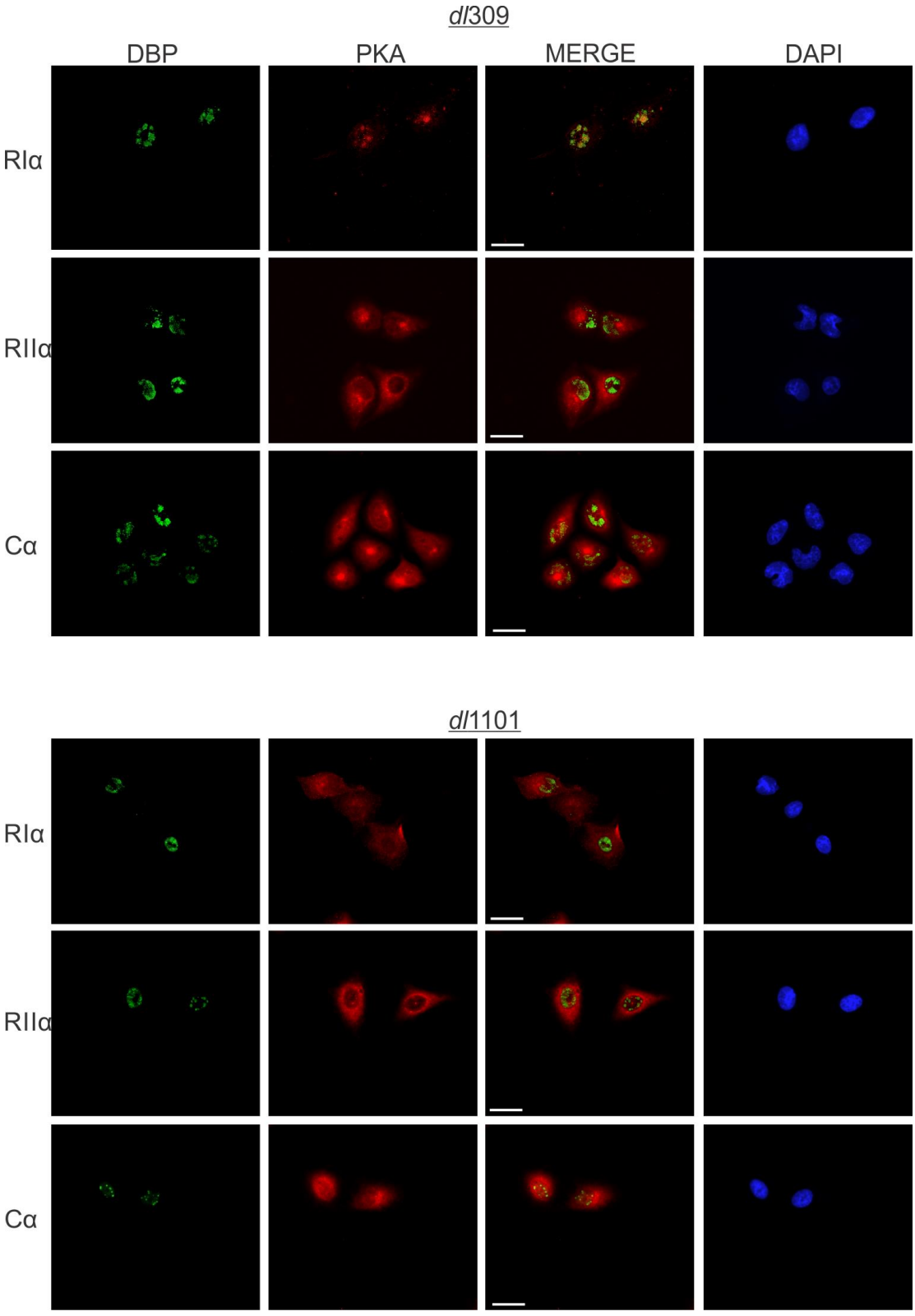

A
PKA RIIα
PKA Cα
PKA
DAPI
MERGE
E1A
UI
d/309
d/312
d/1101
B
Untreated
E1A Knockdown
PKA
DAPI
Merge
E1A
RIα
RIIα
Cα
siRNA:
scr
E1A 1
E1A 2
E1A 3
triple
E1A
actin

Figure 2.9. Co-staining of PKA and viral replication centres during HAdV-5 infection.

A549 cells were infected with the indicated virus (MOI 5) and were subsequently fixed, permeabilized and stained with antibodies specific for the indicated PKA subunits or HAdV-5 DNA-binding protein (DBP) and DAPI as indicated. Images were acquired on a Nikon Eclipse inverted laser microscope. During WT infection, a portion of nuclear RIα appears to co-stain with the HAdV-5 DBP, suggesting overlap with viral replication centres. This is not observed during infection with mutant virus encoding E1A incapable of binding PKA (*dl*1101). Under both conditions, RIIα appears to remain cytoplasmic, whereas Cα maintains a diffuse nuclear/cytoplasmic localization. Scale bars represent 200μm.


dl309
DBP
PKA
MERGE
DAPI
RIα
RIIα
Cα
dl1101
RIα
RIIα
Cα

ΔE1A or Δ4-25 virus resembled uninfected cells. Thus, the relocalization of RIα is E1A-dependent and requires the AKAP motif. Subcellular localization of RIIα appeared to be unaffected by the presence of E1A and Cα retained its nuclear/cytoplasmic phenotype in both uninfected and infected cells, thereby rendering any conclusions regarding its relocalization difficult (Figure 2.8A).

RIα, but not RIIα, is similarly trafficked into the nucleus of HEK293 cells, which stably express HAdV-5 E1A. Knockdown of E1A in HEK293 cells reduces the amount of RIα in the nucleus, further suggesting that E1A is functioning as an AKAP in these cells to redistribute PKA (Figure 2.8B). Interestingly, HAdV-5 E1A appears to primarily affect type-I PKA, whereas the previously reported effect of HAdV-12 E1A was restricted to type-II PKA.

2.3.6 E1A uses PKA to enhance HAdV gene expression

Previous studies indicated that E1A and cAMP synergize to activate viral gene expression (Engel et al., 1991, 1988; Gedrich et al., 1992; Hardy and Shenk, 1988; B. H. Lee and Mathews, 1997; Müller et al., 1989). To determine if the E1A-PKA interaction contributes to HAdV early gene transcription, A549 cells were first treated with control siRNA or siRNA specific for each PKA subunit and then infected with WT (*dl*309), ΔE1A (*dl*312), or Δ4-25 (*dl*1101) HAdV-5. Cells were harvested 20 hours post-infection, cDNA was prepared and the expression of a panel of HAdV early genes known to be activated by E1A was determined by quantitative real-time PCR. Knockdown of RIα, RIIα, or Cα did not affect expression of the E1A (Figure 2.10A) or E1B (Figure 2.10B) transcription units for any of the tested viruses. However, mRNA levels were significantly reduced for both the E3 (Figure 2.10D) and E4 (Figure 2.10E) transcription units in WT virus infected cells treated with siRNA for each of the PKA subunits, demonstrating that PKA plays a role in the regulation of these transcription units. Importantly, cells infected with the Δ4-25 virus also showed decreased expression of E3 and E4 as compared to WT infection, and this was not further reduced by knockdown of any PKA subunit (Figure 2.10D, 2.10E). This is fully consistent with the inability of this mutant E1A protein to bind PKA and relocalize it to the nucleus. Mechanistically,

Figure 2.10. The interaction between E1A and PKA subunits is required for full expression of HAdV-5 E3 and E4 transcripts.

A549 cells were treated with control siRNA or siRNA specific for PKA subunits (shown in the inset panel) and infected with either WT HAdV-5 or the indicated mutants. RT-qPCR was performed with a panel of HAdV-5 early genes, normalized to GAPDH and fold change to control treated cells was plotted. Results for the HAdV-5 E1A (A), E1B (B), E2 (C), E3 (D) and E4 (E) transcription units are shown. A statistically significant decrease from control-treated cells is indicated (*$p<0.05$) n=3.

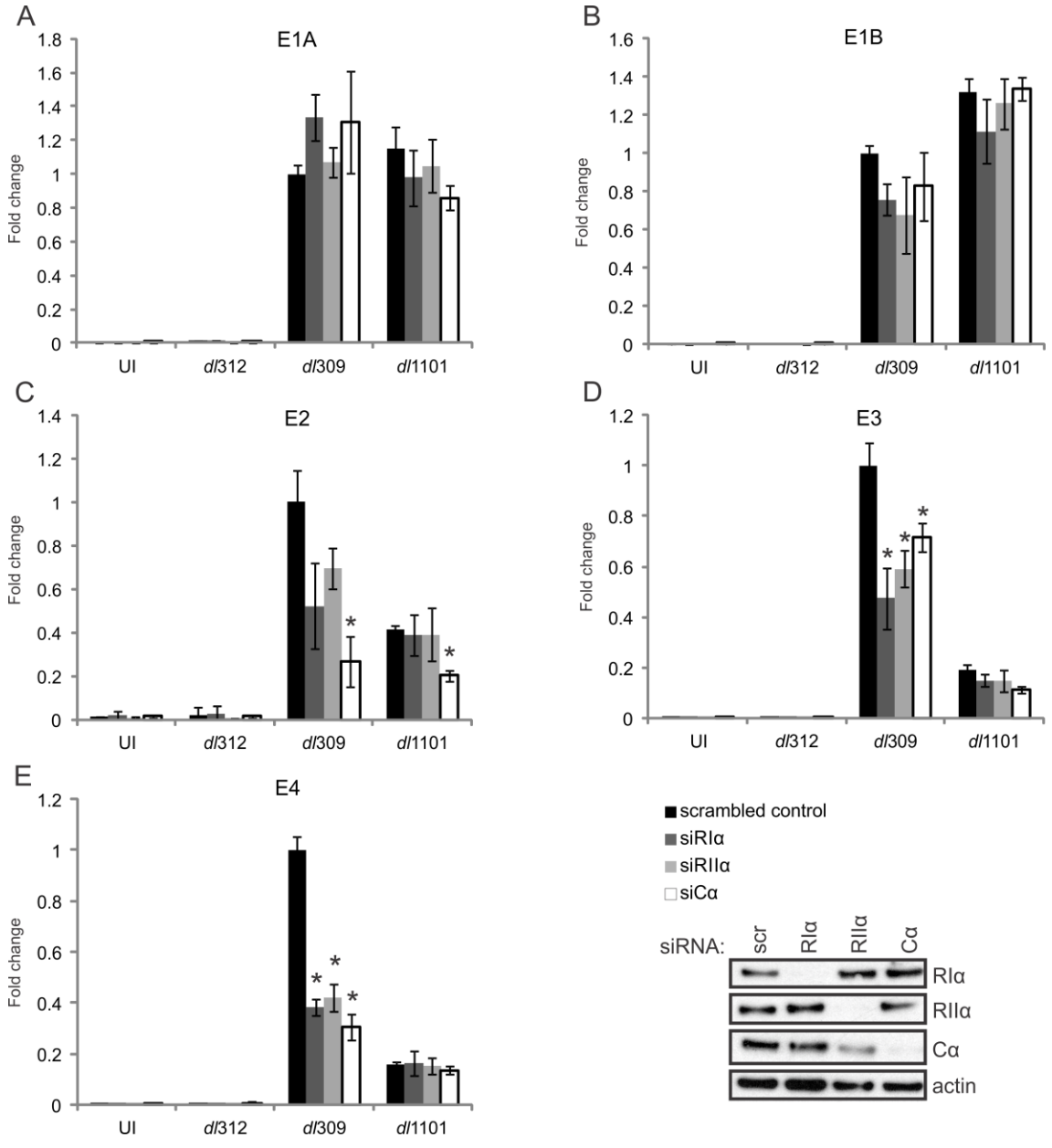
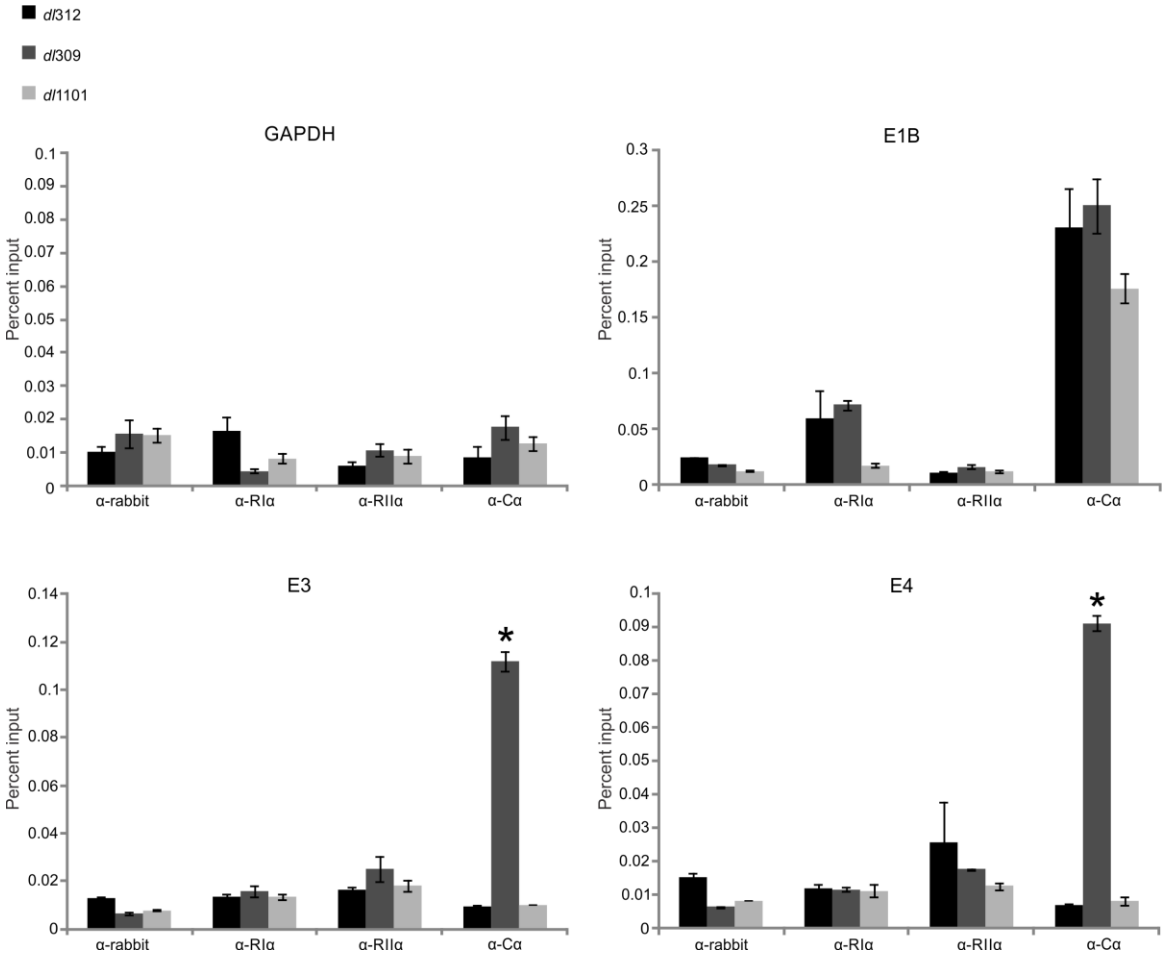

A
E1A
Fold change
UI
d/312
d/309
d/1101
B
E1B
C
E2
D
E3
E
E4
scrambled control
siRIα
siRIIα
siCα
siRNA:
scr
RIα
RIIα
Cα
actin

Figure 2.11. PKA is recruited to HAdV early gene promoters in an E1A-dependent manner.

A549 cells were infected with the indicated viruses at an MOI of 5 and harvested 20 hours post-infection. Chromatin immunoprecipitation (ChIP) was performed with antibodies specific for the indicated proteins. DNA was probed via qPCR for the presence of multiple HAdV early gene promoters (E1B, E3, and E4) and a cellular GAPDH promoter previously shown to be unaffected by E1A in similar conditions (Fonseca *et al.* 2013). Data was normalized to input samples and compared to a non-specific control antibody and ΔE1A-infected cells. A statistically significant increase from ΔE1A-infected cells for each specific ChIP reaction is indicated (* $p<0.05$, $n=3$). In WT-infected cells (*dl*309), the catalytic subunit (Cα) is specifically recruited to the HAdV E3 and E4 promoters whose transcription was shown to be affected by the E1A-PKA interaction. This recruitment is E1A-dependent as neither ΔE1A HAdV (*dl*312) nor virus incapable of binding PKA (*dl*1101; Δ4-25) could recruit Cα. In contrast, Cα is not recruited to the GAPDH promoter and while it was present on the E1B promoter, this was independent of E1A and does not appear to affect transcription (Figure 2.10). Interestingly, neither regulatory subunit of PKA was directly recruited to the HAdV genome. Instead, it appears E1A uses the interaction with the PKA regulatory subunits to retask the catalytic component of the holoenzyme to sites of action in the nucleus.


dl312
dl309
dl1101
GAPDH
E1B
E3
E4
Percent input
α-rabbit
α-RIα
α-RIIα
α-Cα
*

chromatin immunoprecipitation (ChIP) experiments showed that PKA's catalytic subunit (Cα) was recruited to the HAdV E3 and E4 promoter regions in an E1A-dependent manner (Figure 2.11). In contrast, E1A did not specifically recruit Cα to the E1B or GAPDH promoters, whose transcription was unaffected by the E1A-PKA interaction (Figure 2.10). These results strongly support a mechanism of early gene activation that relies on the AKAP function of E1A.

Although knockdown of PKA regulatory subunits had no statistically significant effect on E2 transcripts, knockdown of the catalytic subunit reduced E2 expression for both WT and Δ4-25 virus (Figure 2.10C). This suggests an independent effect for PKA on this transcription unit that does not rely on the AKAP motif.

To extend the observations that PKA plays a role in HAdV gene expression, we further examined PKA's role in HAdV-5 protein production (Figure 2.12). A549 cells were treated with control siRNA or siRNA specific for each PKA subunits and infected with WT HAdV-5. Cell lysates were collected at 12, 24, and 36 hours post-infection. Viral protein production was assayed by western blot using antibodies against an array of HAdV-5 proteins representing both early and late transcription units. Compared to control-treated cells, knockdown of PKA subunits had no effect on the production of HAdV-5 E1A proteins. In contrast, knockdown of the individual PKA subunits caused a notable reduction in several early proteins. These included a reduction in E3-19K at each time point examined, a reduced level of E4orf6 expression at 24 hours post-infection and a delay in expression of the E2-encoded DBP. E1B-55K was also reduced, most notably in the RIα knockdown. Interestingly, many of the late proteins also exhibited lower expressions levels in PKA-knockdown cells, including hexon, penton, protein V, and protein VII. This confirms a role for PKA in regulating HAdV-5 gene expression.

2.3.7 E1A uses PKA to enhance viral replication

To establish the biological significance of E1A's role as a viral AKAP, we also assessed the effect of the E1A-PKA interaction on viral replication (Figure 2.13). A549 cells were treated with either control siRNA or siRNA specific for each PKA subunit and infected

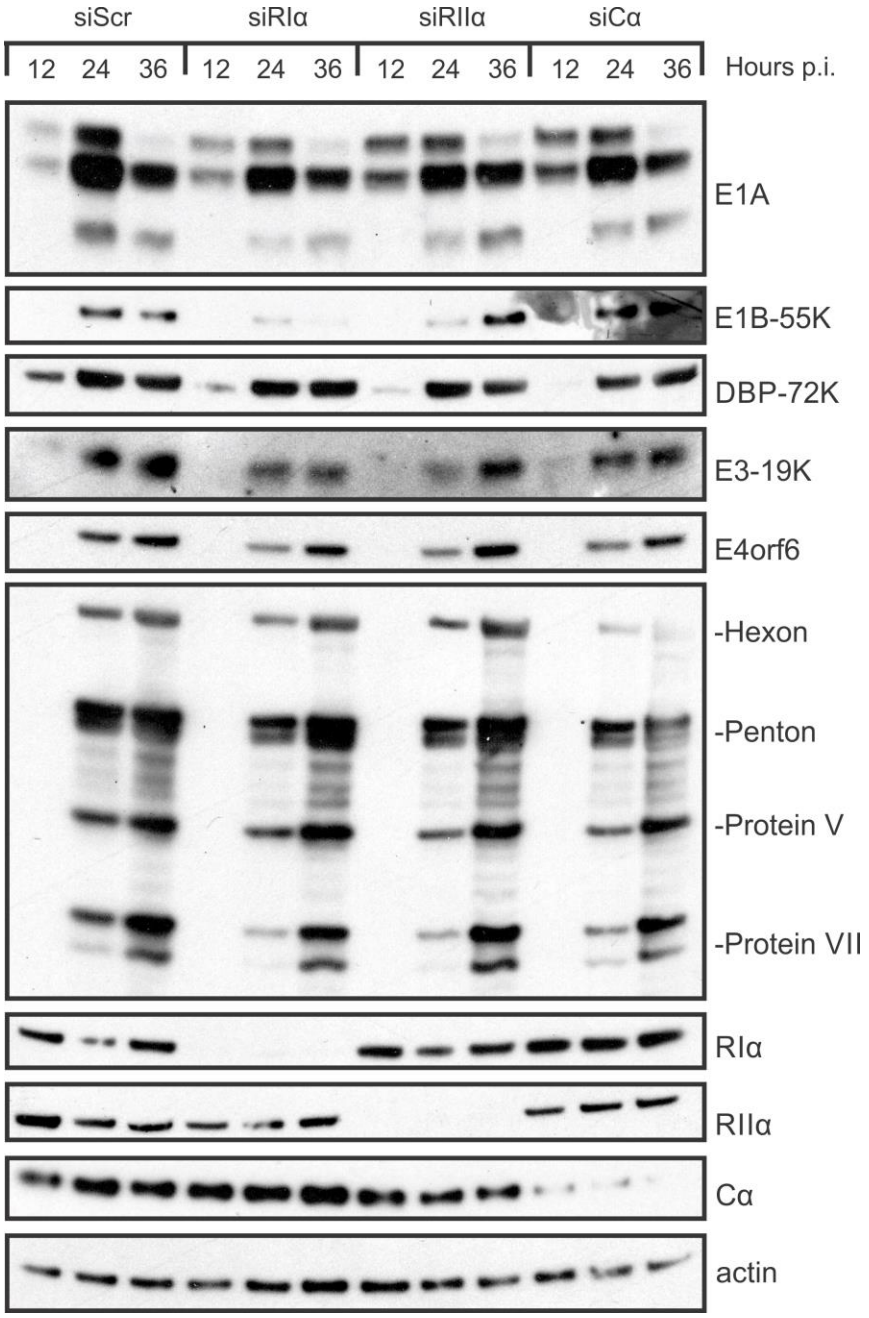


Figure 2.12. PKA is required for WT levels of HAdV-5 protein production.

A549 cells were treated with control siRNA or siRNA specific for PKA subunits and infected with WT HAdV-5 (*dl*309; MOI of 5). Cells were harvested at 12, 24, and 36 hr post-infection and viral protein production was assayed by western blot using antibodies against representative proteins from an array of HAdV-5 transcription units.

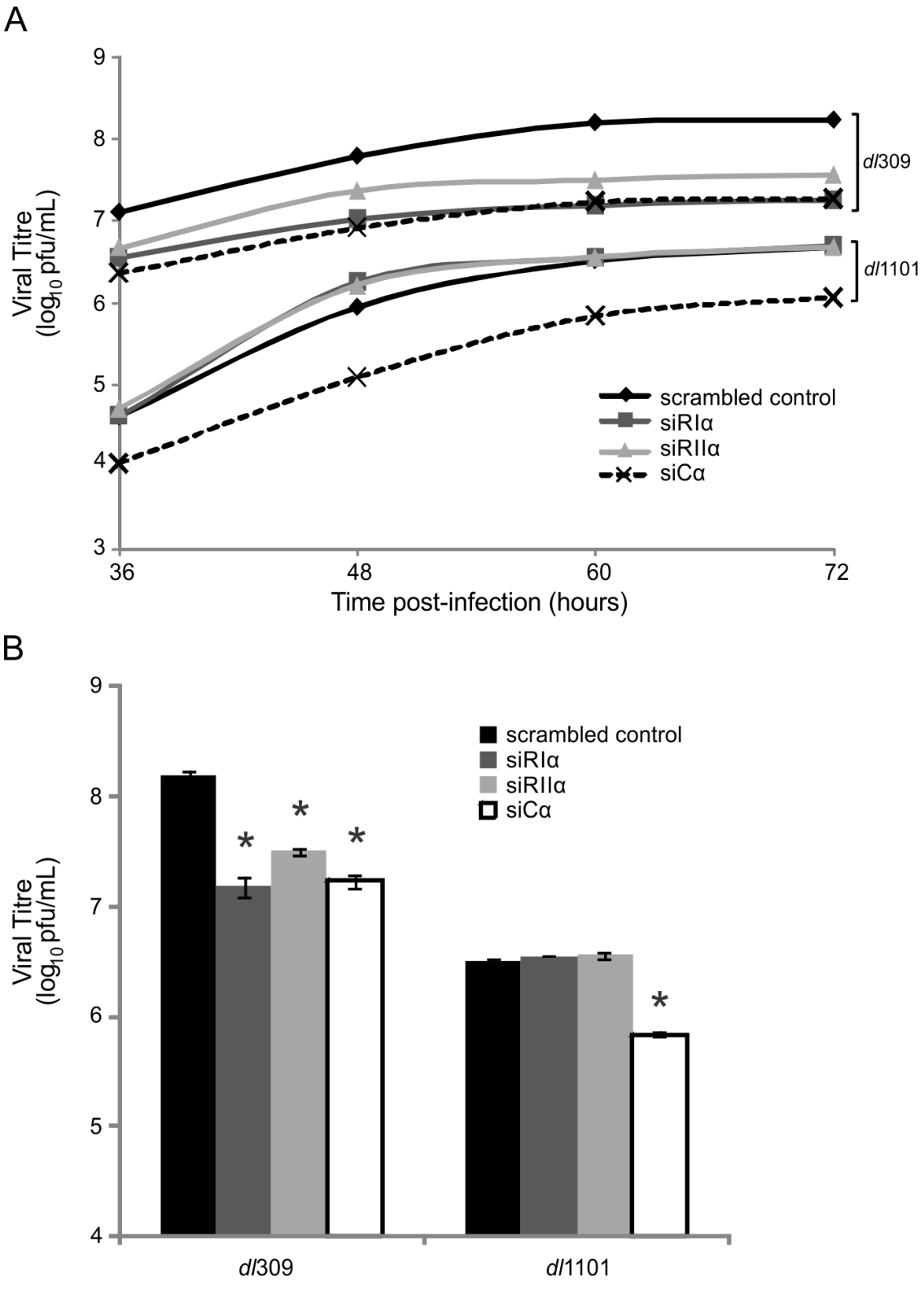
with either WT or Δ4-25 HAdV-5. Production of infectious virus progeny was assayed at various time points over 72 hours by plaque assay. The production of WT virus was reduced by knockdown of each PKA subunit when compared to control-treated cells. Although the production of the Δ4-25 virus was reduced as compared with WT infection, it was not further reduced by knockdown of either RIα or RIIα. This again suggests that the lack of PKA-binding by this E1A mutant is functionally equivalent to PKA knockdown. These results indicate that HAdV replication requires PKA activity and that E1A's interaction with PKA's regulatory subunits is required for WT-levels of replication. Interestingly, we observed a reduction in progeny production for both WT and Δ4-25 virus in cells treated with Cα-specific siRNA. However, the observed reduction compared to control-siRNA treated cells was more severe in the WT infection, suggesting an additional role for PKA in HAdV-5 infection that is E1A-independent and specific for PKA's catalytic subunit. Altogether, these results confirm that the targeting of PKA by the AKAP motif in E1A is a critical aspect in the HAdV-5 replicative cycle.

2.4 Discussion

Cellular AKAPs function as scaffolds that target PKA and other signaling enzymes to specified subcellular locations. These multivalent anchoring proteins serve as important focal points for the processing and integration of intracellular signaling (Colledge and Scott, 1999; Taskén and Aandahl, 2004). We report here that the adenovirus E1A oncoproteins function as the first known viral AKAPs. Intriguingly, E1A interacts with both the RIα and RIIα subunits of PKA in a way that precisely mimics that of cellular dual-specificity AKAPs. Specifically, we found that E1A bound to the N-terminal D/D domain of the regulatory subunit dimer of PKA, which is the same exact domain targeted by cellular AKAPs (Taylor et al., 2012; Wong and Scott, 2004). We identified a short conserved sequence in HAdV-5 E1A spanning residues 14-28 that was necessary and sufficient for interaction with either RIα or RIIα. Like the PKA interaction domains of cellular AKAPs, this region of E1A is predicted to form an amphipathic α-helix. This apparent structural mimicry allows E1A to bind PKA with an affinity comparable to cellular AKAPs, such that E1A can successfully compete with endogenous cellular AKAPs for PKA interaction during infection (Figure 2.14).

Figure 2.13. Interactions between E1A and PKA are required for full HAdV-5 progeny production.

A549 cells were treated with control siRNA or siRNA specific for PKA subunits and infected with either WT HAdV-5 or a virus encoding E1A unable to bind PKA (Δ4-25). Cells were collected at various time points up to 72 hr post-infection. Production of infectious progeny virus was quantitatively assayed by plaque formation on HEK293 cells (A). Data are shown over 36-72 hr. Growth of WT virus was decreased by knockdown of each PKA subunit. Growth of HAdV E1A Δ4-25 was not affected by knockdown of PKA regulatory subunits, but was affected by knockdown of PKA Cα (though to a lesser extent than WT HAdV-5). (B) Total viral progeny production at 60 hours post-infection when virus replication appeared to peak in most conditions. All values are represented as mean ± SEM. The statistically significant reductions in viral titres compared to control-treated cells are denoted (*$p<0.01$).


A
Viral Titre (log10 pfu/mL)
9
8
7
6
5
4
3
36
48
60
72
Time post-infection (hours)
dl309
dl1101
scrambled control
siRIα
siRIIα
siCα
B
Viral Titre (log10 pfu/mL)
9
8
7
6
5
4
scrambled control
siRIα
siRIIα
siCα
*
*
*
*
dl309
dl1101

In support of our *in vivo* and *in vitro* results, molecular modeling based on a known structure of the AKAP/PKA interaction predicts that E1A binds the exact same surface of the PKA regulatory subunit in a fashion virtually identical to that determined for cellular AKAPs (Figure 2.3). Substitution of specific residues predicted by this model to make contacts reduced the interaction *in vivo*, supporting the validity of this structural model of molecular mimicry.

Functionally, as observed for cellular AKAPs, E1A relocalizes PKA to target sites of action. In the case of E1A, the interaction with PKA induces a specific relocalization to the nucleus, which contributes to viral gene expression and efficient virus propagation during infection. Competition by E1A with cellular AKAPs for PKA interaction may also influence cellular gene expression, which may provide some insight into the previous observations that E1A influences cAMP signaling (Engel et al., 1991, 1988; Gedrich et al., 1992; B. H. Lee and Mathews, 1997; Miller et al., 1995; Müller et al., 1989). The E1A region mapped as necessary and sufficient for PKA-binding also overlaps with regions previously implicated in E1A's ability to function as a transforming oncoprotein (Chinnadurai, 2011). Whether PKA contributes to the immortalizing activity of E1A remains unknown, though PKA itself has been investigated in a variety of cancer-related functions (Gold et al., 2013; Palorini et al., 2016; Pattabiraman et al., 2016)

Our results also demonstrate that PKA is a conserved target of the E1A proteins from multiple HAdV species, suggesting that this interaction is functionally important for the virus. The E1A proteins from all HAdV types tested bound PKA strongly, with the exception of HAdV-4 E1A; modeling of an interaction between HAdV-4 E1A and PKA predicts that key electrostatic and hydrophobic contacts are absent, which are necessary for the HAdV-5 E1A PKA interaction (Figures 2.3H, 2.4). Interestingly, HAdV-4 is unique as it is the sole member of species E HAdV and arose from an interspecies recombination event between chimpanzee and human adenovirus (Dehghan et al., 2013).

As mentioned above, during HAdV-5 infection, E1A was able to out-compete endogenous cellular AKAP7 for PKA interaction; however, there exist a plethora of other, diverse AKAPs with varying affinities for PKA. For example, the *in silic*o-

designed ‘super AKAPs’ RIAD and sAKAPis (Carlson et al., 2006; Gold et al., 2006) blocked the binding of E1A to the PKA RIα and RIIα subunits, respectively. Thus, the affinity of the E1A/PKA interaction is not high enough to compete with synthetic AKAPs with sub-nanomolar affinities for PKA. Consequently, these inhibitors are potential tools for further study of E1A function in the context of its role as a viral AKAP.

During HAdV-5 infection, a substantial fraction of the RIα subunit was trafficked from the cytoplasm into the nucleus in an E1A-dependent manner. We also observed signal overlap between RIα and HAdV DBP (Figure 2.9), suggesting co-localization with viral replication centres. Interestingly, the HAdV-5 E1A-mediated shift in RIα localization is the opposite finding reported for E1A from HAdV-12, which relocalized RIIα only (Fax et al., 2001). While both E1As bound to both type-I and –II PKA in Co-IP assays, our studies suggest that in biologically-relevant conditions they each may exhibit higher affinity or preference for one PKA flavour over another, a property shared by many cellular AKAPs (Gold et al., 2006; Taylor et al., 2012; Wong and Scott, 2004). The binding affinities and potential preferences of E1A proteins from the other HAdV species during infection remains to be fully explored. It also remains to be determined if type-I and type-II PKA are completely interchangeable, or if there are functional consequences driving the preference of each virus for each regulatory subunit type.

Interestingly, nuclear localization of the PKA holoenzyme is considered relatively unusual, but has been studied in detail in HEK-293 cells (Sample et al., 2012). We confirmed nuclear localization of RIα in these cells, which constitutively express HAdV-5 E1A (Graham et al., 1977). Our results suggest that nuclear localization of PKA in HEK-293 cells is a likely consequence of the AKAP function of E1A. Furthermore, our data suggests that the results of studies of PKA function in these cells may be confounded by the impact of viral manipulation of this pathway.

The targeting of PKA by E1A is required for maximal expression of the HAdV-5 E3 and E4 transcription units. It appears that E1A is using the regulatory subunits of PKA as a bridge to bind Cα, redistributing it to associate with other E1A binding partners at preferred sites within the nucleus, such as the HAdV early gene promoters (Figure 2.11).

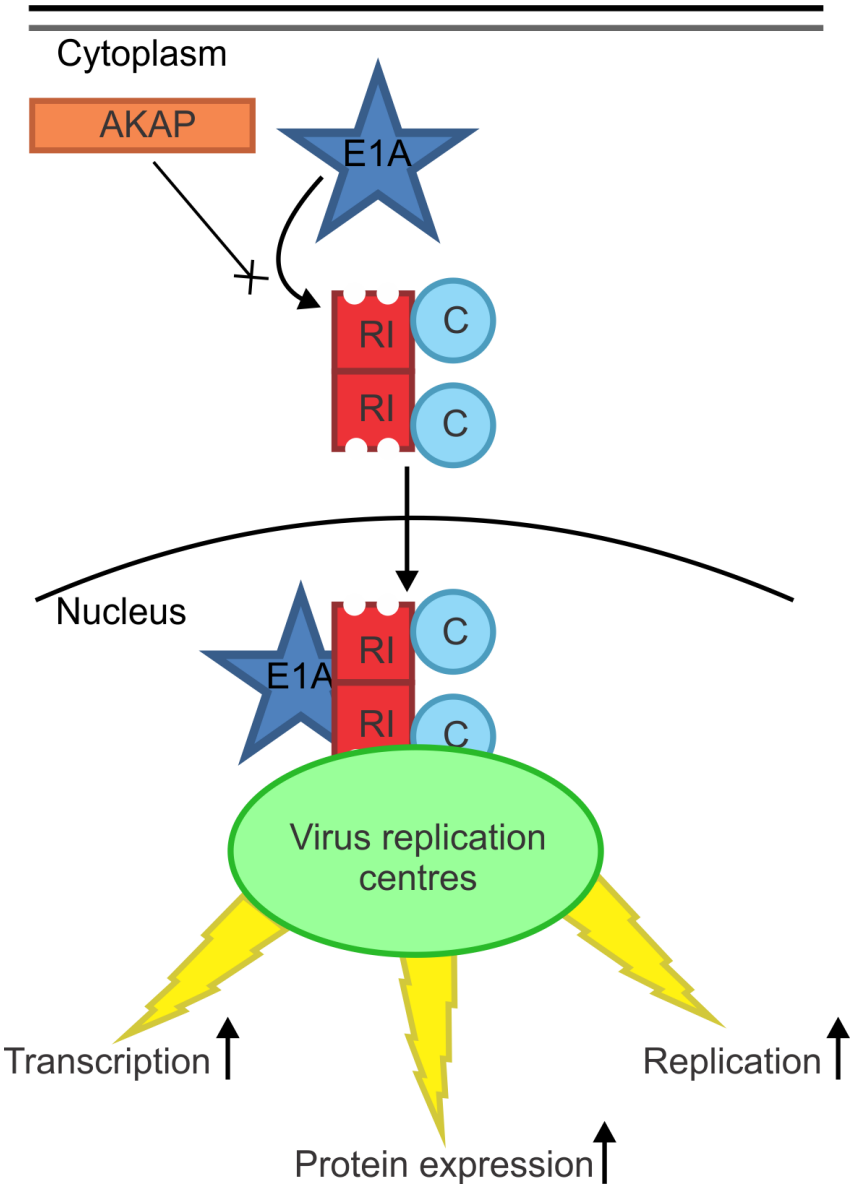


Figure 2.14. E1A functions as a viral AKAP.

During infection, the viral E1A protein interacts with the PKA holoenzyme. This occurs via structural mimicry of the PKA interaction domain of cellular AKAPs. As a consequence, competition occurs between the viral AKAP and cellular AKAPs for interaction with PKA. The interaction of E1A with PKA leads to a relocalization of a subset of PKA to the nucleus, likely to HAdV early gene promoters within virus replication centres. This interaction and relocalization is required for efficient viral transcription, protein expression and progeny production.

This could establish new localized connections with cAMP-regulated transcriptional machinery, such as CREB or ATF, at viral or cellular loci. This may help explain the previously-observed ability of E1A to cooperate with cAMP in transcriptional activation (Engel et al., 1991, 1988; Müller et al., 1989).

The importance of PKA during a productive infection is further underscored by our observation that siRNA-mediated downregulation of PKA subunits reduces progeny production by WT HAdV-5. It is likely that the observed defect in the virus' ability to express numerous crucial transcripts and proteins in the absence of PKA (or the AKAP function of E1A) contributes greatly to this. It is also possible that the E1A-PKA interaction affects cellular tasks that influence HAdV replication, given that PKA and cAMP have been previously shown to extensively modulate cellular transcription, protein expression, and cell signaling (Guo et al., 2012; Sands and Palmer, 2008; Zambon et al., 2005; Zhang et al., 2005). As expected, growth of a virus expressing an E1A mutant unable to bind PKA (Δ4-25) was reduced relative to WT. Importantly, knockdown of regulatory subunits RIα and RIIα did not further reduce the overall replication of this mutant, confirming that the lack of the E1A-PKA interaction contributes to its growth defect. Interestingly, loss of Cα expression negatively affected overall viral replication for both WT and Δ4-25 viruses, suggesting an E1A-independent effect of Cα on the HAdV life cycle. This may be related to reports that PKA activity is involved in dynein-mediated transport of species C HAdV virions to the nucleus during the establishment of infection (Scherer et al., 2014; Suomalainen et al., 2001).

Although E1A is presently unique in its ability to function as a viral AKAP, the important role of PKA in cellular homeostasis makes it an attractive target for modulation during infection by other viruses. For example, the Herpes simplex virus-1 US3 kinase interacts with and activates PKA to block apoptosis (Benetti and Roizman, 2004). Varicella-zoster virus also upregulates PKA expression and modulates phosphorylation of PKA substrates to improve replication (Desloges et al., 2008). More typically, PKA is recruited to phosphorylate viral proteins, altering their stability, folding or ability to interact with other targets (Barnitz et al., 2010; Cartier et al., 2003; Majeau et al., 2007; Okabe et al.,

2006). As one well characterized example, the E6 oncoprotein from human papillomavirus (HPV) is phosphorylated by PKA during infection, allowing it to interact with numerous cellular proteins (Boon et al., 2015; Delury et al., 2013). While E1A does not appear to be a substrate for PKA, its unique mechanism of commandeering this enzyme via mimicry highlights the diverse ways in which viruses can repurpose the same cellular factors. It is also interesting that rather than encoding an entire PKA ortholog or an entire viral protein to subvert PKA function, HAdV uses a short 15 amino acid fragment of the versatile E1A protein to retask PKA for the benefit of the virus. The fact that the AKAP mimic motif in E1A also overlaps regions required for targeting other cellular regulatory proteins (Fonseca et al., 2012; Meng et al., 2005; Rasti et al., 2005) further demonstrates the incredible effect of selective pressure on maximizing the impact of the relatively limited coding capacity of HAdV.

In summary, we conclusively identify E1A as the first known viral AKAP. We demonstrate that the N-terminus of E1A has evolved to mimic the appearance, structure and function of the PKA interaction domain of cellular AKAPs. Furthermore, we have established that the AKAP function of E1A plays a biologically significant role in redirecting PKA to the nucleus during infection, where it is repurposed to enhance HAdV early gene expression and viral progeny production.

2.5 References

Banky, P., Huang, L.J., Taylor, S.S., 1998. Dimerization/docking domain of the type Ialpha regulatory subunit of cAMP-dependent protein kinase. Requirements for dimerization and docking are distinct but overlapping. J. Biol. Chem. 273, 35048–55.

Barnitz, R.A., Wan, F., Tripuraneni, V., Bolton, D.L., Lenardo, M.J., 2010. Protein kinase A phosphorylation activates Vpr-induced cell cycle arrest during human immunodeficiency virus type 1 infection. J. Virol. 84, 6410–24.

Benetti, L., Roizman, B., 2004. Herpes simplex virus protein kinase US3 activates and functionally overlaps protein kinase A to block apoptosis. Proc. Natl. Acad. Sci. U. S. A. 101, 9411–6.

Boon, S.S., Tomaić, V., Thomas, M., Roberts, S., Banks, L., 2015. Cancer-causing human papillomavirus E6 proteins display major differences in the phospho-regulation of their PDZ interactions. J. Virol. 89, 1579–86.

Brockmann, D., Esche, H., 2003. The multifunctional role of E1A in the transcriptional regulation of CREB/CBP-dependent target genes. Curr. Top. Microbiol. Immunol. 272, 97–129.

Buchan, D.W.A., Minneci, F., Nugent, T.C.O., Bryson, K., Jones, D.T., 2013. Scalable web services for the PSIPRED Protein Analysis Workbench. Nucleic Acids Res. 41, W349-57.

Carlson, C.R., Lygren, B., Berge, T., Hoshi, N., Wong, W., Taskén, K., Scott, J.D., 2006. Delineation of type I protein kinase A-selective signaling events using an RI anchoring disruptor. J. Biol. Chem. 281, 21535–45.

Cartier, C., Hemonnot, B., Gay, B., Bardy, M., Sanchiz, C., Devaux, C., Briant, L., 2003. Active cAMP-dependent protein kinase incorporated within highly purified HIV-1 particles is required for viral infectivity and interacts with viral capsid protein. J. Biol. Chem. 278, 35211–9.

Chinnadurai, G., 2011. Opposing oncogenic activities of small DNA tumor virus transforming proteins. Trends Microbiol. 19, 174–83.

Colledge, M., Scott, J.D., 1999. AKAPs: from structure to function. Trends Cell Biol. 9, 216–221.

Dehghan, S., Seto, J., Liu, E.B., Walsh, M.P., Dyer, D.W., Chodosh, J., Seto, D., 2013. Computational analysis of four human adenovirus type 4 genomes reveals molecular evolution through two interspecies recombination events. Virology 443, 197–207.

Delury, C.P., Marsh, E.K., James, C.D., Boon, S.S., Banks, L., Knight, G.L., Roberts, S., 2013. The role of protein kinase A regulation of the E6 PDZ-binding domain during the differentiation-dependent life cycle of human papillomavirus type 18. J. Virol. 87, 9463–72.

Desloges, N., Rahaus, M., Wolff, M.H., 2008. The phosphorylation profile of protein kinase A substrates is modulated during Varicella-zoster virus infection. Med. Microbiol. Immunol. 197, 353–60.

Egan, C., Jelsma, T.N., Howe, J.A., Bayley, S.T., Ferguson, B., Branton, P.E., 1988. Mapping of cellular protein-binding sites on the products of early-region 1A of human adenovirus type 5. Mol. Cell. Biol. 8, 3955–9.

Engel, D.A., Hardy, S., Shenk, T., 1988. cAMP acts in synergy with E1A protein to activate transcription of the adenovirus early genes E4 and E1A. Genes Dev. 2, 1517–28.

Engel, D.A., Muller, U., Gedrich, R.W., Eubanks, J.S., Shenk, T., 1991. Induction of c-fos mRNA and AP-1 DNA-binding activity by cAMP in cooperation with either the adenovirus 243- or the adenovirus 289-amino acid E1A protein. Proc. Natl. Acad. Sci. U. S. A. 88, 3957–61.

Fax, P., Carlson, C.R., Collas, P., Taskén, K., Esche, H., Brockmann, D., 2001. Binding of PKA-RIIalpha to the Adenovirus E1A12S oncoprotein correlates with its nuclear translocation and an increase in PKA-dependent promoter activity. Virology 285, 30–41.

Ferrari, R., Berk, A.J., Kurdistani, S.K., 2009. Viral manipulation of the host epigenome for oncogenic transformation. Nat. Rev. Genet. 10, 290–4.

Ferrari, R., Gou, D., Jawdekar, G., Johnson, S.A., Nava, M., Su, T., Yousef, A.F., Zemke, N.R., Pellegrini, M., Kurdistani, S.K., Berk, A.J., 2014. Adenovirus small E1A employs the lysine acetylases p300/CBP and tumor suppressor Rb to repress select host genes and promote productive virus infection. Cell Host Microbe 16, 663–76.

Ferrari, R., Pellegrini, M., Horwitz, G.A., Xie, W., Berk, A.J., Kurdistani, S.K., 2008. Epigenetic reprogramming by adenovirus e1a. Science 321, 1086–8.

Ferreon, A.C.M., Ferreon, J.C., Wright, P.E., Deniz, A.A., 2013. Modulation of allostery by protein intrinsic disorder. Nature 498, 390–4.

Fonseca, G.J., Thillainadesan, G., Yousef, A.F., Ablack, J.N., Mossman, K.L., Torchia, J., Mymryk, J.S., 2012. Adenovirus evasion of interferon-mediated innate immunity by direct antagonism of a cellular histone posttranslational modification. Cell Host Microbe 11, 597–606.

Gedrich, R.W., Bayley, S.T., Engel, D.A., 1992. Induction of AP-1 DNA-binding activity and c-fos mRNA by the adenovirus 243R E1A protein and cyclic AMP requires domains necessary for transformation. J. Virol. 66, 5849–59.

Gold, M.G., Gonen, T., Scott, J.D., 2013. Local cAMP signaling in disease at a glance. J. Cell Sci. 126, 4537–43.

Gold, M.G., Lygren, B., Dokurno, P., Hoshi, N., McConnachie, G., Taskén, K., Carlson, C.R., Scott, J.D., Barford, D., 2006. Molecular basis of AKAP specificity for PKA regulatory subunits. Mol. Cell 24, 383–95.

Goujon, M., McWilliam, H., Li, W., Valentin, F., Squizzato, S., Paern, J., Lopez, R., 2010. A new bioinformatics analysis tools framework at EMBL-EBI. Nucleic Acids Res. 38, W695–W699.

Graham, F.L., Smiley, J., Russell, W.C., Nairn, R., 1977. Characteristics of a Human Cell Line Transformed by DNA from Human Adenovirus Type 5. J. Gen. Virol. 36, 59–72.

Guo, Y., Wilderman, A., Zhang, L., Taylor, S.S., Insel, P.A., 2012. Quantitative Proteomics Analysis of the cAMP/Protein Kinase A Signaling Pathway. Biochemistry 51, 9323–32.

Hardy, S., Shenk, T., 1988. Adenoviral control regions activated by E1A and the cAMP

response element bind to the same factor. Proc. Natl. Acad. Sci. U. S. A. 85, 4171–5.

Jones, N., Shenk, T., 1979. Isolation of adenovirus type 5 host range deletion mutants defective for transformation of rat embryo cells. Cell 17, 683–9.

Kelley, L.A., Mezulis, S., Yates, C.M., Wass, M.N., Sternberg, M.J.E., 2015. The Phyre2 web portal for protein modeling, prediction and analysis. Nat. Protoc. 10, 845–858.

Kozakov, D., Beglov, D., Bohnuud, T., Mottarella, S.E., Xia, B., Hall, D.R., Vajda, S., 2013. How good is automated protein docking? Proteins 81, 2159–66.

Lee, B.H., Mathews, M.B., 1997. Transcriptional coactivator cAMP response element binding protein mediates induction of the human proliferating cell nuclear antigen promoter by the adenovirus E1A oncoprotein. Proc. Natl. Acad. Sci. U. S. A. 94, 4481–6.

Lee, B.H., Mathews, M.B., 1997. Transcriptional coactivator cAMP response element binding protein mediates induction of the human proliferating cell nuclear antigen promoter by the adenovirus E1A oncoprotein. Proc. Natl. Acad. Sci. 94, 4481–4486.

Magico, A.C., Bell, J.B., 2011. Identification of a classical bipartite nuclear localization signal in the Drosophila TEA/ATTS protein scalloped. PLoS One 6, e21431.

Majeau, N., Bolduc, M., Duvignaud, J.-B., Fromentin, R., Leclerc, D., 2007. Effect of cAMP-dependent protein kinase A (PKA) on HCV nucleocapsid assembly and degradation. Biochem. Cell Biol. 85, 78–87.

Meng, X., Webb, P., Yang, Y.-F., Shuen, M., Yousef, A.F., Baxter, J.D., Mymryk, J.S., Walfish, P.G., 2005. E1A and a nuclear receptor corepressor splice variant (N-CoRI) are thyroid hormone receptor coactivators that bind in the corepressor mode. Proc. Natl. Acad. Sci. U. S. A. 102, 6267–72.

Miller, M.E., Engel, D.A., Smith, M.M., 1995. Cyclic AMP signaling is required for function of the N-terminal and CR1 domains of adenovirus E1A in Saccharomyces cerevisiae. Oncogene 11, 1623–30.

Miyake-Stoner, S.J., O'Shea, C.C., 2014. Metabolism goes viral. Cell Metab. 19, 549–50.

Müller, U., Roberts, M.P., Engel, D.A., Doerfler, W., Shenk, T., 1989. Induction of transcription factor AP-1 by adenovirus E1A protein and cAMP. Genes Dev. 3, 1991–2002.

Newlon, M.G., Roy, M., Morikis, D., Hausken, Z.E., Coghlan, V., Scott, J.D., Jennings, P.A., 1999. The molecular basis for protein kinase A anchoring revealed by solution NMR. Nat. Struct. Biol. 6, 222–7.

Okabe, M., Enomoto, M., Maeda, H., Kuroki, K., Ohtsuki, K., 2006. Biochemical

characterization of suramin as a selective inhibitor for the PKA-mediated phosphorylation of HBV core protein in vitro. Biol. Pharm. Bull. 29, 1810–4.

Palorini, R., Votta, G., Pirola, Y., De Vitto, H., De Palma, S., Airoldi, C., Vasso, M., Ricciardiello, F., Lombardi, P.P., Cirulli, C., Rizzi, R., Nicotra, F., Hiller, K., Gelfi, C., Alberghina, L., Chiaradonna, F., 2016. Protein Kinase A Activation Promotes Cancer Cell Resistance to Glucose Starvation and Anoikis. PLoS Genet. 12, e1005931.

Pattabiraman, D.R., Bierie, B., Kober, K.I., Thiru, P., Krall, J.A., Zill, C., Reinhardt, F., Tam, W.L., Weinberg, R.A., 2016. Activation of PKA leads to mesenchymal-to-epithelial transition and loss of tumor-initiating ability. Science (80-.). 351, aad3680-aad3680.

Pelka, P., Ablack, J.N.G., Fonseca, G.J., Yousef, A.F., Mymryk, J.S., 2008. Intrinsic structural disorder in adenovirus E1A: a viral molecular hub linking multiple diverse processes. J. Virol. 82, 7252–63.

Pelka, P., Miller, M.S., Cecchini, M., Yousef, A.F., Bowdish, D.M., Dick, F., Whyte, P., Mymryk, J.S., 2011. Adenovirus E1A directly targets the E2F/DP-1 complex. J. Virol. 85, 8841–51.

Rasti, M., Grand, R.J.A., Mymryk, J.S., Gallimore, P.H., Turnell, A.S., 2005. Recruitment of CBP/p300, TATA-binding protein, and S8 to distinct regions at the N terminus of adenovirus E1A. J. Virol. 79, 5594–605.

Rozenblatt-Rosen, O., Deo, R.C., Padi, M., Adelmant, G., Calderwood, M.A., Rolland, T., Grace, M., Dricot, A., Askenazi, M., Tavares, M., Pevzner, S.J., Abderazzaq, F., Byrdsong, D., Carvunis, A.-R., Chen, A.A., Cheng, J., Correll, M., Duarte, M., Fan, C., Feltkamp, M.C., Ficarro, S.B., Franchi, R., Garg, B.K., Gulbahce, N., Hao, T., Holthaus, A.M., James, R., Korkhin, A., Litovchick, L., Mar, J.C., Pak, T.R., Rabello, S., Rubio, R., Shen, Y., Singh, S., Spangle, J.M., Tasan, M., Wanamaker, S., Webber, J.T., Roecklein-Canfield, J., Johannsen, E., Barabási, A.-L., Beroukhim, R., Kieff, E., Cusick, M.E., Hill, D.E., Münger, K., Marto, J.A., Quackenbush, J., Roth, F.P., DeCaprio, J.A., Vidal, M., 2012. Interpreting cancer genomes using systematic host network perturbations by tumour virus proteins. Nature 487, 491–5.

Sample, V., DiPilato, L.M., Yang, J.H., Ni, Q., Saucerman, J.J., Zhang, J., 2012. Regulation of nuclear PKA revealed by spatiotemporal manipulation of cyclic AMP. Nat. Chem. Biol. 8, 375–82.

Sands, W.A., Palmer, T.M., 2008. Regulating gene transcription in response to cyclic AMP elevation. Cell. Signal. 20, 460–6.

Sarma, G.N., Kinderman, F.S., Kim, C., von Daake, S., Chen, L., Wang, B.-C., Taylor, S.S., 2010. Structure of D-AKAP2:PKA RI complex: insights into AKAP specificity and selectivity. Structure 18, 155–66.

Scherer, J., Yi, J., Vallee, R.B., 2014. PKA-dependent dynein switching from lysosomes to adenovirus: a novel form of host-virus competition. J. Cell Biol. 205, 163–77.

Schmittgen, T.D., Livak, K.J., 2008. Analyzing real-time PCR data by the comparative C(T) method. Nat. Protoc. 3, 1101–8.

Shah, G.A., O'Shea, C.C., 2015. Viral and Cellular Genomes Activate Distinct DNA Damage Responses. Cell 162, 987–1002.

Soria, C., Estermann, F.E., Espantman, K.C., O'Shea, C.C., 2010. Heterochromatin silencing of p53 target genes by a small viral protein. Nature 466, 1076–81.

Suomalainen, M., Nakano, M.Y., Boucke, K., Keller, S., Greber, U.F., 2001. Adenovirus-activated PKA and p38/MAPK pathways boost microtubule-mediated nuclear targeting of virus. EMBO J. 20, 1310–9.

Taskén, K., Aandahl, E.M., 2004. Localized effects of cAMP mediated by distinct routes of protein kinase A. Physiol. Rev. 84, 137–67.

Taylor, S.S., Ilouz, R., Zhang, P., Kornev, A.P., 2012. Assembly of allosteric macromolecular switches: lessons from PKA. Nat. Rev. Mol. Cell Biol. 13, 646–58.

Wong, W., Scott, J.D., 2004. AKAP signalling complexes: focal points in space and time. Nat. Rev. Mol. Cell Biol. 5, 959–70.

Zambon, A.C., Zhang, L., Minovitsky, S., Kanter, J.R., Prabhakar, S., Salomonis, N., Vranizan, K., Dubchak, I., Conklin, B.R., Insel, P.A., 2005. Gene expression patterns define key transcriptional events in cell-cycle regulation by cAMP and protein kinase A. Proc. Natl. Acad. Sci. U. S. A. 102, 8561–6.

Zhang, X., Odom, D.T., Koo, S.-H., Conkright, M.D., Canettieri, G., Best, J., Chen, H., Jenner, R., Herbolsheimer, E., Jacobsen, E., Kadam, S., Ecker, J.R., Emerson, B., Hogenesch, J.B., Unterman, T., Young, R.A., Montminy, M., 2005. Genome-wide analysis of cAMP-response element binding protein occupancy, phosphorylation, and target gene activation in human tissues. Proc. Natl. Acad. Sci. U. S. A. 102, 4459–64.

Chapter 3

3 Mimicry of cellular A-kinase anchoring proteins is a conserved and critical function of E1A across various human adenovirus species

"When you run the marathon, you run against the distance, not against the other runners and not against the time."

– *Haile Gebrselassie*

3.1 Introduction

The E1A oncoprotein of human adenovirus (HAdV) is the first viral protein produced upon infection and performs numerous crucial tasks required for viral command of the host cell (Frisch and Mymryk, 2002; Pelka et al., 2008). By binding to and modulating the activities of important cellular regulatory proteins, E1A alters the transcriptional landscape of infected cells to favour viral replication (Ferrari et al., 2014, 2008; Hearing and Shenk, 1986). The targets of E1A are diverse in both form and function, highlighting its modular and multifunctional nature. As a relatively small protein, E1A is densely packed with protein interaction motifs ranging in size from a mere few to dozens of amino acids (Ferreon et al., 2013; Pelka et al., 2008). Additionally, many of these motifs are functional mimics of cellular counterparts suggesting E1A has coevolved with its host to be an effective competitor for its targeted binding partners.

The N-terminus of HAdV-5 E1A (~aa1-40) alone is capable of interacting with roughly a dozen cellular proteins and contains strong transcriptional activation ability (Bondesson et al., 1994; Pelka et al., 2008; Yousef et al., 2009). Indeed, viral genes as well as cellular genes involved in cell cycle, signalling, differentiation, and immunity are modulated by the protein-protein interactions from this small portion of a single viral protein. Recently, protein kinase A (PKA) was shown to be a target of HAdV-5 E1A via viral mimicry (King et al., 2016a). PKA primarily exists as a tetrameric holoenzyme consisting of two catalytic subunits (C) bound by a regulatory subunit (R) homodimer (Taylor et al., 2013). Multiple isoforms of both C- and R-subunits exist, providing

additional levels of functional specificity based on cell-type specific expression, additional binding partners, and substrate recognition. Cα specifically was manipulated by HAdV-5 E1A via E1A's ability to bind both the RIα- and RIIα-type regulatory subunits. This was mediated through the N-terminus of HAdV-5 E1A which contains a binding motif similar to that found in PKA's canonical cellular interacting partner(s), A-kinase anchoring proteins (AKAPs). AKAPs are polyvalent and highly dynamic scaffold proteins that provide spatiotemporal control of PKA by localizing it within the cell (Banky et al., 1998; Gold et al., 2006; Newlon et al., 1999). Their hallmark PKA-binding motif is an amphipathic α-helix that directly binds with high-affinity to the hydrophobic groove in the D/D domain of PKA R-subunit dimers. Most AKAP-PKA interactions are specific for either RIα or RIIα subunits but dual-specificity AKAPs exist as well. In addition to binding PKA, AKAPs typically contain motifs directing their subcellular localization to specific intracellular compartments or organelles. AKAPs enable tight and efficient control of cAMP signaling by simultaneously tethering interactions between PKA, substrates, and up- or down-stream regulators of the pathway. PKA serves several signalling roles in cell biology, including transcriptional regulation, making it an attractive target for manipulation by a viral hub like E1A (Guo et al., 2012; Sands and Palmer, 2008; Zambon et al., 2005; Zhang et al., 2005). E1A's own amphipathic α-helix motif allowed it to outcompete endogenous AKAPs to bind with PKA and relocalize it to the nucleus. The alteration of PKA compartmentalization by this 'viral AKAP' (vAKAP) was biologically significant as it enhanced viral transcription, protein synthesis, and progeny production.

The mechanism behind E1A-mediated manipulation of PKA was well-characterized in the context of HAdV-5 infection. However, while protein-protein binding was shown to be largely conserved between PKA and E1A proteins of different HAdV species, very little is known regarding how E1A from other HAdVs may affect this important cellular kinase. The N-terminal sequence of E1A, containing the PKA-binding α-helix, is not as conserved as other portions of this protein (Avvakumov et al., 2004) and it has been shown that altering a single or few amino acids can disrupt the interaction between E1A and PKA (King et al., 2016a). Whether all species of HAdV utilize E1A as a vAKAP has yet to be determined. There may also exist varying preferences for PKA subunit binding

or relocalization, and this may confer differential effects on replication for distinct HAdVs.

Here we analyze representative E1A proteins from seven HAdV species for their abilities to act as vAKAPs. We found that while not all species of E1A could engage in this form of mimicry, the mechanism described for HAdV-5 E1A is largely conserved between several distinct HAdVs. Building upon previous observations, different E1As exhibit a preference for one type of PKA regulatory subunit (RIα or RIIα) over the other. While exploring this finding, we identified individual residuesin the PKA-binding motifs of vAKAPs that when altered by site-directed mutagenesis can convert the preferred interaction to the alternative regulatory subunit. Additionally, we provide new mechanistic insights into how HAdV alters cellular cAMP signalling and show viruses capable of manipulating PKA have an enhanced ability to drive cAMP-regulated transcription. An E1A-PKA interaction is also a conserved requirement for wild-type (WT) levels of viral genome replication and infectious progeny production. Together, our results characterize a shared mechanism for viral manipulation of a cellular kinase which enhances the life cycle of numerous human adenoviruses.

3.2 Materials and Methods

3.2.1 Cell lines and transfections

Human A549 (provided by Russ Wheeler, Molecular Pathology/Genetics London Health Sciences Centre), HEK293, HT1080, and HeLa (purchased from the American Type Culture Collection) were grown at 37°C with 5% CO_2 in DMEM (Multicell) supplemented with 10% fetal bovine serum (Gibco). Plasmids were transfected into HT1080 and HeLa cells using XtremegeneHP (Roche) following the manufacturer's recommendation. After 24 hours of incubation, transfected cells were used for downstream experiments.

3.2.2 Viruses and infection of cells

Wild-type (WT) HAdV-5 (*dl*309) was previously described (Jones and Shenk, 1979) and mutants *dl*312 and *dl*1101 are derived from the same background to express no E1A and

E1AΔ4-25 respectively. HAdV-5 *dl*520 and *dl*1131/520 express only the "12S" product of E1A with the latter containing an additional C-terminal deletion (219-289) (Mymryk and Bayley, 1993). WT serotypes of HAdV-3 (strain GB Lot 11W), -4 (strain RI-67 Lot 3W), -9 (Strain Hicks Lot 1W), and -12 (Strain Huie Lot 8W) were purchased from the ATCC via Cedarlane. A549 cells were infected at a multiplicity of infection (MOI) of 5 plaque-forming units/mL (for HAdV-5) or 25 for all other virus species to attain similar levels of infection. Cell cultures were infected at ~50% confluence and subconfluent cells were collected at indicated time points for downstream experiments. For plaque assays, HEK293 cells were infected with serially-diluted samples for 1 hour at 37°C before being overlayed with DMEM containing 1% SeaPlaque Agarose (Lonza).

3.2.3 RNAi knockdown

Downregulation of PKA subunits RIα, RIIα, and Cα was performed using Silencer Select siRNA (Thermo), specifically s286 (PRKAR1A), s11086 (PRKAR2A), and s11065 (PRKACA). Four hours after seeding, siRNA was delivered to A549 and HT1080 cells via transfection with Silentfect (BioRad) according to the manufacturer's instructions. A scrambled siRNA was used as a negative control. Treated cells were used for experiments 48 hours post-transfection.

3.2.4 Plasmids

All constructs were expressed in vectors under control of the CMV promoter, with the exception of the luciferase reporter which was driven by the cAMP-responsive rat PEPCK promoter (Klemm et al., 1996). WT RIα, RIIα, and Cα along with ΔD/D mutants were previously described (King et al., 2016a). WT full-length HAdV-52 E1A was synthesized using IDT's gene synthesis platform from a published genome sequence (Jones et al., 2007). E1A fragments, truncations, and deletion mutants for all species were expressed as fusions to EGFP and either previously described (King et al., 2016a) or derived via PCR and cloned into pEGFP-C2. Chimeras (HAdV-4 chimera and HAdV-5 chimera) and point mutants (HAdV-5 D21E, E26T, V27L and HAdV-3 E22D, T27E, L28V) were cloned similarly. A list of all plasmids used in this chapter can be found in table 3.1.

Table 3.1. List of plasmids used in this chapter

Clone	Backbone(s)	Notes
13S Ad3 E1A	pEGFP-C2	Species B
13S Ad4 E1A	pEGFP-C2	Species E
13S Ad5 E1A	pEGFP-C2, pcDNA3	Species C
13S Ad9 E1A	pEGFP-C2	Species D
13S Ad12 E1A	pEGFP-C2	Species A
13S Ad40 E1A	pEGFP-C2	Species F
*13S Ad52 E1A	pEGFP-C2	Species G, Jones et al., 2007
Ad5 E1A 1-82	pEGFP-C2	N-terminus and CR1
*Ad3 E1A Δ1-82	pEGFP-C2	
Ad3 E1A 1-82	pEGFP-C2	N-terminus and CR1
*Ad9 E1A Δ1-81	pEGFP-C2	
Ad9 E1A 1-81	pEGFP-C2	N-terminus and CR1
*Ad12 E1A Δ1-79	pEGFP-C2	
Ad12 E1A 1-79	pEGFP-C2	N-terminus and CR1
*Ad40 E1A Δ1-77	pEGFP-C2	
Ad40 E1A 1-77	pEGFP-C2	N-terminus and CR1
*Ad52 E1A Δ1-75	pEGFP-C2	
*Ad52 E1A 1-75	pEGFP-C2	N-terminus and CR1
*Ad3 E1A 1-13	pEGFP-C2	
*Ad3 E1A 11-28	pEGFP-C2	
*Ad3 E1A 28-82	pEGFP-C2	
*Ad3 E1A Δ11-28	pEGFP-C2	
*Ad9 E1A 1-14	pEGFP-C2	
*Ad9 E1A 12-29	pEGFP-C2	
*Ad9 E1A 30-81	pEGFP-C2	
*Ad9 E1A Δ12-29	pEGFP-C2	
*Ad12 E1A 1-13	pEGFP-C2	
*Ad12 E1A 12-27	pEGFP-C2	
*Ad12 E1A 28-79	pEGFP-C2	
*Ad12 E1A Δ12-27	pEGFP-C2	
*Ad12 E1A Δ37-49	pEGFP-C2	
*Ad12 E1A Δ12-27,37-49	pEGFP-C2	
*Ad40 E1A 1-12	pEGFP-C2	
*Ad40 E1A 12-26	pEGFP-C2	
*Ad40 E1A 25-77	pEGFP-C2	
*Ad40 E1A Δ12-26	pEGFP-C2	
PKA RIα	pcDNA-HA, pcDNA-MYC	
PKA RIIα	pcDNA-HA, pcDNA-MYC	
PKA Cα	pcDNA-HA	
PKA RIα Δ1-63	pcDNA-HA	ΔD/D
PKA RIIα Δ1-45	pcDNA-HA, pcDNA-MYC	ΔD/D
Ad5 E1A Δ16-28	pEGFP-C2	
*Ad5 chimera E1A	pEGFP-C2	Ad5 backbone with Ad4 N-terminus
*Ad4 chimera E1A	pEGFP-C2	Ad4 backbone with Ad5 N-terminus
*Ad5 E1A D21E E26T V27L	pEGFP-C2	
*Ad3 E1A E22D T27E L28V	pEGFP-C2	
PEPCK-luc	pGL3-Basic	From Joshua Hamilton

*Novel clone generated for this study

Table 3.2. List of primary antibodies used in this chapter

Reactivity	Purpose	Description	Company
RIα	Western	Mouse monoclonal	BD
RIα	IF	Rabbit polyclonal	Thermo
RIIα	Western	Mouse monoclonal	BD
RIIα	IF	Rabbit polyclonal	Santa Cruz
Cα	Western	Mouse monoclonal	BD
Cα	IF	Rabbit polyclonal	Santa Cruz
Actin	Western	Rabbit polyclonal	Sigma
EGFP	Western, IP	Rabbit polyclonal	Clontech
9E10 (MYC)	Western, IP	Mouse monoclonal	In-house
3F10 (HA)	Western	Rat monoclonal	Roche
CREB1	Western	Rabbit polyclonal	Santa Cruz
CREB1-pSer133	Western, IF	Rabbit polyclonal	Santa Cruz
M73 (E1A)	Western, IF	Mouse monoclonal	In-house
M37 (E1A)	IF	Mouse monoclonal	In-house
cAMP	IF	Rabbit monoclonal	Abcam
B68 (DBP)	IF	Mouse monoclonal	In-house

3.2.5 Western blotting and co-immunoprecipitation

Cells were lysed in NP40 lysis buffer (150mM NaCl, 50mM Tris-HCL pH 7.5, 0.1% NP-40) with protease inhibitor cocktail. Protein concentrations were determined using BioRad protein assay reagent using BSA as a standard. Co-immunoprecipitations were carried out at 4°C for 4 hours. 2% of sample was kept as input control. After washing with NP40 buffer, complexes were boiled in 25 µL of LDS sample buffer for 5 minutes. Samples were separated on NuPage Bis-Tris gels (Life Technologies) and transferred onto a PVDF membrane (Amersham). Membranes were blocked in 5% skim milk or 3% bovine serum albumin (BSA) constituted in TBS with 0.1% Tween-20. Horseradish peroxidise-conjugated secondary antibody was detected using Luminata Forte or Crescendo substrate (Millipore). Primary antibodies used in this chapter can be found in table 2.2.

3.2.6 Immunofluorescence microscopy

Cells were fixed in 3.7% paraformaldehyde, permeabilized on ice using 0.2% Triton X-100, and blocked using 3% BSA in phospho-buffered saline (PBS). Samples were incubated in the primary antibody for 1 hour at room temperature or 4°C overnight and another hour at room temperature with secondary antibody (Alexa Fluor 594 or 488 α-rabbit; Life technologies). Samples were mounted with Prolong Gold reagent containing DAPI (Life technologies). Confocal images were acquired using a Fluoview 1000 laser scanning confocal microscope (Olympus Corp). Quantification of total cellular signal and nuclear signal was conducted using ImageJ. Cells were normalized for both cytoplasmic and nuclear size and %nuclear signal was determined as previously described (Cohen et al., 2014).

3.2.7 Virus replication assay

A549 cells were treated with siRNAs followed by HAdV infection for 1 hour at 37°C at the indicated MOI. Total cell DNA was purified at 6 and 48 hours post-infection using a DNeasy Blood and Tissue Kit (QIAGEN). Viral DNA levels were quantified by qPCR with Power SYBR Green (Thermo) using a forward primer that recognizes a conserved

Table 3.3. List of qPCR primers used in this chapter

Target	Region	Location	Sequence	Size
GAPDH-F	Cellular cDNA	566-587	ACTGCTTAGCACCCCTGGCCAA	79
GAPDH-R	Cellular cDNA	620-644	ATGGCATGGACTGTGGTCATGAGTC	
E1A-All-F	Promoter	Variable	AGAGGCCACTCTTGAGTGC	Variable
Ad3E1A-R	Exon1	702-721	TACAGATCGTGCAGCGTAGG	271
Ad4E1A-R	Exon1	690-709	AGCGAAGGTGTCTCAAATGG	206
Ad5E1A-R	Exon1	697-715	CGTCACGTCTAAATCATAC	224
Ad9E1A-R	Exon1	711-730	GGGCATCTACCTCCAGATCA	226
Ad12E1A-R	Exon1	641-659	CGGCAGACTCCACATCAAG	221

Table 3.4. List of cloning primers used in this chapter

Clone	Forward	Reverse	Notes
13S Ad52 E1A	ATTGGAATTCATGAGGCTGGTCCCC	CATCGTCGACTTAGGTCAGCTTA	Jones et al., 2007
Ad3 E1A Δ1-82	ATCCGAATTCATGAACCCTCCTCCTGAGACA	GGATGTCGACTCATTGCCTTGGCAGTTT	
Ad9 E1A Δ1-81	ATCCGAATTCATGACTCCACTTCATACAC	AGTGTCGACGTCTAGTTCCTGGGGCG	
Ad12 E1A Δ1-79	ATTCGAATTCATGCCGGAGCCTCCTGTA	CAGGTCGACTTAATTACATCTAGGGCGTTTC	
Ad40 E1A Δ1-77	ATTGGAATTCATGAGCGGTTCGGGTGAT	GTGGTCGACTCAGGAGCACTTGGGGCGC	
Ad52 E1A Δ1-75	ATTGGAATTCATGTCTCACAGCAGTGA	CATCGTCGACTTAGGTCAGCTTA	
Ad52 E1A 1-75	ATTGGAATTCATGAGGCTGGTCCCC	CATCGTCGACCTAACCCTCCTCAGCCG	
Ad3 E1A 1-13	TACGAATTCATGAGACACCTGCGCTTC	GGATGTCGACTTAGGAGATAACCT	
Ad3 E1A 11-28	ATCCGAATTCATGGTTATCTCCAGTGAGAC	CATCGTCGACTTAGGTATTTACCACAAA	
Ad3 E1A 28-82	ATCCGAATTCATGCTAATGGGAGACGAC	AGTGTCGACTTATATGTCCAATCCTTCATCGG	
Ad3 E1A Δ11-28	TTCCTGCCACAGGAGCTAATGGGAGACGAC	GTCGTCTCCCATTAGCTCCTGTGGCAGGAA	Internal
	TACGAATTCATGAGACACCTGCGCTTC	GGATGTCGACTCATTGCCTTGGCAGTTT	Flanking
Ad9 E1A 1-14	CTGGAATTCATGAGACACCTGCGCC	CATCGTCGACTTACTCACCGGGCACAGT	
Ad9 E1A 12-29	ATTCGAATTCATGCCCGGTGAGCTGGCT	CATCGTCGACTTACTCCAATACTGTAT	
Ad9 E1A 30-81	ATTCGAATTCATGGACGAACTGCATCCA	ATAGTCGACTTAAGATTCGTTGGCTATGTCAG	
Ad9 E1A Δ12-29	CTGCCTTCAACTGTGGACGAACTGCATCCA	TGGATGCAGTTCGTCCACAGTTGAAGGCAG	Internal
	CTGGAATTCATGAGACACCTGCGCC	AGTGTCGACGTCTAGTTCCTGGGGCG	Flanking
Ad12 E1A 1-13	CTACGAATTCATGAGAACTGAAATGACTCCC	CAGCGTCGACTTACTGATACGACAG	
Ad12 E1A 12-27	ATCCGAATTCATGTATCAGGAAGCTGAC	CATCGTCGACCTAAAAAAAGTTGTCCA	
Ad12 E1A 28-79	ATCCGAATTCATGAACGAGGTACCCAGT	TGAGTCGACTAAAAACAACCCCTCACTGGC	
Ad12 E1A Δ12-27	CCCTTGGTCCTGTCGAACGAGGTACCCAGT	ACTGGGTACCTCGTTCGACAGGACCAAGGG	Internal
	CTACGAATTCATGAGAACTGAAATGACTCCC	CAGGTCGACTTAATTACATCTAGGGCGTTTC	Flanking
Ad12 E1A Δ37-49	AGTGATGATGATCTTGAGTCTGCCGGTGAA	TTCACCGGCAGACTCAAGATCATCATCACT	Internal
	CTACGAATTCATGAGAACTGAAATGACTCCC	CAGGTCGACTTAATTACATCTAGGGCGTTTC	Flanking
Ad12 E1A Δ12-27,37-49 (same as Δ37-49 with Δ12-27 as template)	AGTGATGATGATCTTGAGTCTGCCGGTGAA	TTCACCGGCAGACTCAAGATCATCATCACT	Internal
	CTACGAATTCATGAGAACTGAAATGACTCCC	CAGGTCGACTTAATTACATCTAGGGCGTTTC	Flanking
Ad40 E1A 1-12	TCAGAATTCATGAGAATGCTGCCGG	CATCGTCGACCTACCAGTTCCCG	

Ad40 E1A 12-26	ATTGGAATTCATGTGGGATGACATGT	CATCGTCGACCTAAAACACATATTCA	
Ad40 E1A 25-77	ATTGGAATTCATGGTGTTTGATTTC	TCAGTCGACTTACTCTGCAGCGCTCTCAG	
Ad40 E1A Δ12-26	TTTACCGGGAACGATTTCCCTGAACCT	AGGTTCAGGGAAATCGTTCCCGGTAAA	Internal
	TCAGAATTCATGAGAATGCTGCCGG	GTGGTCGACTCAGGAGCACTTGGGGCGC	Flanking
Ad5 chimera E1A	CGAGGAGGCTTCATTTCCGCCGGCGC	GCGCCGGCGGAAATGAAGCCTCCTCG	Internal
	TCCGAATTCATGAGGCACCTAAGAGAC	ACTGTCGACTTATGGCCTGGGACGTTTACAGCTC	Flanking
Ad4 chimera E1A	ATTGACTTACTCACTAGCCCTAGCTCAGA	TCTGAGCTAGGGCTAGTGAGTAAGTCAAT	Internal
	CGACGAATTCATGAGACATATTATCTGC	GTGGTCGACTTAATGCCTGGGGCGTTTCC	Flanking
Ad5 E1A D21E E26T V27L	TTTGGAGCAGCTGATCGAAACCCTACTGG	CCAGTAGGGTTTCGATCAGCTGCTCCAAA	Internal
	CGACGAATTCATGAGACATATTATCTGC	ACTGTCGACTTATGGCCTGGGACGTTTACAGCTC	Flanking
Ad3 E1A E22D T27E L28V	TACTGGACTTTGTGGTAAATGAGGTAATGGGA	TCCCATTACCTCATTTACCACAAAGTCCAGTA	Internal
	TACGAATTCATGAGACACCTGCGCTTC	GGATGTCGACTCATTGCCTTGGCAGTTT	Flanking

sequence in E1A in combination with a serotype-specific reverse primer. Values are normalized to GAPDH and the fold-increase of viral copy number at 48 hours was calculated by normalizing to 'input' viral DNA at 6 hours post-infection. Viral replication efficiency in the presence of PKA knockdowns was presented as the relative value compared to scrambled control siRNA-treated cells, which were normalized to 1. A list of all primers used in this chapter can be found in tables 3.3 and 3.4.

3.2.8 Luciferase reporter assay

HT1080 cells were co-transfected with a constitutive β-galactosidase reporter plasmid for normalization, a plasmid containing the luciferase gene driven by the rat PEPCK promoter, and the indicated EGFP-E1A fusion constructs. The reporter was validated by 6 hours of 0.5μM forskolin (fsk) treatment and siRNA-mediated knockdown of PKA protein. 24 hours post-transfection, cells were lysed in 200 μL of the supplied lysis buffer (Promega). For detection of luciferase production, 50 μL of lysate was mixed with 50μL of the provided Luciferase Substrate (Promega) immediately before detection of light was measured using a Berthold Lumat LB 9507. Relative light values were set to an empty plasmid control, were normalized to protein levels via Bradford Assay, and further normalized via β-galactosidase activity as detected by ONPG (Ortho-Nitrophenyl-β-D-Galactopyranoside).

3.2.9 PKA activity assay

PKA activity in cell lysates was measured using the Protein Kinase A Colorimetric Activity Kit (Thermo). $1x10^6$ A549 cells were seeded overnight then either treated with fsk for 6 hours or infected with the indicated virus. After harvesting at the indicated timepoints, PKA activity of the cell lysates was assayed using the protocol outlined by the manufacturer. Activity in uninfected cells were normalized to 1 and compared to all other conditions.

3.2.10 Statistical analysis

All experiments were carried out with three biological replicates performed in duplicate. Graphs represent mean and standard error of the mean (S.E.M.) of all biological

replicates. For western blots, a representative image was selected. Statistical significance of numerical differences was calculated using t-tests (Figure 3.11) or one-way ANOVA and Holm-Sidak post-hoc comparisons (Figures 3.4, 3.12, 3.13) between experimental conditions.

3.3 Results

3.3.1 Protein kinase A subunits are conserved binding targets of the N-terminal region of E1A across numerous HAdV species

The full-length isoforms ("13S") of E1A proteins from representative viruses of six HAdV species (A-F) were previously tested for protein-protein interactions with PKA subunits. E1A from HAdV-12 (species A), HAdV-3 (species B), HAdV-5 (species C), HAdV-9 (species D), and HAdV-40 (species F) could associate with PKA via co-immunoprecipitation (Co-IP) while HAdV-4 (species E) could not (King et al., 2016a). However, the detailed mechanisms behind these individual interactions have only been further explored for HAdV-5 and HAdV-12 (Fax et al., 2001; King et al., 2016a). Additionally, a seventh species of HAdV (G) has been recently classified, containing a lone member, HAdV-52 (Jones et al., 2007). To further investigate these E1A-PKA interactions, HT1080 fibrosarcoma cells were transfected with vectors expressing PKA subunits along with either WT E1A from the seven HAdV species or the indicated fragments/portions of E1A (Figure 3.1).

Co-immunoprecipitation analysis revealed that RIα, RIIα, and Cα all associated with the majority of the E1A proteins tested. E1A from HAdV-5 was used as a positive control while the non-binding E1A from HAdV-4 was used as a negative control. E1A from HAdV-4 and the less-characterized HAdV-52 were the only non-binding types observed. For the E1As which bound (HAdV-3, -5, -9, -12, -40), the N-terminal portion of these proteins was both necessary and sufficient for conferring these interactions, though this was notably weakest for HAdV-40 E1A. These data show for the first time where PKA binds on each of these unique E1As, and the observations align with those previous for both HAdV-5 and -12 (Fax et al., 2001; King et al., 2016a). Additionally, this region of E1A has been previously shown to be associated with alterations in cAMP signalling

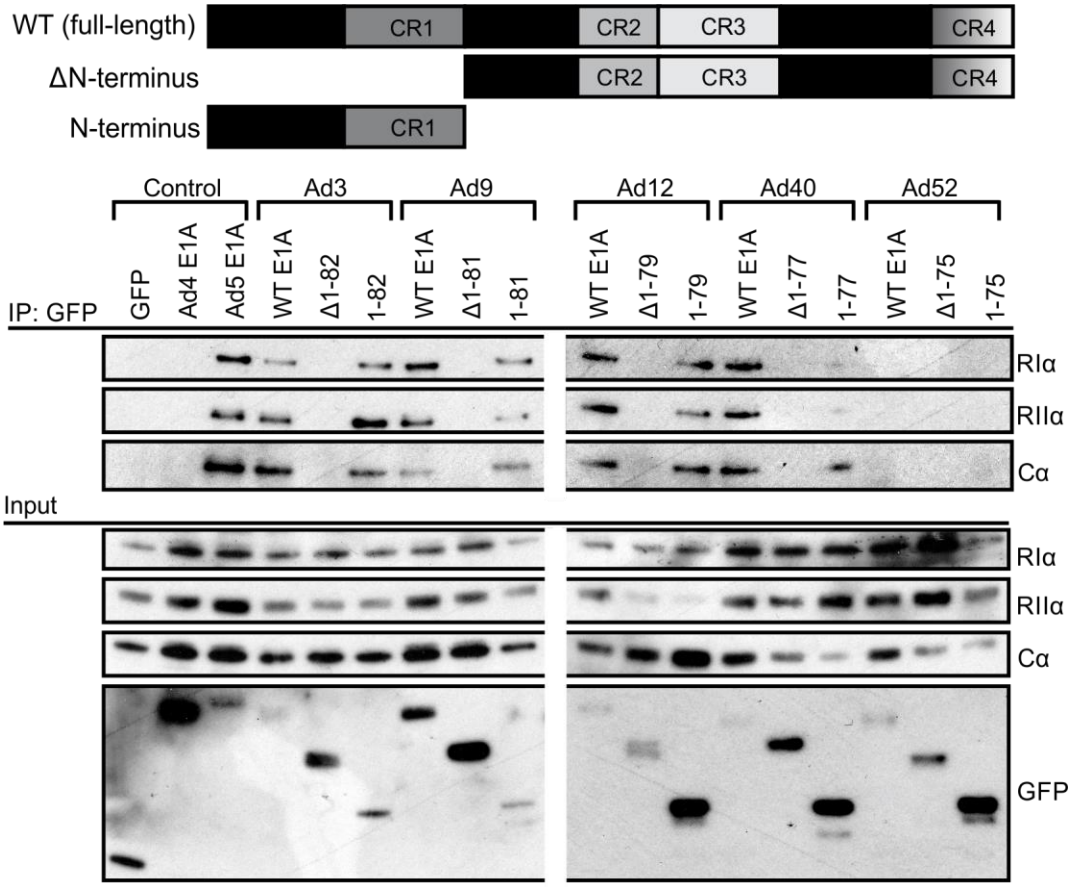


Figure 3.1. Protein Kinase A subunits are conserved binding targets of HAdV E1A's N-terminus.

HT1080 cells were co-transfected with PKA subunits and various WT or mutant E1A constructs from 7 HAdV types (shown in the inset panel). Whole cell lysates were harvested for co-immunoprecipitation. WT E1A from HAdV-3, -5, -9, -12, and -40 associates with RIα, RIIα, and Cα, whereas E1A from HAdV-4 and -52 do not. Deletion of the entire E1A N-terminus ablated the interactions, whereas the N-terminus alone was sufficient for binding.

(Engel et al., 1991, 1988; Hardy and Shenk, 1988; Lee and Mathews, 1997; Müller et al., 1989) and has been characterized as being a conserved transactivation domain (Bondesson et al., 1994; Yousef et al., 2009).

3.3.2 E1A proteins that bind PKA use an AKAP-like sequence motif

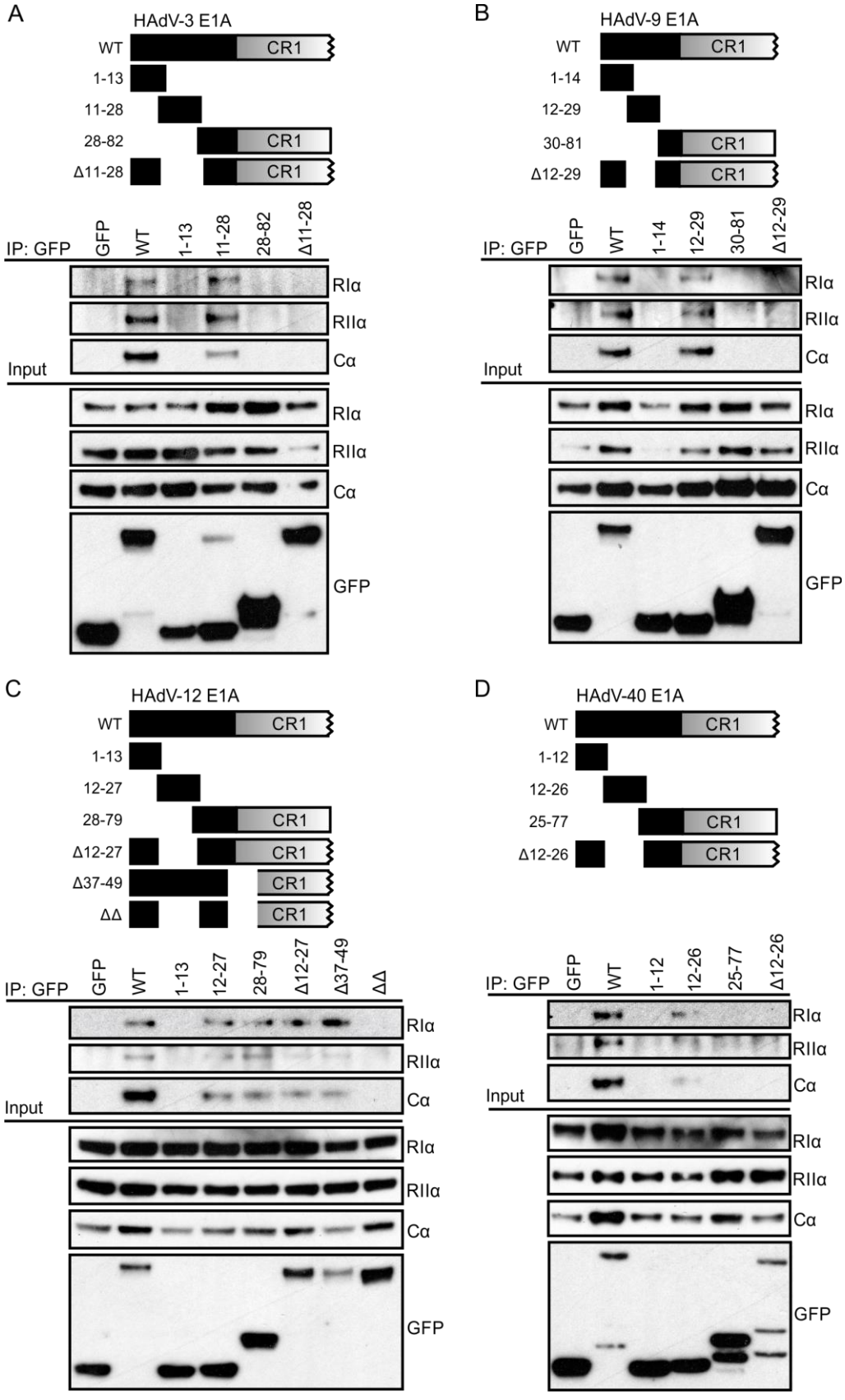
The minimal region of E1A required to bind PKA was previously delineated for HAdV-5 (King et al., 2016a). To determine the regions within E1A for the other HAdVs whose E1As have putative viral AKAP activity, we carried out more detailed mutational analyses for each distinct E1A (Figure 3.2A-D). Cells were co-transfected with vectors expressing PKA subunits and either WT or the indicated portions of E1A for several HAdV species. For HAdV-3 (Figure 3.2A), -9 (Figure 3.2B), -12 (Figure 3.2C), and -40 (Figure 3.2D), overlapping deletions or truncations within the N-terminus were tested. HAdV-3, -9, and -40 each contained a small region that was both necessary and sufficient for associating with PKA subunits (amino acids 11-28, 12-29, 12-26 respectively). Each of these regions bears strong similarity to the PKA-binding motif within HAdV-5 E1A, as well as those of cellular AKAPs (9). Also like HAdV-5 E1A and cellular AKAPs, these motifs are predicted to contain α-helical secondary structure (Pelka et al., 2008). HAdV-12 E1A appeared to have two regions contributing to its binding with PKA (amino acids 12-27 and 37-49) and deletion of both was required to fully abrogate this interaction. While unexpected, this observation does align with that of a previous report on this E1A's interaction with PKA (Fax et al., 2001) and may suggest additional, unconventional means of manipulating PKA by this E1A type. Taken together, these results demonstrate that the utilization of AKAP mimicry is a conserved function of the E1As which bind to PKA.

3.3.3 E1A binds to PKA via the PKA docking-dimerization domain

Cellular AKAPs bind PKA via the docking-dimerization (D/D) domain of PKA regulatory subunits (RIα and RIIα) (Banky et al., 1998; Newlon et al., 1999). Given the predicted sequence and structural similarities between cellular AKAPs and the E1As that bind PKA, we tested if the D/D domain was necessary for the observed E1A-PKA interactions (Figure 3.3). Cells were co-transfected with WT E1A from seven HAdV

Figure 3.2. E1A proteins that bind PKA use an AKAP-like sequence motif.

HT1080 cells were co-transfected with PKA subunits and E1A constructs from either HAdV-3, -9, -12, or -40 (A-D). Mutation schemes are shown in the inset panels. Cell lysates were harvested and co-immunoprecipitation analysis of each E1A protein revealed that amino acids 11-28 for HAdV-3 (A), aa12-29 for HAdV-9 (B), aa12-27, 37-49 for HAdV-12 (C), and aa12-26 for HAdV-40 (D) were necessary and sufficient for binding PKA subunits.


A
HAdV-3 E1A
WT
1-13
11-28
28-82
Δ11-28
CR1
IP: GFP
GFP
WT
1-13
11-28
28-82
Δ11-28
RIα
RIIα
Cα
Input
GFP
B
HAdV-9 E1A
WT
1-14
12-29
30-81
Δ12-29
IP: GFP
GFP
WT
1-14
12-29
30-81
Δ12-29
C
HAdV-12 E1A
WT
1-13
12-27
28-79
Δ12-27
Δ37-49
ΔΔ
D
HAdV-40 E1A
WT
1-12
12-26
25-77
Δ12-26

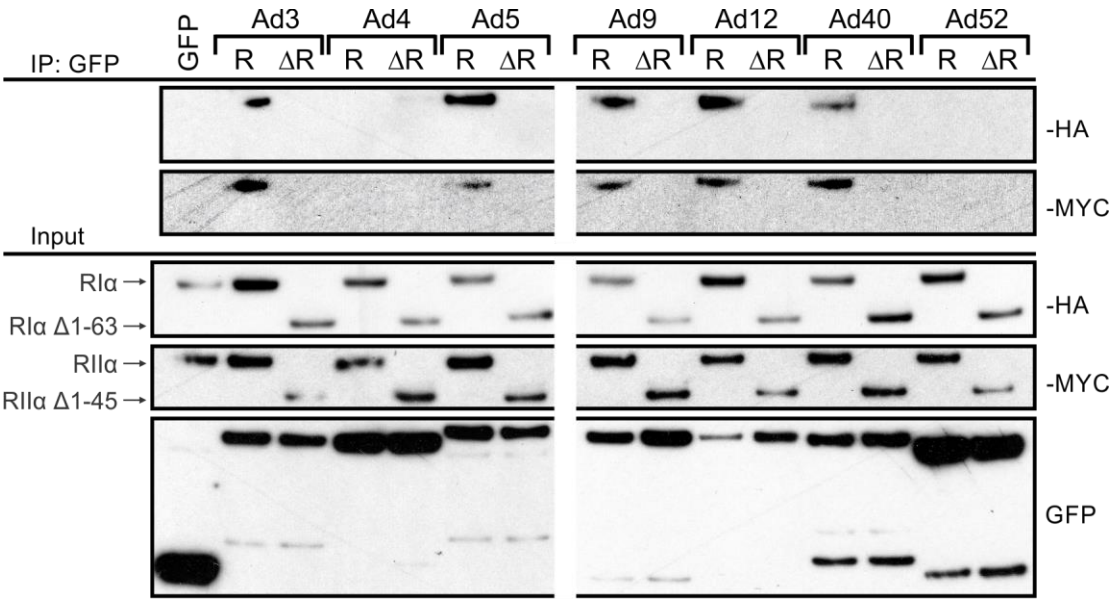


Figure 3.3. HAdV species whose E1A proteins bind PKA do so via PKA's docking-dimerization domain.

HT1080 cells were co-transfected with WT E1A from 7 species of HAdV along with either both WT PKA regulatory subunits ("R") or both subunits lacking their D/D domains ("ΔR"). Whole cell lysates were harvested for co-immunoprecipitation. Deletion of the D/D domains in either HA-tagged RIα or MYC-tagged RIIα abrogated the interactions with E1A.

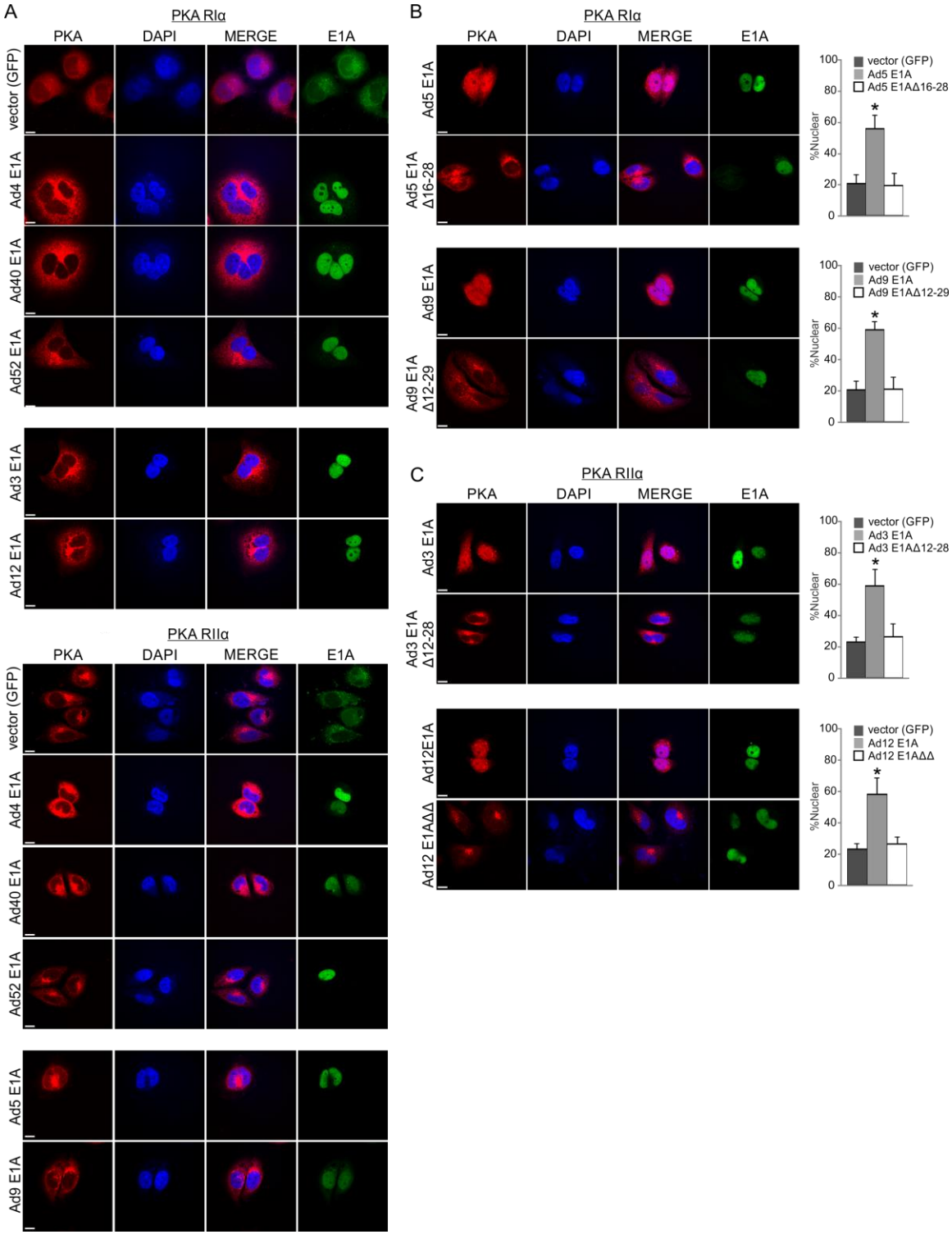
species along with either WT PKA regulatory subunits or mutants lacking their respective D/D domains (RIαΔ1-63 and RIIαΔ1-45). As expected, neither HAdV-4 nor -52 E1A interacted with any PKA construct tested. E1A from HAdV-3, -5, -9, -12, and -40 co-immunoprecipitated with WT PKA regulatory subunits but failed to associate with those lacking the D/D domains. Thus, multiple E1As not only resemble AKAPs themselves, but also require the same site on the PKA regulatory subunits that is targeted by cellular AKAPs and the previously characterized HAdV-5 E1A for interaction.

3.3.4 Distinct E1A proteins relocalize PKA regulatory subunits in a type-specific manner

The subcellular redistribution of PKA subunits was previously explored with E1A from HAdV-5 and HAdV-12. Those two proteins were found to have contrasting preferences for the type-I and type-II regulatory subunits, respectively (Fax et al., 2001; King et al., 2016a). Here we sought to extend our investigation into how E1A from different HAdVs affect PKA by utilizing both infection and transfection-based approaches. HeLa cells were transiently transfected with EGFP-tagged WT E1A proteins from all seven HAdV species and proteins were subsequently detected via autofluorescence (EGFP) or immunofluorescence staining (PKA) (Figure 3.4A-C). While PKA Cα appeared nuclear-cytoplasmic and broadly similar in all immunofluorescence experiments (data not shown), notable differences emerged between RIα and RIIα. As expected, neither HAdV-4 nor HAdV-52 E1A, which did not bind PKA, showed any ability to alter the subcellular localization of PKA subunits RIα or RIIα (Figure 3.4A). The relocalization phenotypes for both HAdV-5 (RIα specific) and HAdV-12 E1A (RIIα specific) were reproduced as positive controls. This confirmed that different E1As exhibit a type ‘preference’ for functionally altering the PKA subunits they bind (Figure 3.4B, C). Similar to HAdV-5, E1A from HAdV-9 caused the RIα subunit to be redirected from the cytoplasm into the nucleus (Figure 3.4B). In contrast, E1A from HAdV-3 caused the RIIα subunit to be relocalized, similarly to E1A from HAdV-12 (Figure 3.4C). HAdV-40 E1A did not appear to relocalize either PKA regulatory subunit (Figure 3.4A).

Figure 3.4. Distinct E1A proteins relocalize PKA regulatory subunits in a type-specific manner.

HeLa cells were transfected with EGFP-tagged WT or mutant E1A constructs from 7 species of HAdV. Cells were fixed, permeabilized, and proteins were detected via autofluorescence (E1A) or by immunofluorescence (PKA). Scale bars represent 100μm and the targeted PKA subunit is indicated above each group of panels. A) EGFP alone or E1A from HAdV-4, -52, and -40 showed no relocalization of either PKA regulatory subunit. E1A from HAdV-3 and -12 showed no relocalization of RIα, whereas HAdV-5 and -9 E1A showed no relocalization of RIIα. B) HAdV-5 and -9 E1A caused a shift of RIα from the cytoplasm to the nucleus that was ablated when these E1A proteins had their AKAP-like motifs deleted. Conversely, (C) HAdV-3 and -12 E1A caused a shift of RIIα from the cytoplasm to the nucleus that was ablated when their PKA-binding regions were removed. Quantification of nuclear signal relative to total cellular signal is also shown next to representative images for the indicated E1A species (values are displayed as mean ± SD *$p<0.001$, $n=50$).


A
PKA RIα
PKA
DAPI
MERGE
E1A
vector (GFP)
Ad4 E1A
Ad40 E1A
Ad52 E1A
Ad3 E1A
Ad12 E1A
PKA RIIα
PKA
DAPI
MERGE
E1A
vector (GFP)
Ad4 E1A
Ad40 E1A
Ad52 E1A
Ad5 E1A
Ad9 E1A
B
PKA RIα
PKA
DAPI
MERGE
E1A
Ad5 E1A
Ad5 E1A Δ16-28
Ad9 E1A
Ad9 E1A Δ12-29
vector (GFP)
Ad5 E1A
Ad5 E1AΔ16-28
vector (GFP)
Ad9 E1A
Ad9 E1AΔ12-29
%Nuclear
C
PKA RIIα
PKA
DAPI
MERGE
E1A
Ad3 E1A
Ad3 E1A Δ12-28
Ad12E1A
Ad12 E1AΔΔ
vector (GFP)
Ad3 E1A
Ad3 E1AΔ12-28
vector (GFP)
Ad12 E1A
Ad12 E1AΔΔ
%Nuclear

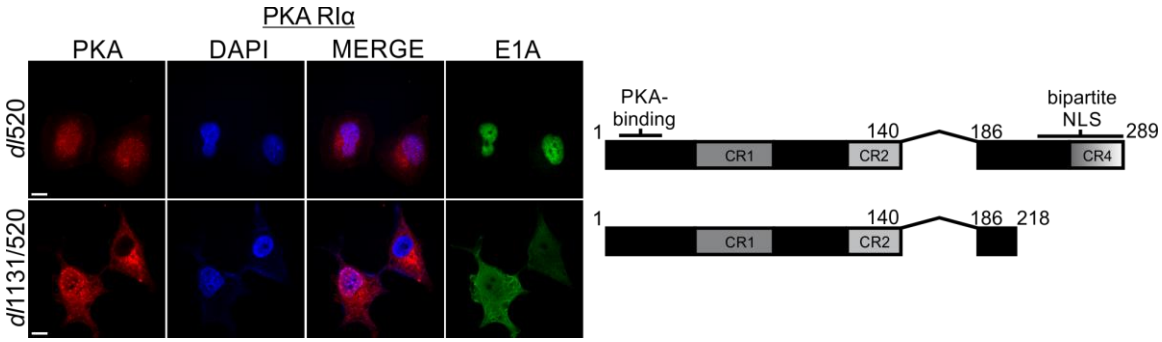


Figure 3.5. The C-terminus of E1A, which contains the nuclear localization signal, is required for efficient retasking of PKA.

A549 cells were infected with either HAdV-5 *dl*520 or *dl*1131/520 (the E1A proteins expressed by these viruses are shown in the adjacent panel). After 24 hours, cells were fixed, permeabilized, and stained for confocal immunofluorescence. E1A lacking the C-terminal bipartite NLS was deficient for both its nuclear localization and recompartmentalization of RIα. Scale bars represent 100μm.

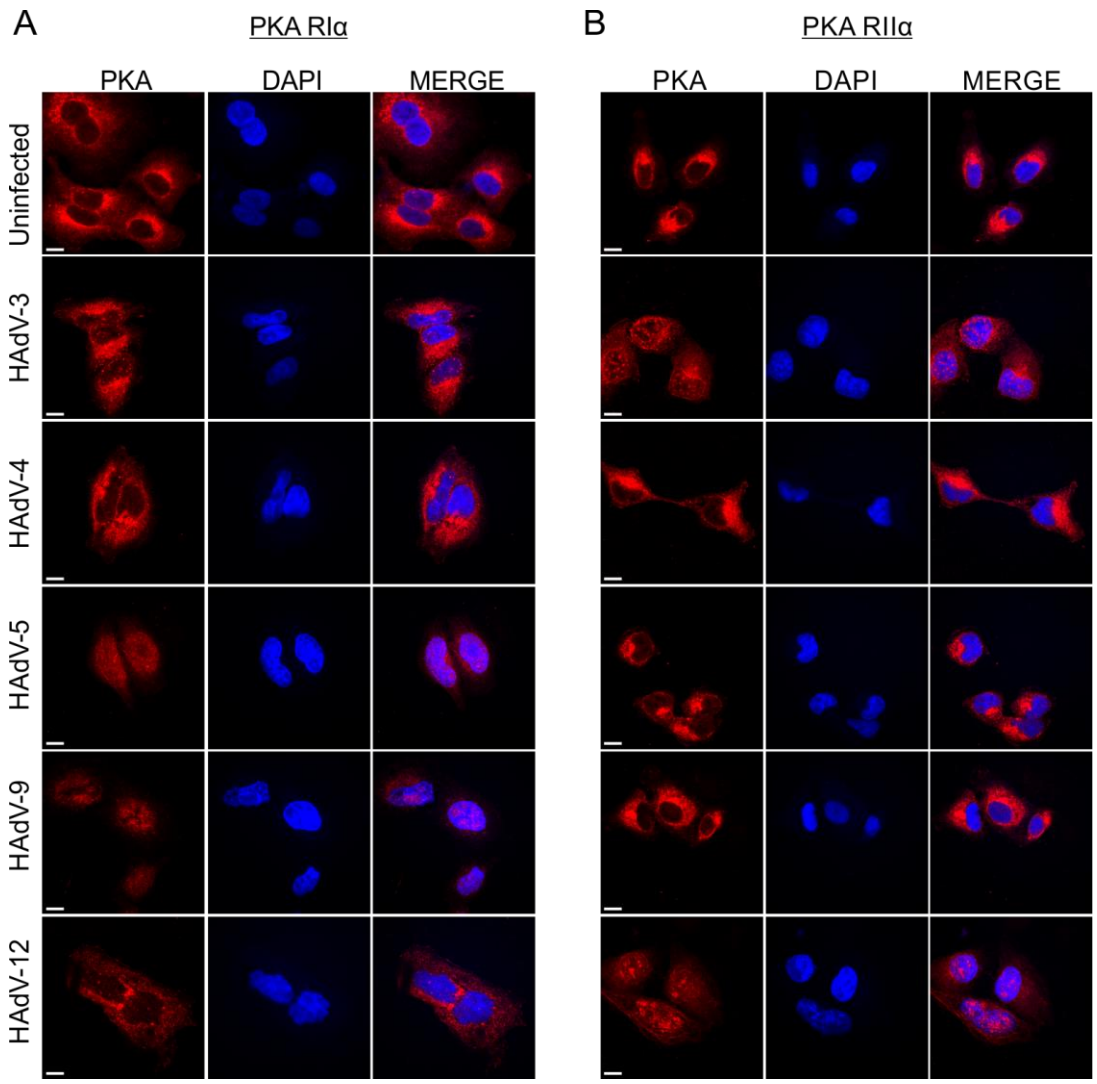


Figure 3.6. Localization of PKA regulatory subunits in cells infected with various HAdV species.

A549 cells were infected with either WT HAdV-3, -4, -5 (*dl*309), -9, or -12 at an MOI of 5 (for HAdV-5) or 25 (for all other viruses). After 24 hours cells were fixed, permeabilized, and stained for confocal immunofluorescence. RIα appears to be more nuclear in HAdV-5 or -9 infected cells compared to uninfected cells. In contrast, RIIα appears more nuclear in HAdV-3 or -12 infected cells. Scale bars represent 100μm.

Mutant E1As containing deletions which abrogate PKA-binding for each species were tested side-by-side with the cognate WT E1A control (Figure 3.4A-C). As expected, the removal of the previously-established binding motifs within each E1A protein abrogated the protein's ability to relocalize PKA in transfected conditions. The relocalization of PKA regulatory subunits was therefore E1A-dependent and required the AKAP-like motif necessary for PKA interaction, as previously demonstrated with HAdV-5 E1A. Using HAdV-5 as a model, we also demonstrated that the nuclear localization signal (NLS) of E1A is needed to contribute to PKA relocalization, but not CR3 (Figure 3.5). While WT "12S" virus (*dl*520) relocalized RIα despite the absence of CR3, a mutant lacking both CR3 and the C-terminal bipartite NLS (*dl*1131/520) was deficient for relocalization of RIα. This suggests that binding of the regulatory subunit by E1A is not sufficient for efficient recompartmentalization of PKA and that relocalization requires cooperation with other functions located in the C-terminus of E1A. PKA localization was also examined in A549 cells infected with WT strains of HAdV-3, -4, -9, and -12 (Figure 3.6). The observed staining phenotype matched that of the transfection-based experiments, suggesting that *in vitro* results faithfully reflect what occurs during an *in vivo* infection.

The curious observation that E1A proteins are able to associate with both RIα and RIIα in Co-IP experiments, yet only relocalize one isoform in vivo led us to test whether differences in binding affinity for distinct subunits contributed to this selectivity. First, we established a Co-IP-based squelching assay where untagged HAdV-5 E1A was titrated in as a competitor against EGFP-tagged HAdV-5 E1A (Figure 3.7A). As expected, higher levels of competition eventually abrogated the ability of EGFP-E1A to Co-IP with PKA regulatory subunits. We then applied these conditions to similarly test HAdV-5 E1A against EGFP-tagged E1A from HAdV-3, -9, -12, and -40 (Figure 3.7B). HAdV-3 and -12 E1As were more resistant to competition from HAdV-5 E1A when examining their associations with RIIα; conversely, their interactions with RIα were more susceptible to depletion in this competition assay. Together these data suggest again that HAdV-3 and -12 have relatively higher affinity for RIIα while HAdV-5 prefers RIα. HAdV-9 E1A was susceptible to competition in a manner similar to how HAdV-5 was

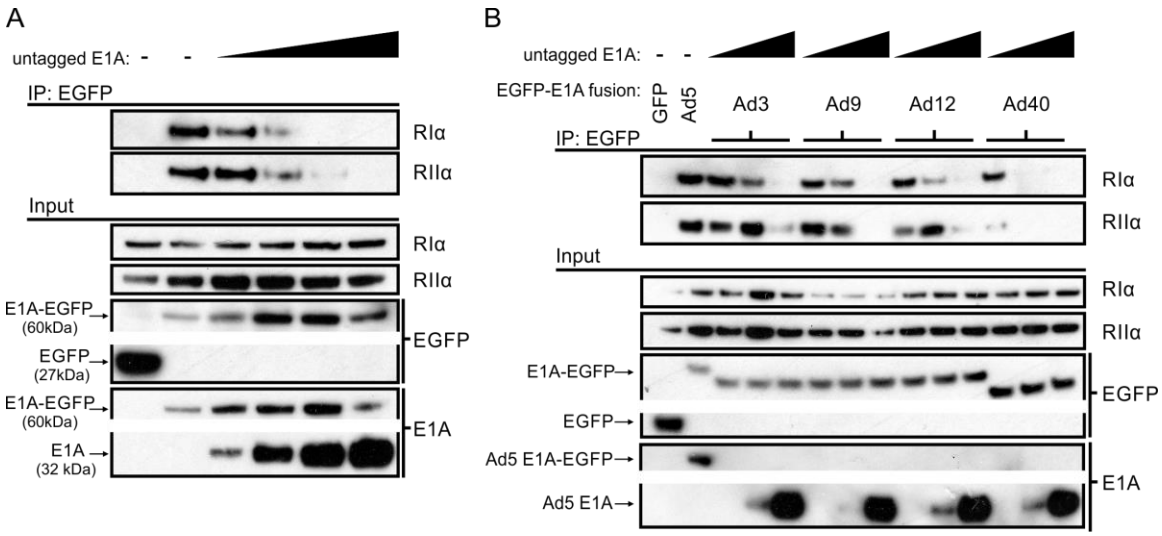


Figure 3.7. Different E1A species display an affinity preference for either RIα or RIIα.

A) HT1080 cells were co-transfected with EGFP-tagged HAdV-5 E1A and PKA regulatory subunits. Untagged HAdV-5 E1A was titrated in as a competitor against EGFP-E1A for binding PKA. Whole cell lysates were harvested for Co-IP using an antibody against EGFP. Increasing amounts of untagged E1A squelched EGFP-E1A's interactions with RIα and RIIα. B) Conditions optimized from (A) were used in a larger competition panel with EGFP-tagged E1A from HAdV-3, -9, -12, and -40. HT1080 cells were similarly co-transfected with the indicated E1A species, PKA subunits, and increasing amounts of untagged HAdV-5 E1A. Co-IP analysis revealed varying resistances and susceptibilities to competition from HAdV-5 E1A for binding either RIα or RIIα.

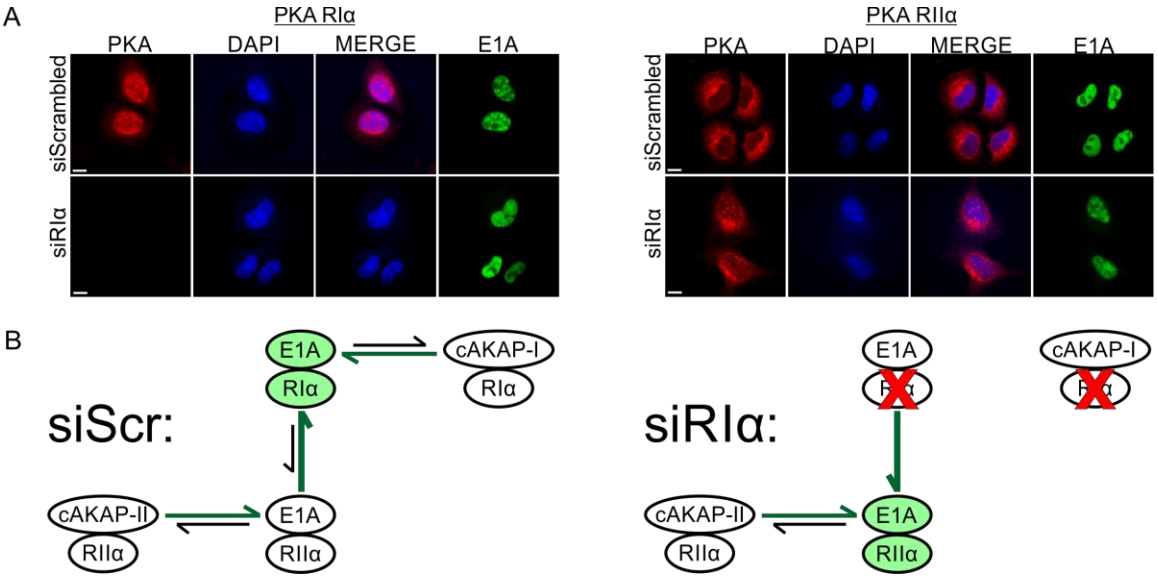


Figure 3.8. Depriving HAdV-5 E1A of type-I PKA causes E1A to retask type-II PKA instead.

A) A549 cells were treated with the indicated control or RIα-specific siRNAs and subsequently infected with WT (*dl*309) HAdV-5. Only in the absence of RIα protein does E1A relocalize some RIIα to the nucleus. Scale bars represent 100µm. B) Equilibrium models of how E1A may compete against cellular AKAPs (cAKAP) and determine which PKA isoform it relocalizes to the nucleus.

against itself (Figure 3.7A) reinforcing the similarity between these two E1A species. HAdV-40 E1A was the most susceptible to competition relative to other E1A proteins indicating that its association with PKA is the weakest.

While E1As clearly have a preference to relocalize and bind one specific PKA isoform, the ability to functionally affect the other isoform remains a possibility. Using HAdV-5 as a model, we infected A549 cells pretreated with either a scrambled control siRNA or siRNA knocking-down RIα, HAdV-5 E1A's preferred regulatory subunit, with WT (*dl*309) virus (Figure 3.8A). In control cells, HAdV-5 E1A behaved as expected by relocalizing RIα into the nucleus while leaving RIIα in the cytoplasm. However, when cells were deprived of RIα protein, some RIIα was localized within the nucleus along with E1A, a finding only observed previously for HAdV-3 and -12 E1A. This suggests the siRNA-mediated removal of the RIα isoform of PKA shifted the binding equilibrium of E1A with RIIα *in vivo*, and that the increased interaction was sufficient to allow some of this isoform to be relocalized (Figure 3.8B). While the observed recompartmentalization was not quite as dramatic as with RIα, this may be due to HAdV-5 E1A's apparent lower affinity for this particular subunit.

3.3.5 E1A's ability to act as a vAKAP is modular and malleable by mutagenesis

To test if the 'viral AKAP' function of E1A was modular, we created a set of EGFP-tagged constructs that combined various portions of E1A from HAdV-4 and HAdV-5 to form chimeric proteins. Specifically, the N-terminal domains of each protein were swapped for the analogous region in the other. When these constructs were tested alongside WT counterparts for binding PKA subunits via Co-IP, the chimera with the HAdV-5 N-terminus and HAdV-4 C-terminus gained the protein-protein interactions exhibited by WT HAdV-5 E1A, whereas the reciprocal chimera lost them (Figure 3.9A). Subsequently, these chimeras were transfected into HeLa cells and PKA subcellular localization was examined via immunofluorescence staining (Figure 3.9B). As with the Co-IP assays, the HAdV-4 chimera gained the ability to relocalize the PKA RIα subunit when its N-terminus was replaced with that from HAdV-5. In contrast, the HAdV-5

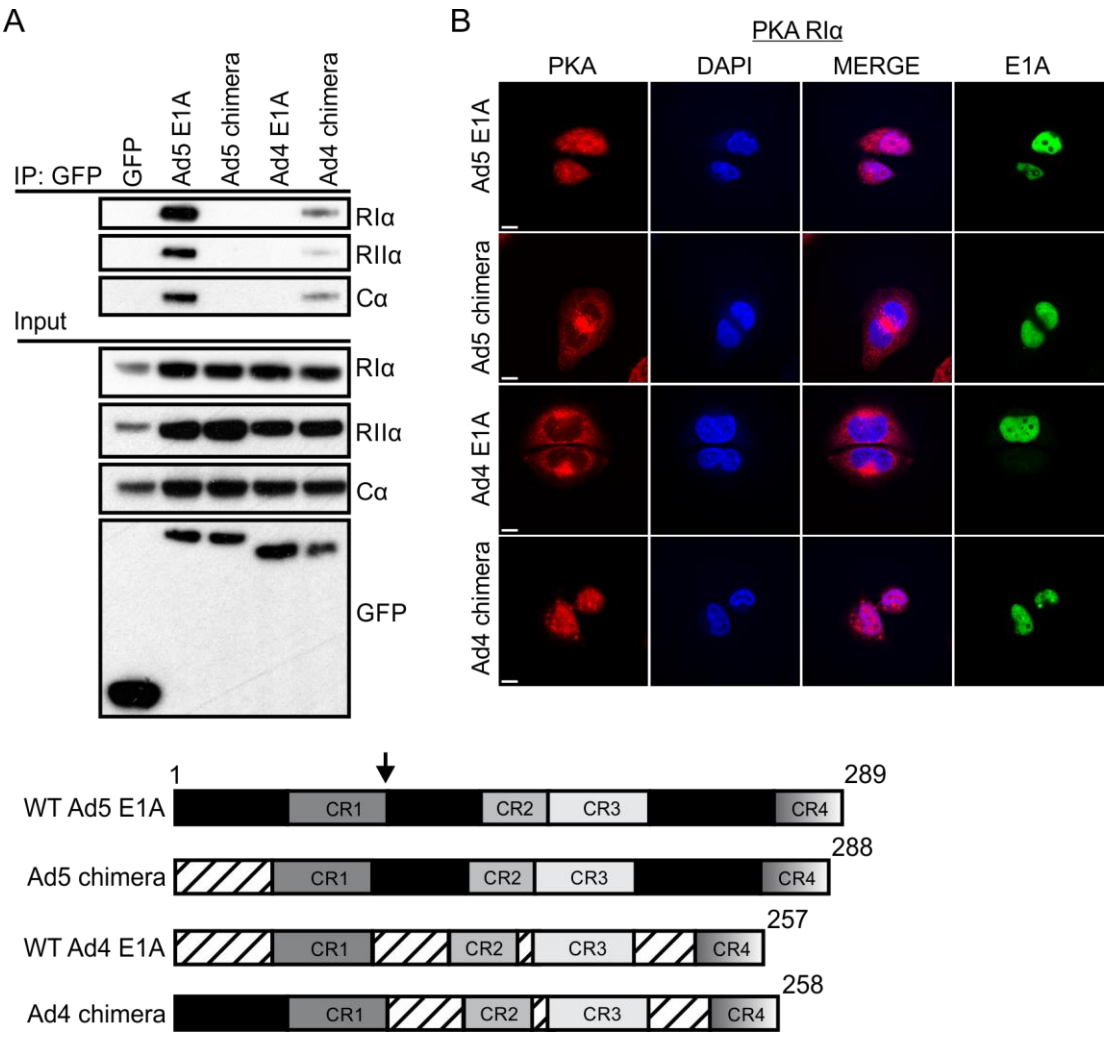
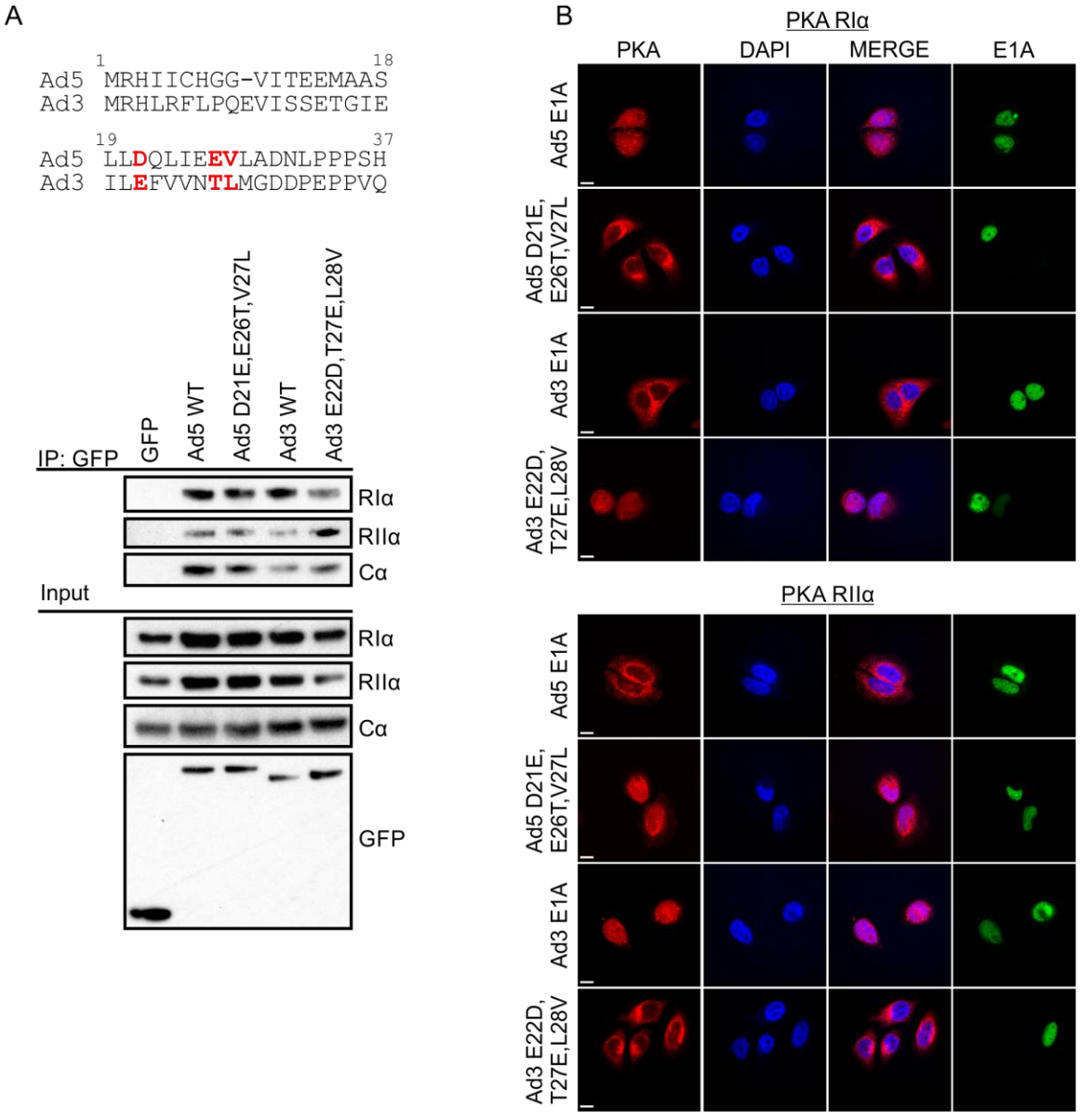


Figure 3.9. Viral AKAP mimicry is a modular function of E1A.

A) HT1080 cells were co-transfected with PKA subunits and either WT or chimeric E1A constructs and cell lysates were harvested for Co-IP. The inset panel displays the construction of the HAdV-4 and -5 E1A chimeras (arrow indicates where N-terminal swap occurred). The HAdV-4 chimera gained the ability to bind PKA subunits whereas this was lost for the HAdV-5 chimera. B) HeLa cells were transfected with the indicated EGFP-tagged E1A constructs. Cells were fixed, permeabilized, and proteins were detected via autofluorescence (E1A) or by immunofluorescence (PKA). Cells transfected with WT HAdV-5 or the HAdV-4 chimera exhibited a shift of RIα from the cytoplasm into the nucleus. In contrast, WT HAdV-4 or the HAdV-5 chimera showed no noticeable relocalization of RIα. Scale bars represent 100μm.

Figure 3.10. Specific residues in the AKAP-like motif of E1As induce preference for specific PKA subunits.

A) HT1080 cells were co-transfected with PKA subunits and the indicated E1A constructs and cell lysates were harvested for Co-IP. Both WT HAdV-3 and -5 along with mutants (HAdV-5 D21E, E26T, V27L and HAdV-3 E22D, T27E, L28V) retain similar levels of binding to PKA. The mutation scheme in the inset panel shows residue positions previously-demonstrated as crucial in HAdV-5 along with the analogous positions in HAdV-3. B) HeLa cells were transfected with the indicated EGFP-tagged E1A constructs. Cells were fixed, permeabilized, and proteins were detected via autofluorescence (E1A) or by immunofluorescence (PKA). Cells transfected with WT HAdV-3 or the HAdV-5 D21E, E26T, V27L mutant exhibited a shift of RIIα from the cytoplasm into the nucleus. In contrast, WT HAdV-5 or the HAdV-3 E22D, T27E, L28V mutant exhibited a relocalization of RIα. Scale bars represent 100μm.


A
1 18
Ad5 MRHIICHGG-VITEEMAAS
Ad3 MRHLRFLPQEVISSETGIE
19 37
Ad5 LLDQLIEEVLADNLPPPSH
Ad3 ILEFVVNTLMGDDPEPPVQ
GFP
Ad5 WT
Ad5 D21E,E26T,V27L
Ad3 WT
Ad3 E22D,T27E,L28V
IP: GFP
RIα
RIIα
Cα
Input
RIα
RIIα
Cα
GFP
B
PKA RIα
PKA
DAPI
MERGE
E1A
Ad5 E1A
Ad5 D21E, E26T,V27L
Ad3 E1A
Ad3 E22D, T27E,L28V
PKA RIIα
Ad5 E1A
Ad5 D21E, E26T,V27L
Ad3 E1A
Ad3 E22D, T27E,L28V

chimera with the HAdV-4 N-terminus behaved like the EGFP negative control and WT HAdV-4 E1A.

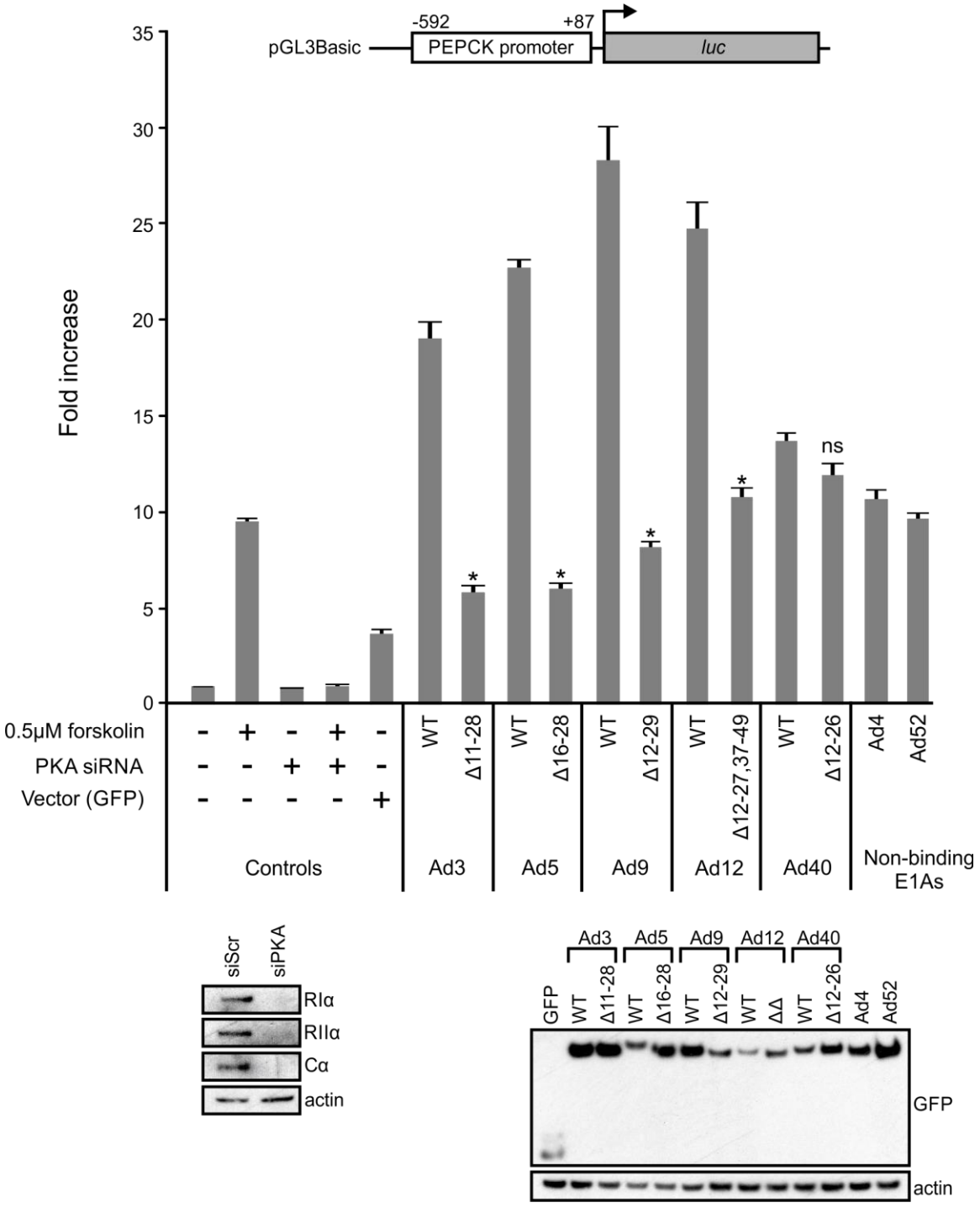
To explore this concept of modularity further, we aimed to test if swapping individual residues within the AKAP-like motif of different E1As could alter their preference for relocalizing one type of PKA subunit over the other. By comparing the sequences of the AKAP-like motifs between HAdV-3 and HAdV-5 E1A, triple point mutants for both E1As were designed (HAdV-5 D21E, E26T, V27L and HAdV-3 E22D, T27E, L28V) in an effort to exchange specificity. These relatively conservative mutations are embedded within the predicted α-helix portions of both proteins and are at analogous positions that were previously demonstrated to be crucial for HAdV-5 E1A binding (King et al., 2016a). When tested alongside WT E1A counterparts for binding via Co-IP, both mutants retained the interaction with PKA subunits similar to that of WT E1A (Figure 3.10A). Next, the mutants were transfected into HeLa cells and examined for their ability to relocalize individual PKA subunits (Figure 3.10B). Both triple mutants experienced a change in preference for which PKA subunit they relocalized to the nucleus. When mutated in this way, HAdV-5 E1A, which previously preferred RIα, now altered RIIα distribution like WT E1A from HAdV-3 and -12. When HAdV-3 E1A, which normally preferred RIIα, was mutated in this fashion, it relocalized RIα like WT E1A from HAdV-5 or -9. Thus, it appeared that the preference of E1As for specific PKA subunits can be altered using targeted mutagenesis of select residues.

3.3.6 Interaction with PKA is required for full E1A-mediated transactivation of a PKA-driven promoter

It was previously shown that the interaction between E1A and PKA could serve to enhance viral gene expression during HAdV-5 infection (King et al., 2016a). Additionally, co-transfected HAdV-12 E1A and PKA could activate the HAdV-12 E2 promoter (Fax et al., 2001). However, many cellular genes are also known to be regulated by PKA via signaling through cAMP (Guo et al., 2012; Sands and Palmer, 2008; Zambon et al., 2005; Zhang et al., 2005). Also, E1A has been demonstrated to affect cAMP-responsive cellular genes in certain contexts (Gedrich et al., 1992; Lee and

Figure 3.11. Interaction with PKA is required for full E1A-mediated transactivation of a PKA-driven promoter.

HT1080 cells were transfected with a reporter consisting of the luciferase gene driven by the rat PEPCK promoter (sequence shown in inset panel) along with various WT or mutant E1A constructs from 7 HAdV species. A 6-hour treatment of 0.5μM forskolin (fsk) and siRNA-mediated PKA knockdowns were performed to demonstrate this reporter's cAMP-responsiveness and PKA-dependent regulation. Protein levels of each E1A construct are also shown. Luciferase activity was measured for each condition and light units were set relative to an empty plasmid control. Each of the 7 WT E1As tested could transactivate this reporter, with those shown to bind PKA having the strongest effect. For the 5 species that interacted with PKA, deletion of their PKA-binding motifs conferred a statistically-significant reduction on E1A's ability to induce this reporter (with the exception of HAdV-40). Values are displayed as displayed as mean ± S.E.M. *$p<0.05$, $n=3$.


pGL3Basic
-592
+87
PEPCK promoter
luc
Fold increase
35
30
25
20
15
10
5
0
ns
*
0.5μM forskolin
PKA siRNA
Vector (GFP)
WT
Δ11-28
WT
Δ16-28
WT
Δ12-29
WT
Δ12-27,37-49
WT
Δ12-26
Ad4
Ad52
Controls
Ad3
Ad5
Ad9
Ad12
Ad40
Non-binding E1As
siScr
siPKA
RIα
RIIα
Cα
actin
Ad3
Ad5
Ad9
Ad12
Ad40
GFP
WT
Δ11-28
WT
Δ16-28
WT
Δ12-29
WT
ΔΔ
WT
Δ12-26
Ad4
Ad52
GFP
actin

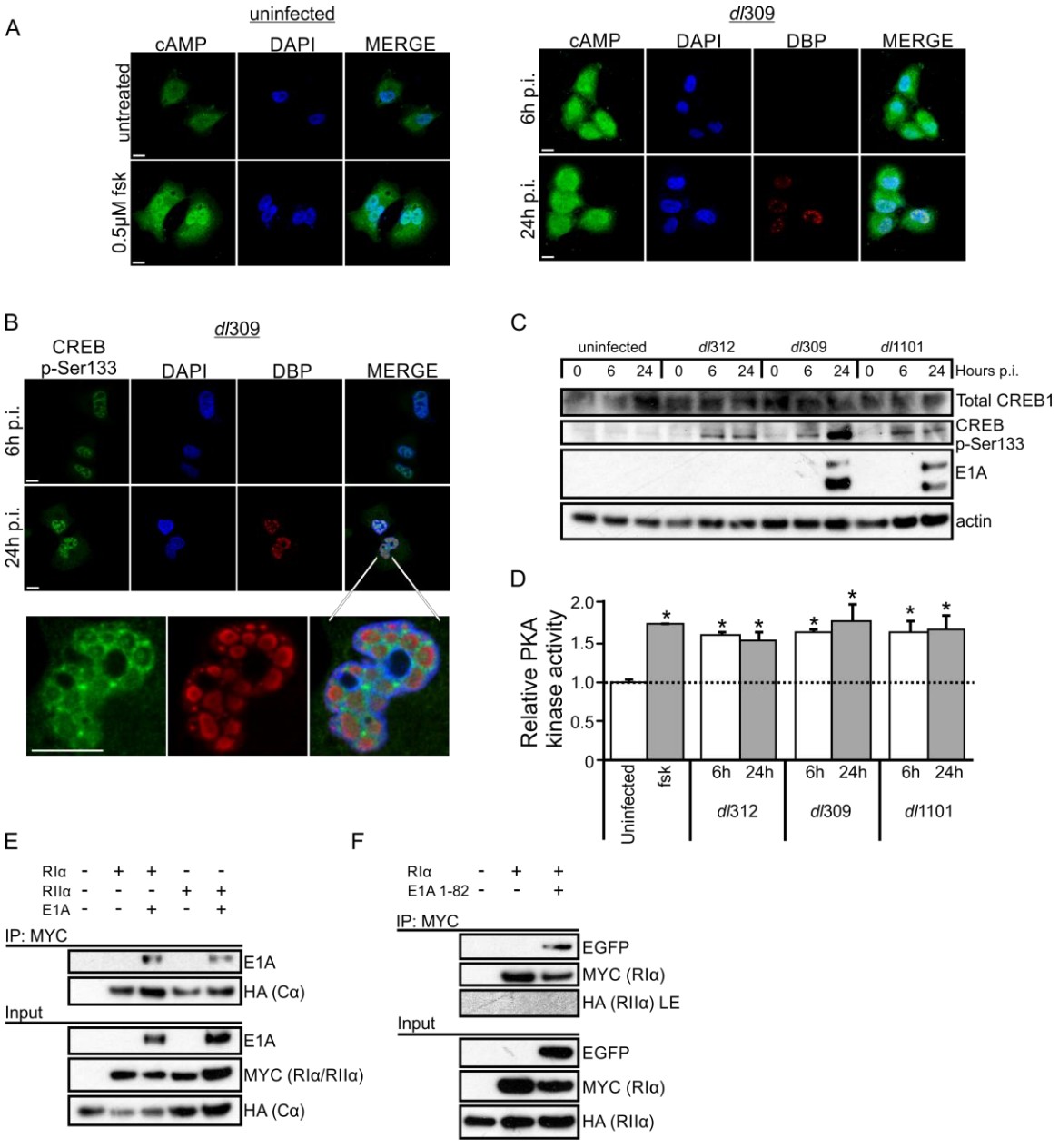
Mathews, 1997; Miller et al., 1995). We aimed to determine if the E1A proteins from different HAdVs could insert themselves into this signaling pathway and alter PKA/cAMP-regulated transactivation. HT1080 cells were co-transfected with a luciferase reporter demonstrated to be cAMP- and PKA-regulated (driven by the rat phosphoenolpyruvate carboxykinase [PEPCK] promoter) along with various E1A constructs (Figure 3.11). Initial experiments confirmed that this reporter is both cAMP-inducible via forskolin treatment (an adenylyl cyclase activator that increases intracellular levels of cAMP), as well as dependent on PKA protein as shown by siRNA-mediated knockdown. E1As shown to bind PKA were then compared to a species-specific deletion mutant lacking the PKA binding site for their effects on PEPCK-dependent transcription. Although all seven species of E1A were able to transactivate this reporter to levels higher than the EGFP (vector control) alone, those previously shown to both bind and relocalize PKA functioned as the strongest transactivators (HAdV-3, -5, -9, -12). Deletion of the AKAP-like PKA-binding motifs in these E1As reduced their ability to drive expression of this reporter construct several-fold. Transactivation by HAdV-40 E1A was weaker relative to the other E1As and deletion of its putative PKA-binding region did not affect this function. Thus, similarly to the negative result in the relocalization assays (Figure 3.4A), HAdV-40 appears to bind PKA in the Co-IP assays, but this interaction does not appear to functionally impact PKA-dependent transcription in vivo.

3.3.7 HAdV infection stimulates the cAMP pathway

Using HAdV-5 as a model, we further investigated how HAdV infection and E1A expression may cooperate with cellular cAMP signalling. A549 cells infected with either WT (*dl*309), ΔE1A (*dl*312), or E1AΔ4-25 (*dl*1101) all showed increased levels of cellular cAMP over uninfected cells as visualized by immunofluorescence (WT virus is shown as representative) (Figure 3.12A). This was accompanied by higher levels of phosphorylated CREB1 on Ser133 (a known PKA substrate) (Figures 12B, C) and higher levels of PKA kinase activity (Figure 12D). Each of these findings was examined at timepoints before and after the onset of E1A protein production. Notably, levels of PKA kinase activity and cAMP were similar across all timepoints post-infection for all viruses, indicating that they are independent of E1A and more likely a biological response to the

Figure 3.12. HAdV-mediated stimulation of cAMP signalling allows WT E1A to retarget PKA activity.

A549 cells were infected with either WT (*dl*309), ΔE1A (*dl*312), or E1AΔ4-25 (*dl*1101) HAdV-5 (MOI 5) and examined at the indicated timepoints post-infection using confocal immunofluorescence (A, B), western blot (C), or kinase assay (D). A) Cellular cAMP levels were examined before and after the onset of viral replication (as indicated by staining for HAdV-5 ssDNA-binding protein [DBP]). Production of cAMP is noticeably higher relative to uninfected cells both before and after E1A is expressed. Results with WT virus are shown as representative of all infected conditions. B) Increased levels of phosphorylated CREB1 on Ser133 are present relative to uninfected cells and are shown for WT virus, localizing adjacent to and overlapping with virus replication centres. Scale bars represent 100μm. C) Western blot analysis of phosphorylated CREB1 detected increased levels in all infected conditions, which was highest in WT-infected cells after production of E1A protein. D) Cell lysates were measured for PKA kinase activity, and displayed similarly higher levels of activity for all infected conditions. Values are displayed as mean ± S.E.M. *$p<0.05$, n=3. E) HT1080 cells were co-transfected with PKA regulatory and catalytic subunits along with HAdV-5 E1A. Co-IP analysis showed that E1A does not dissociate the PKA holoenzyme. F) HT1080 cells were co-transfected with RIα and/or RIIα in the presence or absence of the HAdV-5 E1A N-terminus and subjected to Co-IP. No heterodimerization of PKA regulatory subunits was detectable even with a long exposure (LE) western blot.


A
uninfected
cAMP
DAPI
MERGE
untreated
0.5μM fsk
dl309
cAMP
DAPI
DBP
MERGE
6h p.i.
24h p.i.
B
dl309
CREB p-Ser133
DAPI
DBP
MERGE
6h p.i.
24h p.i.
C
uninfected
dl312
dl309
dl1101
0 6 24 0 6 24 0 6 24 0 6 24 Hours p.i.
Total CREB1
CREB p-Ser133
E1A
actin
D
Relative PKA kinase activity
2.0
1.5
1.0
0.5
0
Uninfected
fsk
6h 24h
dl312
6h 24h
dl309
6h 24h
dl1101
E
RIα - + + - -
RIIα - - - + +
E1A - - + - +
IP: MYC
E1A
HA (Cα)
Input
E1A
MYC (RIα/RIIα)
HA (Cα)
F
RIα - + +
E1A 1-82 - - +
IP: MYC
EGFP
MYC (RIα)
HA (RIIα) LE
Input
EGFP
MYC (RIα)
HA (RIIα)

infection itself. We also showed that E1A itself is not causing aberrant interactions between PKA subunits (Figures 3.12E, F). The coexpression of E1A did not inhibit the association between regulatory and catalytic PKA components (consistent with what we know of where E1A and PKA interact with each other) nor did its N-terminus induce any noticeable heterodimerization of type-I and -II regulatory subunits. The most striking observation from these experiments was that only in WT-infected cells and after the onset of E1A protein expression did we observe the highest levels of phosphorylated CREB1 on Ser133. Given that the cAMP and kinase activity levels are similar between cells expressing either WT E1A or a mutant unable to bind PKA, it is likely the increased phosphorylation of this PKA-targeted transcription factor is due to WT E1A's vAKAP function. In these conditions, high levels of phosphorylated CREB1 were localized immediately adjacent to and overlapping with virus replication centres (Figure 3.12B).

3.3.8 Requirement of PKA for viral replication is conserved across numerous HAdV species

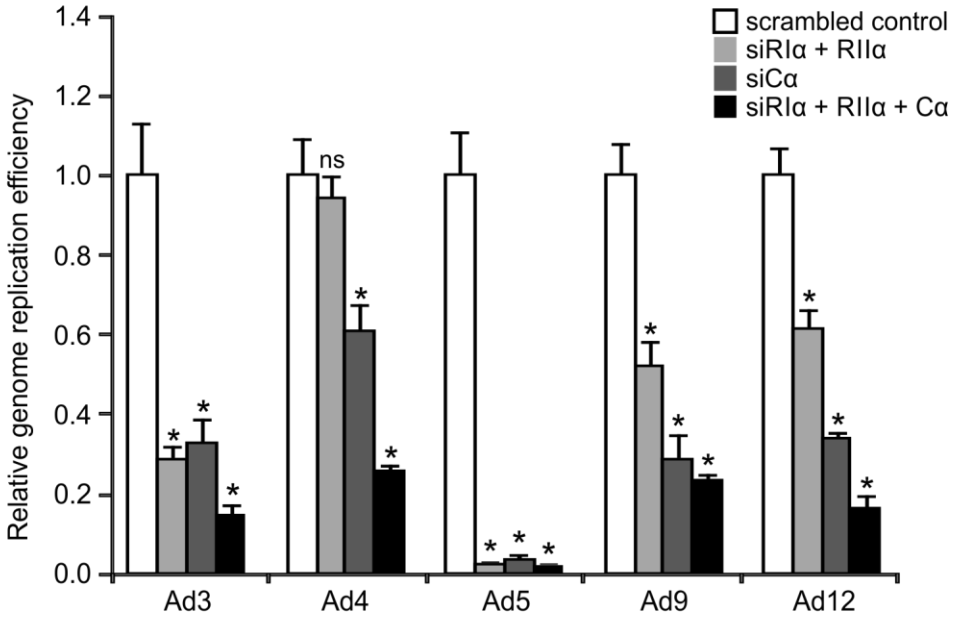
To firmly establish the biological significance of these distinct E1As as vAKAPs, we assessed PKA's role in multiple steps of the viral replicative cycle. A549 cells were treated with either control siRNA or siRNA specific for the indicated PKA subunits and infected with WT HAdV-3, -4, -9, -12 (MOI 25) or HAdV-5 (MOI 5) (Figure 3.13A, B). The efficiency of viral genome replication was assessed by qPCR of genomic viral DNA at 48 hours post-infection compared to an "input" sample at 6 hours post-infection and normalized to cellular GAPDH (Figure 3.13A). For HAdV-3, -5, -9, and -12, knockdown of the PKA regulatory subunits resulted in an inhibition of viral DNA replication. This was not observed for HAdV-4, whose E1A does not bind PKA. This suggests that viruses that bind and relocalize PKA benefit from the interaction and require it for full, WT-levels of DNA replication. Knockdown of the catalytic subunit of PKA resulted in an inhibition for all viruses, including HAdV-4. This may be due to an additional role for this protein in the HAdV replicative cycle that is Cα-specific and E1A-independent.

Production of infectious progeny virus was assayed at 72 hours post-infection by plaque assay (Figure 3.13B). The production of WT progeny for each HAdV serotype tested

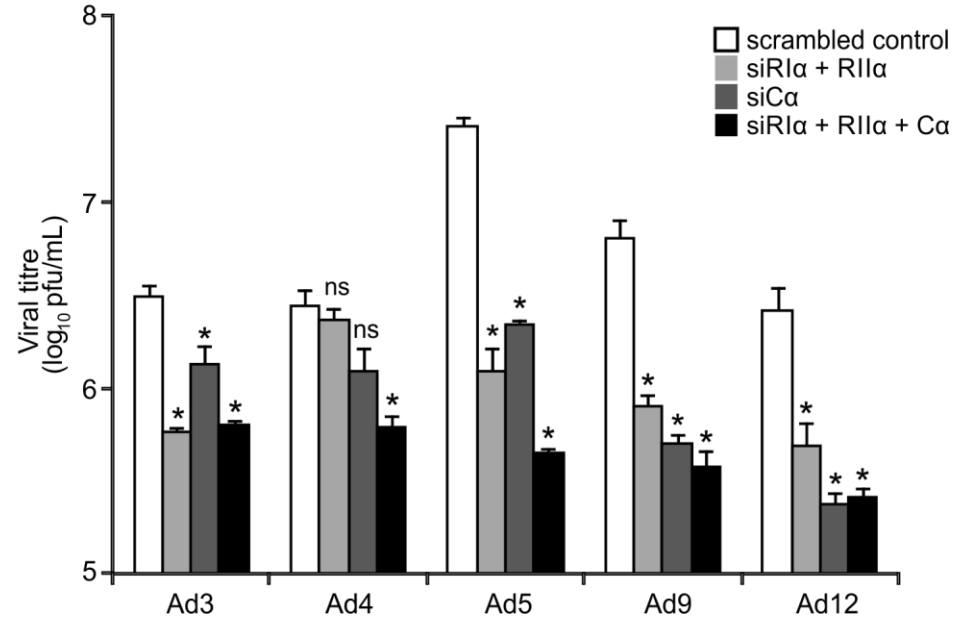
Figure 3.13. Requirement of PKA for viral replication is conserved across numerous HAdV species.

A549 cells were treated with control siRNA or siRNA specific for PKA subunits and infected with either HAdV-5 (MOI 5) or HAdV-3, -4, -9, or -12 (MOI 25). Values for each virus were normalized to 1.0 in control-treated cells and displayed as mean ± S.E.M., n = 3. A) DNA was isolated at 6 hours post-infection as a gauge of viral input and at 48 hours post-infection to measure viral genome replication. Relative viral DNA levels were quantified by qPCR reactions using a forward primer that recognizes a conserved sequence in E1A in combination with a species-specific reverse primer. B) Cells were collected at 72 hours post-infection and production of infectious progeny virus was quantitatively assayed by plaque formation on HEK293 cells. For both genome replication and progeny production, knockdown of PKA regulatory subunits caused impairment for HAdV-3, -5, -9, and -12. The non-PKA binding HAdV-4 was unaffected. Knockdown of either the catalytic subunit or all 3 PKA components together impaired all viruses. *$p<0.05$, n=3.

A


scrambled control
siRIα + RIIα
siCα
siRIα + RIIα + Cα
Relative genome replication efficiency
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
ns
*
Ad3
Ad4
Ad5
Ad9
Ad12

B


scrambled control
siRIα + RIIα
siCα
siRIα + RIIα + Cα
Viral titre
(log10 pfu/mL)
8
7
6
5
ns
*
Ad3
Ad4
Ad5
Ad9
Ad12

mirrored the observed phenotypes for genome replication. Only HAdV-4 was unaffected by the knockdown of PKA regulatory subunits, confirming that viruses that target PKA via E1A do so as part of an important aspect of their replicative cycles. As observed for viral genome replication, knocking down both the regulatory and catalytic subunits in combination did affect all HAdVs tested. This finding matches previous observations for HAdV-5 (Scherer et al., 2014; Suomalainen et al., 2001) and again suggests the influence of an alternative, E1A-independent role for the Cα protein in HAdV biology.

3.4 Discussion

Mimicry is an often-exploited strategy utilized by numerous protein products of HAdV to facilitate virus-host interactions (Avgousti et al., 2016; Chahal et al., 2012; King et al., 2016a, 2016b). HAdV E1A in particular is a remarkably well-connected viral hub protein capable of altering the protein interaction network in an infected cell to create an environment conducive for viral replication (Frisch and Mymryk, 2002; Pelka et al., 2008). By embedding itself deeply within numerous signalling pathways, E1A can exert tremendous influence upon cellular machinery in order to enhance various aspects of the HAdV life cycle. We previously demonstrated that HAdV-5 E1A uses mimicry to act as a viral AKAP, facilitating an interaction with PKA that increased viral transcription, protein synthesis, and progeny production (King et al., 2016a). In the present work, we have determined that despite some mechanistic flexibility, the manipulation of PKA via E1A is a conserved and important function for numerous, distinct species of HAdV.

Even with having substantial differences in primary sequence, E1A proteins are often remarkably consistent in their functional similarities (Marshall et al., 2014). Our findings establish that 5 representative members of the 7 species of HAdV express E1A proteins that make protein-protein interactions with PKA. This apparent conservation suggests that the E1A-PKA interaction has played a potentially important role in the evolutionary history of the virus. Indeed, the two HAdVs which don’t bind PKA via E1A (HAdV-4 from species E and HAdV-52 from species G), along with HAdV-40 from species F (which bound with lower affinity relative to other species and didn’t functionally affect PKA) are considered more closely related to simian adenoviruses than the HAdVs that

have E1A which demonstrably function as viral AKAPs (HAdV-3, -5, -9, and -12) (Dehghan et al., 2013; Jones et al., 2007). Along with lacking vAKAP functionality, these non-PKA-binding E1As also have more dramatic differences in both primary sequence and predicted secondary structure in the region responsible for the interaction compared to the other HAdV types (Pelka et al., 2008). In particular, HAdV-52 E1A contains a single amino acid insertion in the core of this motif, which likely distorts the amphipathic nature of the predicted helix. Among primate species, PKA is a well-conserved kinase (Taylor et al., 2013) and it is unknown if closely-related simian adenovirus E1A proteins are capable of interacting with simian PKA. However, we speculate that an interaction is unlikely given the lack of the requisite predicted amphipathic α-helical structure in the E1A N-terminus of these viruses. Therefore, the capability of some HAdV E1As to function as vAKAPs likely arose from their extended coevolution with human as a result of providing a selective benefit for the virus within this particular host.

For all viruses that did bind PKA, the N-terminus of E1A alone is both necessary and sufficient for this interaction, as previously observed within HAdV-5. These E1As also target the D/D domains of PKA regulatory subunits as the binding interface in the same manner as cellular AKAPs (Banky et al., 1998; Newlon et al., 1999). Furthermore, an AKAP-like motif in the E1A N-terminus containing a putative α-helical structure is responsible or at least contributory to this interaction. E1A proteins from HAdV-3, -5, and -9 all contain this motif, which strongly resembles the PKA-binding region of cellular AKAPs. This similarity indicates strong mechanistic conservation. HAdV-12 E1A also contains such a motif, but also utilizes a second, downstream region contributing to this E1A-PKA interaction. This finding confirms the previously unexplored observation that HAdV-12 E1A bound PKA via two different N-terminal regions (Fax et al., 2001). While recent studies of cellular AKAPs have revealed the ever-increasing diversity of PKA-binding motifs (Burgers et al., 2015), this secondary region bears no obvious similarity to the canonical α-helical domain utilized by cellular AKAPs. It is possible that the largely unstructured nature of E1A allows for a second, novel binding site to form in solution, which strengthens PKA-binding, a feature observed with other targets of E1A (Ferreon et al., 2013). This finding also reveals that

viral protein sequences outside canonical AKAP mimics may be capable of regulating PKA in both normal and infected conditions.

During infection, multiple HAdV species induced a subset of PKA regulatory subunits to relocalize from the cytoplasm to the nucleus. Transfection-based experiments using a range of E1A deletion mutants for each HAdV species confirmed that this phenotype was dependent on the PKA-binding motifs of E1A. Different E1As had differing effects on specific PKA regulatory subunit types. While E1A from HAdV-3, -5, -9, -12, and -40 could all bind PKA in separate Co-IP experiments, HAdV-3 and -12 E1A specifically relocalized the RIIα subunit, whereas HAdV-5 and -9 E1A preferred the RIα subunit. HAdV-40 E1A does not display any ability to relocalize PKA, suggesting that the interaction may not be functionally relevant *in vivo*. The relative differences in binding affinities between E1A species and PKA isoforms was confirmed in competition assays, reinforcing the idea of type-preference. These data are consistent with previous reports on E1A-PKA interactions for HAdV-5 and -12 (Fax et al., 2001; King et al., 2016a) and a type-preference is also a feature of many cellular AKAPs (Gold et al., 2006). This is also the first report of both HAdV-3 and -9 E1A acting as 'viral AKAPs' and explores the molecular determinants of this conserved E1A function in the greatest detail to-date.

Our established viral AKAP model suggests that E1A uses the direct interaction with PKA regulatory subunits as a bridge to associate with the catalytic subunit, Cα (King et al., 2016a). Cα was previously shown to be recruited to the promoters of E1A-regulated viral genes and was capable of enhancing transcription from these sites (King et al., 2016a). It is unclear if the preference for either RIα or RIIα confers the same, or different benefits and this could also be context dependent based on cell or tissue-specific expression levels. The ability to target both regulatory types may also serve as an evolutionary redundancy ensuring the crucial interaction with PKA's catalytic component regardless of the availability of individual PKA subunits. To explore this, we deprived cells of RIα protein using siRNA then infected with HAdV-5. In these conditions, where the preferred isoform for HAdV-5 is absent, some relocalization of RIIα occurred. Although the degree of relocalization was reduced, this may be related to HAdV-5's relatively lower binding affinity for RIIα. Nevertheless, this result suggests that the

potential to bind both type-I and -II PKA to at least some extent could be advantageous to HAdV as they may, to a degree, compensate for each other.

While the hydrophobic component of the PKA-binding amphipathic α-helix is a necessary feature in both cellular and viral AKAPs (Gold et al., 2006; King et al., 2016a), adjacent residues confer critical electrostatic interactions and contribute to the preference for either RIα or RIIα (Burgers et al., 2015). We explored this feature in the context of HAdV E1A by successfully creating mutants in HAdV-3 and -5 E1A that reversed each protein's preference for the different PKA regulatory subunits. Using previously-validated residue positions in HAdV-5 E1A that are known to affect PKA-E1A interaction (King et al., 2016a), triple point mutants were created in both proteins that exchanged these residues in their analogous positions. Both the novel HAdV-3 and -5 mutants retained binding to both types of PKA subunits in Co-IP experiments, but their abilities to relocalize RIα and RIIα were reversed. This demonstrates that like cellular AKAPs, individual amino acids in viral AKAPs specify their differential affinities for distinct PKA subunits, enhancing our knowledge of how type-I, type-II, and dual-specificity AKAPs function.

The targeting of PKA by E1A was previously shown to drive transcription of HAdV-5 early genes via recruitment of Cα to viral promoters (King et al., 2016a). E1A has also been shown to synergize with cAMP (Engel et al., 1991, 1988; Hardy and Shenk, 1988; Lee and Mathews, 1997; Müller et al., 1989). Here we sought to expand upon this mechanism by exploring E1A-PKA interactions for various HAdV species in the context of the cAMP signaling pathway. A luciferase reporter driven by the previously-characterized rat PEPCK promoter (Klemm et al., 1996) was constructed and validated to be both cAMP-inducible and PKA-dependent. E1A proteins from all 7 species of HAdV could activate expression from this reporter, which was expected given E1A has multiple transactivation domains and mechanisms for inducing transcription (Ablack et al., 2012; Bondesson et al., 1994; Duyndam et al., 1996; Pelka et al., 2008; Wong and Ziff, 1994; Yousef et al., 2009). The E1A proteins from viruses that could bind and relocalize PKA subunits (HAdV-3, -5, -9, -12) induced this reporter to the greatest extent. Deletion of the PKA-binding regions of these E1As severely handicapped their ability to

transactivate this construct. Residual activation by these mutants was comparable to the levels observed for WT E1A species unable to bind PKA. This confirms that the PKA interaction contributes to E1A-mediated transactivation of this PKA-responsive reporter.

During HAdV-5 infection, notable alterations to the cellular cAMP signalling pathway occurred. Indeed, increased levels of cAMP relative to uninfected cells were observed by immunofluorescence, as were higher levels of PKA kinase activity and phosphorylation of CREB1 on Ser133. Most of these observations were consistent across timepoints before and after the production of E1A protein as well as between different HAdV-5 mutants (WT, ΔE1A, and a mutant incapable of binding PKA). This indicates that the stimulation of cAMP signalling is in response to the HAdV infection and independent of E1A. The notable exception to this trend was that in the presence of WT E1A protein, a far higher level of phosphorylated CREB1 was observed both by western blot and immunofluoresence – where it localized adjacent to and overlapping with virus replication centres. These findings are consistent with our previously-established vAKAP model suggesting that E1A exerts its effects on the cAMP pathway not through broad changes in 2nd messenger levels or PKA catalytic activity, but rather via the localized retasking of PKA to sites of action. While HAdV infection may provoke cAMP signalling, a functional vAKAP is needed to fully take advantage of these conditions. Notably, all E1A proteins tested also bind CBP, an important co-regulator of CREB via an overlapping region of the N-terminus and/or CR3, providing an additional connection between E1A and cAMP regulated transcriptional control (Pelka et al., 2009; Rasti et al., 2005; Shuen et al., 2003).

Altogether, these data suggest that E1A proteins can integrate into the cAMP signaling pathway to drive higher levels of cAMP-modulated gene expression, presumably in a localized fashion. This also suggests the exciting possibility that E1A may be capable of differentially regulating the hundreds of cellular cAMP-responsive genes during infection. This mechanism could contribute to enhancing the infectious cycle for HAdVs that have a functional E1A-PKA interaction or contribute to the different tropisms and disease pathogeneses of distinct HAdV species.

The conserved importance of PKA during infection was established by examining viral genome replication and progeny production in the context of siRNA-mediated PKA knockdowns for multiple HAdV types. For HAdV-4, which does not bind PKA, knockdown of RIα and RIIα did not notably affect either viral DNA replication or progeny virion production. For HAdV-3, -5, -9, and -12, which all have viral AKAPs, the loss of PKA regulatory subunits as targets for E1A reduced the efficiency and productivity of their replicative cycles. As previously established for HAdV-5, the E1A-PKA interaction serves to drive viral gene expression of numerous HAdV genes including E2, E3, and E4 (King et al., 2016a). Many transcripts from these genes encode for crucial protein products involved in viral genome replication, transcription, cell cycle modulation, and virus-host interactions (O'Shea et al., 2005; van Breukelen et al., 2003). Presumably, the shortfall of these critical viral products contributes to the observed reduction in infectious progeny virus. Loss of Cα or all PKA subunits in combination negatively affected all viruses tested, including HAdV-4, which expresses an E1A that does not target PKA. This result suggests an additional, E1A-independent role for Cα in HAdV infection. Cα activity was previously shown to be crucial for transport of species C HAdV virions to the nucleus during the establishment of infection (Scherer et al., 2014; Suomalainen et al., 2001). Our results suggest other species of HAdV may also use this process to kick-start this early stage of their replicative cycles. The conserved targeting of multiple different PKA subunits by various viral components at distinct stages of infection underscores the true importance of this cellular kinase for HAdV.

E1A has been repeatedly shown to be adept at influencing various cellular scaffold and multiprotein complexes (Boyer et al., 1999; Glenewinkel et al., 2016; King et al., 2016a). To date, E1As from various HAdV species are currently the only well-characterized viral AKAPs; however, given the well-connected nature of cellular scaffolds like AKAPs, mimicry and manipulation of similar proteins is a strategy exploited by numerous viral pathogens (Chemes et al., 2015; Via et al., 2015). This allows viruses to control macromolecular networks or nucleate protein-protein interactions to facilitate their own needs (Brito and Pinney, 2017). By studying how viral regulatory proteins integrate into cellular signalling by mimicking scaffolds, we gain comprehension of how viruses overcome the daunting challenge of reorganizing their hosts into environments conducive

for replication. Examining protein-protein interactions between viral and host factors also offers clinically-relevant insights. For instance, the manipulation of orthologous cellular components within distinct host species (such as PKA) by viral proteins can contribute to successful cross-species transmission events. By understanding these events, we learn more about disease tropism or severity. Also, studying the molecular basis of these virus-host interactions provides opportunity to intervene for druggable targets, such as with the E1A-CDK9 interaction for various HAdVs (Prasad et al., 2017).

In summary, we conclusively identified E1A proteins from multiple species of HAdV can function as viral AKAPs. We also confirmed that they structurally and mechanistically resemble each other, although some flexibility exists presumably as a result of different evolutionary histories. We have also advanced the understanding of the functional mechanism of these vAKAPs by demonstrating their ability to integrate into cAMP signaling-dependent transcriptional activation. Furthermore, we demonstrate that vAKAPs play an important, biologically-significant role in enhancing genome replication and progeny production for multiple HAdV species.

3.5 References

Ablack, J.N.G., Cohen, M., Thillainadesan, G., Fonseca, G.J., Pelka, P., Torchia, J., Mymryk, J.S., 2012. Cellular GCN5 Is a Novel Regulator of Human Adenovirus E1A-Conserved Region 3 Transactivation. J. Virol. 86, 8198–8209.

Avgousti, D.C., Herrmann, C., Kulej, K., Pancholi, N.J., Sekulic, N., Petrescu, J., Molden, R.C., Blumenthal, D., Paris, A.J., Reyes, E.D., Ostapchuk, P., Hearing, P., Seeholzer, S.H., Worthen, G.S., Black, B.E., Garcia, B.A., Weitzman, M.D., 2016. A core viral protein binds host nucleosomes to sequester immune danger signals. Nature 535, 173–7.

Avvakumov, N., Kajon, A.E., Hoeben, R.C., Mymryk, J.S., 2004. Comprehensive sequence analysis of the E1A proteins of human and simian adenoviruses. Virology 329, 477–92.

Banky, P., Huang, L.J., Taylor, S.S., 1998. Dimerization/docking domain of the type Ialpha regulatory subunit of cAMP-dependent protein kinase. Requirements for dimerization and docking are distinct but overlapping. J. Biol. Chem. 273, 35048–55.

Bondesson, M., Mannervik, M., Akusjärvi, G., Svensson, C., 1994. An adenovirus E1A transcriptional repressor domain functions as an activator when tethered to a

promoter. Nucleic Acids Res. 22, 3053–60.

Boyer, T.G., Martin, M.E., Lees, E., Ricciardi, R.P., Berk, A.J., 1999. Mammalian Srb/Mediator complex is targeted by adenovirus E1A protein. Nature 399, 276–9.

Brito, A.F., Pinney, J.W., 2017. Protein-Protein Interactions in Virus-Host Systems. Front. Microbiol. 8, 1557.

Burgers, P.P., van der Heyden, M.A.G., Kok, B., Heck, A.J.R., Scholten, A., 2015. A Systematic Evaluation of Protein Kinase A–A-Kinase Anchoring Protein Interaction Motifs. Biochemistry 54, 11–21.

Chahal, J.S., Qi, J., Flint, S.J., 2012. The human adenovirus type 5 E1B 55 kDa protein obstructs inhibition of viral replication by type I interferon in normal human cells. PLoS Pathog. 8, e1002853.

Chemes, L.B., de Prat-Gay, G., Sánchez, I.E., 2015. Convergent evolution and mimicry of protein linear motifs in host-pathogen interactions. Curr. Opin. Struct. Biol. 32, 91–101.

Cohen, M.J., King, C.R., Dikeakos, J.D., Mymryk, J.S., 2014. Functional analysis of the C-terminal region of human adenovirus E1A reveals a misidentified nuclear localization signal. Virology 468.

Dehghan, S., Seto, J., Liu, E.B., Walsh, M.P., Dyer, D.W., Chodosh, J., Seto, D., 2013. Computational analysis of four human adenovirus type 4 genomes reveals molecular evolution through two interspecies recombination events. Virology 443, 197–207.

Duyndam, M.C., van Dam, H., van der Eb, A.J., Zantema, A., 1996. The CR1 and CR3 domains of the adenovirus type 5 E1A proteins can independently mediate activation of ATF-2. J. Virol. 70, 5852–9.

Engel, D.A., Hardy, S., Shenk, T., 1988. cAMP acts in synergy with E1A protein to activate transcription of the adenovirus early genes E4 and E1A. Genes Dev. 2, 1517–28.

Engel, D.A., Muller, U., Gedrich, R.W., Eubanks, J.S., Shenk, T., 1991. Induction of c-fos mRNA and AP-1 DNA-binding activity by cAMP in cooperation with either the adenovirus 243- or the adenovirus 289-amino acid E1A protein. Proc. Natl. Acad. Sci. U. S. A. 88, 3957–61.

Fax, P., Carlson, C.R., Collas, P., Taskén, K., Esche, H., Brockmann, D., 2001. Binding of PKA-RIIalpha to the Adenovirus E1A12S oncoprotein correlates with its nuclear translocation and an increase in PKA-dependent promoter activity. Virology 285, 30–41.

Ferrari, R., Gou, D., Jawdekar, G., Johnson, S.A., Nava, M., Su, T., Yousef, A.F., Zemke, N.R., Pellegrini, M., Kurdistani, S.K., Berk, A.J., 2014. Adenovirus small E1A

employs the lysine acetylases p300/CBP and tumor suppressor Rb to repress select host genes and promote productive virus infection. Cell Host Microbe 16, 663–76.

Ferrari, R., Pellegrini, M., Horwitz, G.A., Xie, W., Berk, A.J., Kurdistani, S.K., 2008. Epigenetic reprogramming by adenovirus e1a. Science 321, 1086–8.

Ferreon, A.C.M., Ferreon, J.C., Wright, P.E., Deniz, A.A., 2013. Modulation of allostery by protein intrinsic disorder. Nature 498, 390–4.

Frisch, S.M., Mymryk, J.S., 2002. Adenovirus-5 E1A: paradox and paradigm. Nat. Rev. Mol. Cell Biol. 3, 441–52.

Gedrich, R.W., Bayley, S.T., Engel, D.A., 1992. Induction of AP-1 DNA-binding activity and c-fos mRNA by the adenovirus 243R E1A protein and cyclic AMP requires domains necessary for transformation. J. Virol. 66, 5849–59.

Glenewinkel, F., Cohen, M.J., King, C.R., Kaspar, S., Bamberg-Lemper, S., Mymryk, J.S., Becker, W., 2016. The adaptor protein DCAF7 mediates the interaction of the adenovirus E1A oncoprotein with the protein kinases DYRK1A and HIPK2. Sci. Rep. 6, 28241.

Gold, M.G., Lygren, B., Dokurno, P., Hoshi, N., McConnachie, G., Taskén, K., Carlson, C.R., Scott, J.D., Barford, D., 2006. Molecular basis of AKAP specificity for PKA regulatory subunits. Mol. Cell 24, 383–95.

Guo, Y., Wilderman, A., Zhang, L., Taylor, S.S., Insel, P.A., 2012. Quantitative Proteomics Analysis of the cAMP/Protein Kinase A Signaling Pathway. Biochemistry 51, 9323–32.

Hardy, S., Shenk, T., 1988. Adenoviral control regions activated by E1A and the cAMP response element bind to the same factor. Proc. Natl. Acad. Sci. U. S. A. 85, 4171–5.

Hearing, P., Shenk, T., 1986. The adenovirus type 5 E1A enhancer contains two functionally distinct domains: one is specific for E1A and the other modulates all early units in cis. Cell 45, 229–36.

Jones, M.S., Harrach, B., Ganac, R.D., Gozum, M.M.A., Dela Cruz, W.P., Riedel, B., Pan, C., Delwart, E.L., Schnurr, D.P., 2007. New adenovirus species found in a patient presenting with gastroenteritis. J. Virol. 81, 5978–84.

Jones, N., Shenk, T., 1979. Isolation of adenovirus type 5 host range deletion mutants defective for transformation of rat embryo cells. Cell 17, 683–9.

King, C.R., Cohen, M.J., Fonseca, G.J., Dirk, B.S., Dikeakos, J.D., Mymryk, J.S., 2016a. Functional and Structural Mimicry of Cellular Protein Kinase A Anchoring Proteins by a Viral Oncoprotein. PLOS Pathog. 12, e1005621.

King, C.R., Tessier, T.M., Mymryk, J.S., 2016b. Color Me Infected: Painting Cellular Chromatin with a Viral Histone Mimic. Trends Microbiol. 24, 774–776.

Klemm, D.J., Colton, L.A., Ryan, S., Routes, J.M., 1996. Adenovirus E1A proteins regulate phosphoenolpyruvate carboxykinase gene transcription through multiple mechanisms. J. Biol. Chem. 271, 8082–8.

Lee, B.H., Mathews, M.B., 1997. Transcriptional coactivator cAMP response element binding protein mediates induction of the human proliferating cell nuclear antigen promoter by the adenovirus E1A oncoprotein. Proc. Natl. Acad. Sci. 94, 4481–4486.

Marshall, K.S., Cohen, M.J., Fonseca, G.J., Todorovic, B., King, C.R., Yousef, A.F., Zhang, Z., Mymryk, J.S., 2014. Identification and characterization of multiple conserved nuclear localization signals within adenovirus E1A. Virology 454–455, 206–214.

Miller, M.E., Engel, D.A., Smith, M.M., 1995. Cyclic AMP signaling is required for function of the N-terminal and CR1 domains of adenovirus E1A in Saccharomyces cerevisiae. Oncogene 11, 1623–30.

Müller, U., Roberts, M.P., Engel, D.A., Doerfler, W., Shenk, T., 1989. Induction of transcription factor AP-1 by adenovirus E1A protein and cAMP. Genes Dev. 3, 1991–2002.

Mymryk, J.S., Bayley, S.T., 1993. Induction of gene expression by exon 2 of the major E1A proteins of adenovirus type 5. J. Virol. 67, 6922–8.

Newlon, M.G., Roy, M., Morikis, D., Hausken, Z.E., Coghlan, V., Scott, J.D., Jennings, P.A., 1999. The molecular basis for protein kinase A anchoring revealed by solution NMR. Nat. Struct. Biol. 6, 222–7.

O'Shea, C., Klupsch, K., Choi, S., Bagus, B., Soria, C., Shen, J., McCormick, F., Stokoe, D., 2005. Adenoviral proteins mimic nutrient/growth signals to activate the mTOR pathway for viral replication. EMBO J. 24, 1211–1221.

Pelka, P., Ablack, J.N.G., Fonseca, G.J., Yousef, A.F., Mymryk, J.S., 2008. Intrinsic structural disorder in adenovirus E1A: a viral molecular hub linking multiple diverse processes. J. Virol. 82, 7252–63.

Pelka, P., Ablack, J.N.G., Torchia, J., Turnell, A.S., Grand, R.J.A., Mymryk, J.S., 2009. Transcriptional control by adenovirus E1A conserved region 3 via p300/CBP. Nucleic Acids Res. 37, 1095–1106.

Prasad, V., Suomalainen, M., Hemmi, S., Greber, U.F., 2017. Cell Cycle-Dependent Kinase Cdk9 Is a Postexposure Drug Target against Human Adenoviruses. ACS Infect. Dis. 3, 398–405.

Rasti, M., Grand, R.J.A., Mymryk, J.S., Gallimore, P.H., Turnell, A.S., 2005.

Recruitment of CBP/p300, TATA-binding protein, and S8 to distinct regions at the N terminus of adenovirus E1A. J. Virol. 79, 5594–605.

Sands, W.A., Palmer, T.M., 2008. Regulating gene transcription in response to cyclic AMP elevation. Cell. Signal. 20, 460–6.

Scherer, J., Yi, J., Vallee, R.B., 2014. PKA-dependent dynein switching from lysosomes to adenovirus: a novel form of host-virus competition. J. Cell Biol. 205, 163–77.

Shuen, M., Avvakumov, N., Torchia, J., Mymryk, J.S., 2003. The E1A proteins of all six human adenovirus subgroups target the p300/CBP acetyltransferases and the SAGA transcriptional regulatory complex. Virology 316, 75–83.

Suomalainen, M., Nakano, M.Y., Boucke, K., Keller, S., Greber, U.F., 2001. Adenovirus-activated PKA and p38/MAPK pathways boost microtubule-mediated nuclear targeting of virus. EMBO J. 20, 1310–9.

Taylor, S.S., Zhang, P., Steichen, J.M., Keshwani, M.M., Kornev, A.P., 2013. PKA: Lessons learned after twenty years. Biochim. Biophys. Acta - Proteins Proteomics 1834, 1271–1278.

van Breukelen, B., Brenkman, A.B., Holthuizen, P.E., van der Vliet, P.C., 2003. Adenovirus type 5 DNA binding protein stimulates binding of DNA polymerase to the replication origin. J. Virol. 77, 915–22.

Via, A., Uyar, B., Brun, C., Zanzoni, A., 2015. How pathogens use linear motifs to perturb host cell networks. Trends Biochem. Sci. 40, 36–48.

Wong, H.K., Ziff, E.B., 1994. Complementary functions of E1a conserved region 1 cooperate with conserved region 3 to activate adenovirus serotype 5 early promoters. J. Virol. 68, 4910–20.

Yousef, A.F., Brandl, C.J., Mymryk, J.S., 2009. Requirements for E1A dependent transcription in the yeast Saccharomyces cerevisiae. BMC Mol. Biol. 10, 32.

Zambon, A.C., Zhang, L., Minovitsky, S., Kanter, J.R., Prabhakar, S., Salomonis, N., Vranizan, K., Dubchak, I., Conklin, B.R., Insel, P.A., 2005. Gene expression patterns define key transcriptional events in cell-cycle regulation by cAMP and protein kinase A. Proc. Natl. Acad. Sci. U. S. A. 102, 8561–6.

Zhang, X., Odom, D.T., Koo, S.-H., Conkright, M.D., Canettieri, G., Best, J., Chen, H., Jenner, R., Herbolsheimer, E., Jacobsen, E., Kadam, S., Ecker, J.R., Emerson, B., Hogenesch, J.B., Unterman, T., Young, R.A., Montminy, M., 2005. Genome-wide analysis of cAMP-response element binding protein occupancy, phosphorylation, and target gene activation in human tissues. Proc. Natl. Acad. Sci. U. S. A. 102, 4459–64.

Chapter 4

4 General Discussion

"One, remember to look up at the stars and not down at your feet. Two, never give up work. Work gives you meaning and purpose and life is empty without it. Three, if you are lucky enough to find love, remember it is there and don't throw it away."

– *Stephen Hawking*

4.1 Thesis Summary

This body of work describes my studies of the HAdV E1A proteins and the molecular determinants of their interaction with cellular PKA. Specifically, I describe the mechanism by which this virus-host interaction occurs and its functional consequences during HAdV infections. In Chapter 2 of this thesis, multiple subunits of PKA were shown to be targeted for binding by E1A from HAdV-5 (Figure 2.1). Using this virus as a model, I carried out extensive mutagenesis to map the interaction interfaces on both E1A (for HAdV-5) and PKA (Figures 2.1-2.5). Using a variety of molecular reagents, I showed that the manner by which E1A bound PKA strongly resembled that of cellular AKAPs (Figure 2.6). I concluded that this interaction represented a novel case of viral mimicry and that E1A is the first known vAKAP. Furthermore, I demonstrated that the interaction was functional during HAdV-5 infections as the interaction allowed E1A to recruit PKA into the nucleus, specifically to HAdV early gene promoters at viral replication centres (Figures 2.7-2.9). E1A's ability to act as a vAKAP enhanced viral transcription, protein synthesis, and infectious progeny production for HAdV-5 (Figures 2.10-2.13). In Chapter 3, I extended these studies to encompass representative E1A proteins from all seven species of HAdV (A-G). The protein-protein interactions between different types of E1A with various PKA subunits (and the mechanism by which they occurred) were largely conserved across most species of HAdV (Figure 3.1-3.3). Interestingly, different E1As appeared to have a preference for one isoform of PKA R-subunit over another, leading to different relocalization phenotypes in transfected and infected cells (Figures 3.4, 3.6-3.10). Both vAKAP mimicry and relocalization of PKA

subunits during infection were demonstrated to be conserved requirements for full, WT-levels of viral replication for 4 distinct HAdV species (Figure 3.13B). I also increased the mechanistic knowledge of how PKA enhances the viral life cycle by showing it contributes both to efficient viral genome replication as well as E1A-mediated activation of cAMP-responsive promoters (Figures 3.5, 3.11-3.13A). Overall, my work has uncovered and characterized a novel example of viral mimicry carried out by the E1A proteins of multiple HAdVs that repurposes a host target to benefit viral infection.

4.2 The interaction between E1A and PKA

The protein-protein interactions between E1A and PKA were first discovered in 2001 (Fax et al., 2001). Specifically, direct interactions between HAdV-12 E1A and both RIα and RIIα were reported, but this was only briefly explored. PKA, already a well-characterized kinase, was shown to have its RIIα subunit relocalized to the nucleus in E1A-transfected cells. This was further shown to contribute to expression of a HAdV-12 E2 reporter construct. Given the ever-increasing array of genetic and molecular tools available for HAdV-5, we sought to further explore the E1A-PKA interaction in the context of this better-characterized HAdV.

Our interaction studies began by reproducing the previously observed HAdV-12 findings using HAdV-5 E1A in both transfected and infected conditions. Using siRNA-mediated gene silencing, we also showed that the interactions with PKA R-subunits conferred an indirect association with PKA's catalytic component, Cα. Through extensive mutagenesis of both E1A and PKA, the binding sites on both partners were mapped in fine detail. I determined that a small portion of the N-terminus of E1A was both necessary and sufficient for binding PKA subunits, both in transient transfection assays and HAdV-5 infections. Deletion or truncations affecting amino acids 14-28 of E1A abrogated the interaction whereas mutations in adjacent or downstream areas had no effect. Structural modelling strongly indicated that the amino acid sequence in this region of E1A formed an amphipathic α-helix that resembled the analogous PKA-binding motifs in cellular AKAPs. Additional deletion and point mutants designed to test this hypothesis (based on known PKA/AKAP structures) confirmed our prediction of structural mimicry to be accurate. E1A also bound to PKA's D/D domain, the identical

binding site of cellular AKAPs, further strengthening the narrative of viral AKAP mimicry.

All experiments in this thesis utilized the α-isoforms of PKA subunits (RIα, RIIα, and Cα) encoded by the human PRKAR1A, PRKAR2A, and PRKACA genes, respectively (Canaves and Taylor, 2002; Skalhegg and Tasken, 2000; Søberg et al., 2013; Turnham and Scott, 2016). An interaction with the β-subunit isoforms was briefly explored both by our lab and the previous HAdV-12 group, but no interaction with either HAdV-5 or HAdV-12 E1A species was observed (Fax et al., 2001; King et al., 2016). This does not rule out the possibility that E1As from the other species of HAdV are able to bind and manipulate PKAβ, but this was not examined. A γ isoform of the catalytic subunit also exists in humans, but is only expressed in the testis (not a tissue site typically infected by HAdVs) (Berk, 2013).

4.3 Mimicry of cellular AKAPs by E1A

Given both the sequence and putative structural similarities of E1A's AKAP-like motif, I hypothesized that E1A was also capable of functioning like a cellular AKAP. This was investigated using both transfection- and infection-based experiments. Because HAdV-5 E1A can bind to both type-I and –II regulatory subunits of PKA, it is potentially a 'dual-specificity' AKAP (Carlson et al., 2006; Gold et al., 2006). We first examined if the PKA-binding motif (aa14-28) in E1A could functionally complement a binding defect in a known dual-specificity AKAP, D-AKAP1. Indeed, a deletion mutant of D-AKAP1 that lacked its PKA-binding ability was rescued back to WT levels when aa14-28 of HAdV-5 E1A were cloned in its place. Next, high-affinity, *in silico*-designed peptide inhibitors of AKAP-PKA interactions were pitted against E1A in transfected conditions to test for their ability to similarly disrupt E1A-PKA binding. As with cellular AKAPs, RIAD and sAKAPis specifically blocked E1A binding to RIα and RIIα respectively. Finally, in HAdV-5-infected cells, I showed that WT E1A expression outcompeted endogenous, dual-specificity AKAP7 for binding PKA subunits whereas a mutant virus lacking E1A's AKAP-like motif could not. Collectively, these experiments established new molecular tools for probing PKA and AKAP complexes in the context of HAdV infection. Additionally, reagents previously used exclusively to study cellular AKAPs are

demonstrably useful for examining viral AKAPs as well. While E1A was functionally analogous to 2 tested AKAPs (D-AKAP1 and AKAP7), there are over 30 cellular AKAPs variably expressed in human cells (Taylor et al., 2012; Welch et al., 2010; Wong and Scott, 2004). How E1A may or may not compete against these other endogenous PKA scaffolds remains to be elucidated.

The mimicry of AKAPs by E1A caused a relocalization of PKA regulatory subunits from the cytoplasm to the nucleus in both transfected and infected cells. This served to bring PKA proteins into closer proximity with viral replication centres and caused an E1A-mediated increase of the catalytic Cα subunit on the promoters of HAdV-5 early genes. The retargeting of PKA to specific sites of action is the hallmark of AKAP functionality. Given that not all PKA protein is trafficked into the nucleus by E1A, it remains to be seen if E1A-PKA interactions serve functions at other cellular loci or compartments. Furthermore, the stoichiometric nature by which E1A acts as a scaffold to concurrently bind multiple components of the cAMP signaling pathway is unclear. E1A's interactions with p300/CBP have been well-studied, and it has also been shown to potentially bind cAMP-regulated transcription factors, such as CREB and ATFs (Eckner et al., 1994; Fax et al., 2000; Ferrari et al., 2014, 2008; Liu and Green, 1994). Including PKA, many of these interactions take place on E1A's N-terminus. If at least some of these interactions occur concurrently, it's possible that these multiple, related interactions affect regulation of each other by either enhancing or occluding binding. However, these precise details have yet to be determined. Novel HAdVs encoding mutant E1As that specifically lack binding to one (or more) of these targets could shed light on which downstream functions result from independence or cooperation of these overlapping interactions.

4.4 Viral retasking of PKA to enhance infection

The physiological consequences of E1A's ability to usurp PKA were examined to gauge how they might benefit HAdV infection. Overall, E1A's role as a vAKAP was required for multiple aspects of the HAdV life cycle including WT levels of viral transcription, genome replication, protein synthesis, and progeny production. For HAdV-5, viral genes that displayed recruitment of Cα to their promoters in an E1A-dependent manner experienced enhanced transcription from these loci. This largely correlated with what

was subsequently observed at the protein level. Ultimately, the production of both viral genomes and infectious progeny virus were reduced in the absence of an E1A-PKA interaction. It's likely that the lack of expression from the HAdV-5 E2 gene (which encodes viral genome replication machinery) contributes to this reduction in viral DNA levels (Challberg and Kelly, 1979; Levine et al., 1976; van der Vliet and Levine, 1973). It's also possible PKA may have a more direct role in viral DNA synthesis at viral replication centres as has been shown for cellular DNA replication, but this has not been investigated (Costanzo et al., 1999). The overall lack of progeny production is likely due to the shortfall of crucial viral proteins that are under-expressed in the absence of E1A being able to repurpose PKA.

Various mechanistic details of PKA's role in the HAdV life cycle remain unclear, however both HAdV-5 and E1A's ability to alter the catalytic activity of Cα was briefly explored. HAdV infection was capable of globally enhancing cellular cMAP levels and PKA kinase activity. The phosphorylation status of PKA targets (such as CREB) appears directly affected by E1A's interaction with PKA, suggesting that its vAKAP function allows HAdV to take advantage of stimulation of cellular cAMP signaling post-infection. It would be also be of interest to test if the phosphorylation status of other PKA-regulated TFs is altered at viral promoters and if this was dependent on E1A's role as vAKAP. Furthermore, the phosphorylation status and downstream functional consequences for other classes of PKA substrates has yet to be examined. It is possible the relocalization of Cα's kinase activity affects both nuclear and non-nuclear processes unrelated to gene expression. Lastly, PKA may be a suitable drug target for inhibiting viral infections as inhibition of broad-specificity kinases has been successfully utilized in the past for ablating virulence of HAdV (Prasad et al., 2017). Given that PKA is utilized by the virus at multiple stages of its life cycle (Bremner et al., 2009; King et al., 2016; Scherer et al., 2014), PKA disruption (by either catalytic or anchoring inhibitors) may slow or abrogate the infection more potently than typical single-target drugs (provided there is no general toxicity to the infected host).

Another unanswered question pertaining to the E1A-PKA interaction is whether or not E1A is a substrate for the kinase. Phosphorylated forms of HAdV-5 E1A and the

targeted residues have been studied in detail. Phosphorylation is limited largely to serine residues, particularly Ser-89, -132, -185, -188, -219, and -231 in HAdV-5 E1A (Dumont et al., 1993; Dumont and Branton, 1992; Mal et al., 1996; Whalen et al., 1997, 1996). E1A is considered to be modified in this fashion predominantly by cyclin-dependent kinases (Cdk), MAPK, and casein kinase II (CKII). However, Ser-219 is a strong substrate for the DYRK1A kinase as well, and this PTM confers a downstream interaction with the FOXK1/2 transcription factors (Glenewinkel et al., 2016; Komorek et al., 2010). Therefore, E1A's phosphorylation status is important to understand, as it affects binding to several key partners (including Rb) and has been shown to affect its transforming ability (Dumont et al., 1993; Mal et al., 1996). Whether PKA itself can phosphorylate Ser or Thr residues on E1A has not been tested. However, we can speculate that it is unlikely given that alternatively-migrating bands were consistently not observed in protein gels from experiments where Cα was either over- or under-expressed. Additionally, there are only two sites in HAdV-5 E1A that somewhat resemble the PKA consensus site; one surrounding Thr-164 and the other encompassing Thr-218 (although neither of these sequences are conserved across any other HAdV species) (Avvakumov et al., 2004; Jones et al., 2007). Threonine is a much lower-affinity substrate than Ser for PKA catalysis, however, and these sites also lack the adjacent hydrophobic residue typically found in PKA substrates (Masterson et al., 2010; Shabb, 2001).

4.5 Conservation of viral AKAPs

Conservation of the E1A-PKA interaction was briefly examined in Chapter 2 but the majority of experiments in that chapter focused only on E1A from HAdV-5. In the follow-up study to these findings (Chapter 3), similar experimental approaches were utilized to examine the N-terminus of E1A from all 7 HAdV species. This portion of E1A was necessary and sufficient to bind RIα, RIIα, and Cα for 5 of 7 HAdV types tested: HAdV-12, -3, -5, -9, and -40 (representing species A, B, C, D, and F respectively). E1A from HAdV-4 and -52 (representing species E and G) did not associate with any PKA subunit. The interaction between PKA and HAdV-40 E1A was also notably weaker in CoIP assays. The overall conservation of the E1A-PKA interaction across highly divergent HAdVs potentially indicates importance and the observed differences between

species likely stem from the distinct evolutionary histories of each virus. Indeed, E1As which did not bind or bound weakly (from species E, F, and G) are more closely related to simian adenoviruses or arose as cross-species recombinants (Dehghan et al., 2013; Jones et al., 2007). These E1A proteins from those viruses are not predicted to contain the same level of helical structure in their N-terminus, likely explaining their lack of interaction with PKA's D/D domain. Consequently, these E1As were incapable of relocalizing PKA subunits in either transfected or infected cells. Also, viral genome replication and progeny production for HAdV-4 was not affected by siRNA-mediated silencing of RIα and RIIα expression. These HAdV species may not have evolved the means or need for manipulating human PKA subunits to enhance their viral life cycles. It is unlikely that related SAdV E1A proteins are capable of binding simian PKA proteins given the lack of predicted structure in the E1A N-terminus of these viruses. Amino acid sequences of PKA R-subunits are incredibly well-conserved among primates (99% identity between humans and macaques, for example) as is the structural basis for AKAPs binding to PKA D/D domains (Carlson et al., 2006; Gold et al., 2006; Kannan et al., 2007; Taylor et al., 2012). It is therefore more likely that the ability for some HAdV E1As to act as vAKAPs arose from extended coevolution with humans as a result of providing some selective benefit for the virus within this host.

For HAdVs that did bind and relocalize PKA subunits, they required PKA for viral genome replication and progeny production, as well as E1A-mediated transactivation of a cAMP-responsive gene. Interestingly, a distinct preference for affecting either RIα or RIIα was observed. Specifically, HAdV-5 and -9 relocalized RIα to the nucleus, whereas HAdV-3 and -12 relocalized RIIα. Preference for one PKA isoform over another is a common feature of cellular AKAPs (Welch et al., 2010; Wong and Scott, 2004), which is evidently also the case for viral AKAPs. This preference could be altered and swapped within different E1As using targeted mutagenesis of critical residues (predicted *in silico*), suggesting the AKAP-like helix in E1A is an evolutionarily flexible motif. The biological significance for preferring either R1α or RIIα is unknown. It may simply be an artefactual byproduct of divergent evolution between these viruses. Alternatively, it may contribute to virulence in different cells or tissues where PKA subunit expression profiles differ (Skalhegg and Tasken, 2000). Along these lines, in cells lacking RIα protein

expression, HAdV-5 E1A was able to switch and relocalize RIIα. It may be that being able to bind both RIα and RIIα to some degree is a redundancy that ensures the crucial downstream association with Cα, no matter what the availability of PKA proteins is.

4.6 Usurping PKA to alter host cell transcription

Significant amounts of research on both E1A and PKA have focused on their respective abilities to regulate cellular gene expression. I focused most of my attention towards effects on viral transcription and the downstream consequences on HAdV replication (Chapters 2 and 3). However, given the amount of relocalized PKA observed during HAdV infection, it seems extremely likely that its interaction with E1A also affects host genes in a manner similar to viral ones. There have been numerous studies which separately surveyed the effects of either E1A or PKA on the human transcriptome, although no published dataset yet exists that examines E1A's effects specifically on PKA-regulated genes (Ferrari et al., 2014, 2008; Horwitz et al., 2008; Radko et al., 2015; Zhang et al., 2005). This is an attractive area of future study as both E1A and PKA can potentially regulate thousands of host genes on their own and E1A has been previously shown to synergize with cAMP signalling to affect transcription (Brockmann and Esche, 2003; Engel et al., 1991, 1988; Hardy and Shenk, 1988; Lee and Mathews, 1997; Müller et al., 1989).

Using NGS, the Mymryk laboratory previously produced a dataset of differentially-regulated genes in primary cells infected by a variety of HAdV-5 mutants (Appendix A). Additionally, information from publicly available datasets was extracted and queried across the entire human genome for cAMP response elements (CRE) in their promoters (Zhang et al., 2005). To gain a foothold into understanding the potential impact of E1A on PKA-regulated genes during HAdV infection, we cross-referenced these data together. Using Python (Appendix B) a subset of genes was selected whose expression was altered more than 2-fold up or down in cells infected with WT HAdV-5 (*dl*309) versus ΔE1A HAdV-5 (*dl*312). These candidate genes were then trimmed to include only those containing a verified CRE (as a surrogate marker for possible PKA-regulation) in either their proximal (<300bp from TSS [transcriptional start site]) or distal (>300bp from TSS)

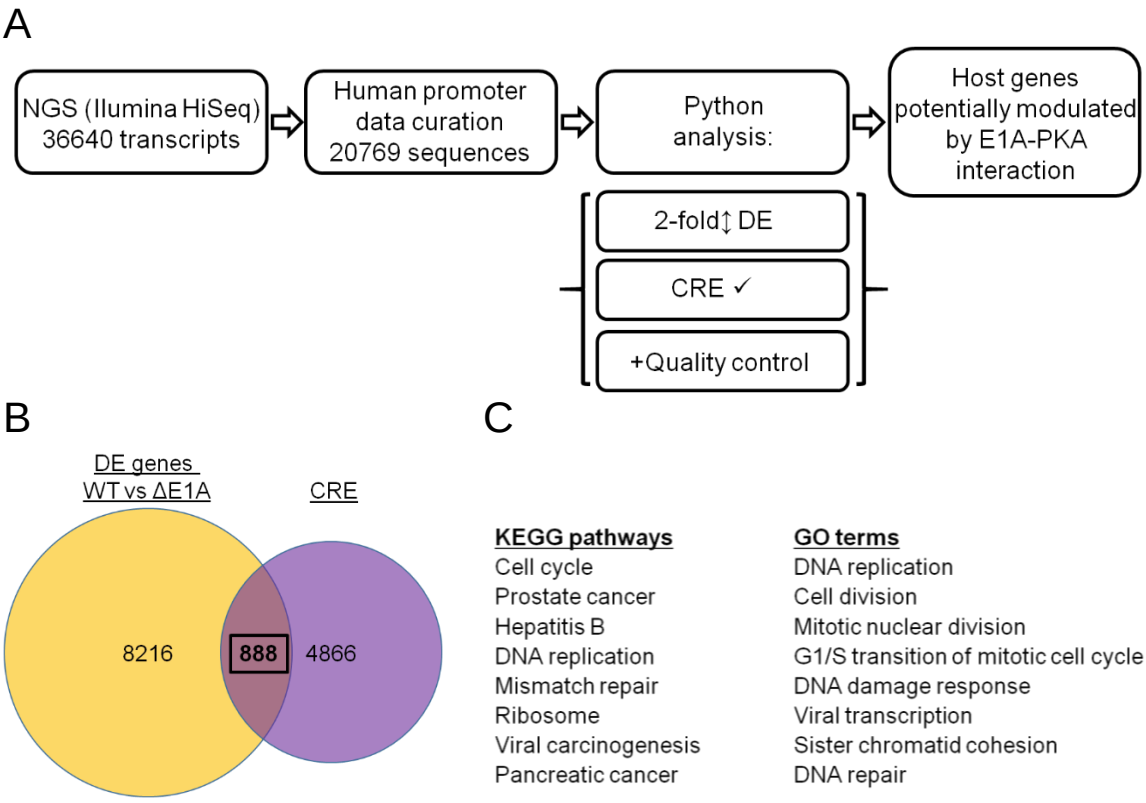


Figure 4.1. Host cell genes potentially regulated via E1A's interaction with PKA.

(A) Workflow of the analyses. Raw NGS data from the Mymryk laboratory was converted into tabular format and information on CREs in human gene promoters was extracted from publicly available datasets (Zhang et al., 2005). All data were subsequently processed using the Python programming language. Host genes that were up- or down-regulated at least 2-fold in WT-infected cells versus ΔE1A-infected cells were selected for further analysis. These candidates were then trimmed to include only those with verified CREs in their upstream regulatory regions. (B) Results of comparison of differentially-expressed genes in WT- versus ΔE1A-infected cells and genes with CREs in their promoters. The number of candidate genes of interest that met all criteria in these analyses (and are therefore potentially modulated by the E1A-PKA interaction) is highlighted in the overlapping region of the Venn diagram. (C) Lists of significantly impacted KEGG pathways and GO terms based on candidate genes.

promoter regions. After removing duplicated or outlier genes that had low read numbers, we obtained a set of putative PKA-regulated host genes was obtained whose expression may be altered by E1A (Figure 4.1).

A total of 888 candidate genes were identified, with 557 being upregulated and 331 being downregulated. As a positive control, a previously-validated gene (proliferating cell nuclear antigen [PCNA]) was found amongst the list of upregulated genes (Lee and Mathews, 1997). This dataset of differentially-expressed genes was then analyzed in the context of pathways obtained from the Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa et al., 2002; Kanehisa and Goto, 2000) and gene ontology from the Gene Ontology Consortium database (Ashburner et al., 2000). Both these analyses were facilitated by the Database for Annotation, Visualization, and Integrated Discovery (DAVID) website (Huang et al., 2009, 2008). The most significantly affected KEGG pathways were those involved in cell cycle, viral infections, and various cancers. Similarly, the most significantly enriched GO terms impacted cellular processes such as transcription, DNA replication, and cell cycle control. These hits strongly align with E1A's known functions during HAdV infection (Bayley and Mymryk, 1994; Berk, 2005; Pelka et al., 2008). They also suggest that an interaction with PKA may contribute to the ability of E1A to fine-tune transcription in infected cells to establish an environment more favourable for HAdV replication.

These analyses should not be used as license to start inefficiently testing individual genes, but rather as a general representation of the likelihood and scale of E1A's ability to modulate cAMP-responsive genes. How E1A uses PKA to modulate host cell transcription to affect downstream processes such as cell cycle or DNA replication remains an exciting and open area of investigation. Using my mapping studies, construction of HAdV mutants encoding E1A that cleanly lack binding to only PKA would be powerful tools aiding future work of this nature. Genome-wide RNAseq experiments on primary cells infected by such viruses would yield powerful results and could validate which hits from these preliminary analyses are real and reproducible. Additional ChIPseq experiments would shed light on which genes showed the presence

of E1A, PKA, or phosphorylated targets of PKA (such as CREB) to their promoters. It's possible that E1A may be inducing a global deregulation of cellular cAMP signaling to modulate hundreds of cellular genes, but this will need to be thoroughly investigated.

4.7 Contribution of E1A-PKA interaction to oncogenic transformation

HAdV E1A (and oncoproteins from other DNA tumour viruses) have repeatedly served as powerful tools for discovering cancer-relevant genes (Rozenblatt-Rosen et al., 2012). E1A's role in oncogenic transformation stems primarily from its ability to drastically rewire host protein-protein interactions (Bayley and Mymryk, 1994; Pelka et al., 2008). Interestingly, the same N-terminal portion of E1A protein that binds PKA is also required for its transformation activity (Chinnadurai, 2011; Jelsma et al., 1989; Subramanian et al., 1988; Whyte et al., 1988). This has been largely linked to E1A's crucial interaction with p300/CBP (Egan et al., 1988; Howe et al., 1990; Howe and Bayley, 1992; Wang et al., 1993). However, many of the mutants used in such studies contain large deletions or point mutations which affect multiple E1A binding partners in this promiscuous portion of the protein (Boyd et al., 2002; Loewenstein et al., 2006; Rasti et al., 2005) (Figure 1.8). Also, some mutants which retain binding to p300 *in vivo* still have reduced transforming ability, confirming that other N-terminal binding partners are involved in E1A-mediated transformation. Therefore, a potential role for the E1A-PKA interaction would need to be thoroughly and cleanly investigated.

While PKA is not typically considered an oncogene or tumour suppressor, it has been studied in the context of several cancer types or as a molecular component in cancer-relevant pathways (Gold et al., 2013; McKenzie et al., 2011; Palorini et al., 2016; Pattabiraman et al., 2016). One way the E1A-PKA interaction could contribute to transformation is through effects on host gene expression (discussed above). Alternatively, E1A-mediated relocalization of PKA to different cellular compartments could affect phosphorylation status of targets unrelated to transcription (such as those involved in DNA synthesis or cytoskeletal remodeling). E1A would of course be valuable as a tool to probe this hypothesis and enhance molecular knowledge of these biological pathways. Specifically using mutants lacking PKA-binding ability,

transformation assays on either baby rat kidney or even human cells would yield clear evidence of a role for this host factor in the transformation process (Massimi and Banks, 2005; Vijayalingam et al., 2016).

4.8 Prevalence of cellular scaffold mimics in the viral universe

Mechanisms that regulate the spatial and temporal control of proteins involved in cellular signaling offer an appealing target for viral manipulation. Cellular scaffold or adaptor proteins typically bind multiple components of signaling pathways (including residue-modifying enzymes and their cognate substrates) and localize them to distinct subcellular loci (Taylor et al., 2012; Welch et al., 2010; Wong and Scott, 2004). These highly-connected hubs thus nucleate the formation of signalosomes that more efficiently perform pleiotropic functions in various signal transduction pathways. Hijacking a host's interaction network by altering or mimicking cellular scaffolds is widespread among viruses, indicative of it being a broadly beneficial strategy (Davey et al., 2011).

In addition to AKAPs, many cellular scaffolds have been shown to be targeted for manipulation or mimicry by a range of diverse viruses such as Epstein-Barr virus (EBV), vaccinia virus (VACV), measles virus (MeV), Kaposi's sarcoma-associated herpes virus (KSHV), HIV, IAV, mumps virus (MuV), and even walleye dermal sarcoma virus (WDSV). These host targets include KSR1 (kinase suppressor of ras; which assembles components of the RAS-ERK pathway) (Lee et al., 2008), NLRPs (NACHT, LRR and PYD domains-containing protein; helps assemble the inflammasome) (Gerlic et al., 2013; Gregory et al., 2011; Komune et al., 2011), RACK1 (receptor of activated protein C kinase; tethers components of protein kinase C [PKC] signaling) among numerous others (Baumann et al., 2000; Daniels et al., 2008; Demirov et al., 2012; Gallina et al., 2001; Kubota et al., 2002; Mondal et al., 2017). E1A itself has been shown to use the cellular adaptor, DCAF7 (DDB1- and CUL4-associated factor 7), to gain access to DYRK1A and HIPK2 kinases (Glenewinkel et al., 2016); E1A has also been reported to bind RACK1 itself (Sang et al., 2001). Clearly, the manipulation of host scaffold proteins is ubiquitous among viruses and offers a large window into examining virus-host interactions at the protein level.

While E1A proteins from various HAdVs are described here as the first (and only) known viral AKAPs, it would not be surprising to discover other functionally analogous viral mimics (Brito and Pinney, 2017; Chemes et al., 2015; Via et al., 2015). Newly-refined bioinformatics tools have recently demonstrated an increased ability to predict and discover AKAPs and PKA-binding motifs (Carlson et al., 2006; Gold et al., 2006). One study was able to translate these *in silico* predictions into the discovery of a new, *bona fide* human AKAP (Burgers et al., 2015). It would be interesting use similar methodology to probe the proteomes of viruses to potentially uncover vAKAPs outside the HAdV family. Additionally, my work has enhanced the knowledge of how flexible AKAP-like motifs can be, increasing the number of undiscovered sequences potentially capable of binding PKA.

4.9 Concluding remarks

HAdV E1A continues to prove itself as an excellent molecular tool for studying protein-protein interactions between viruses and hosts. Investigations into these interactions has contributed greatly to our collective understanding of numerous processes in both viral and cellular biology. These include (but are not limited to) transcription, splicing, cell cycle, innate immunity, nuclear import, transformation, and cancer (Berk, 2005; Cohen et al., 2014; Fonseca et al., 2012; Pelka et al., 2008; Rozenblatt-Rosen et al., 2012). My work described in this thesis advances this research by providing new insights into the molecular biology of both E1A and one of its cellular binding partners, PKA. Mechanistic studies of how this interaction occurs revealed a novel example of viral mimicry by HAdV, and the tools generated have laid the groundwork for future studies to search for other viral AKAPs. Furthermore, my work demonstrated previously-unknown roles for PKA in HAdV biology, including early gene transcription, protein synthesis, genome replication, and progeny production. This was also examined across all seven species of HAdV, revealing new insights into the evolutionary histories of E1A proteins from these viruses. Moving forward, my data have identified potential roles for PKA in E1A-mediated regulation of host gene expression and cellular transformation, which could be the subject of follow-up projects. Therefore, despite over 30 years of intense genetic and biochemical characterization, the E1A protein of HAdV still has much more

to provide for scientists yearning to understand the molecular biology of both viruses and cells.

4.10 References

Ashburner, M., Ball, C.A., Blake, J.A., Botstein, D., Butler, H., Cherry, J.M., Davis, A.P., Dolinski, K., Dwight, S.S., Eppig, J.T., Harris, M.A., Hill, D.P., Issel-Tarver, L., Kasarskis, A., Lewis, S., Matese, J.C., Richardson, J.E., Ringwald, M., Rubin, G.M., Sherlock, G., 2000. Gene Ontology: tool for the unification of biology. Nat. Genet. 25, 25–29.

Avvakumov, N., Kajon, A.E., Hoeben, R.C., Mymryk, J.S., 2004. Comprehensive sequence analysis of the E1A proteins of human and simian adenoviruses. Virology 329, 477–92.

Baumann, M., Gires, O., Kolch, W., Mischak, H., Zeidler, R., Pich, D., Hammerschmidt, W., 2000. The PKC targeting protein RACK1 interacts with the Epstein-Barr virus activator protein BZLF1. Eur. J. Biochem. 267, 3891–901.

Bayley, S.T., Mymryk, J.S., 1994. Adenovirus e1a proteins and transformation (review). Int. J. Oncol. 5, 425–44.

Berk, A., 2013. Adenoviridae. In: Fields Virology. pp. 1704–1731.

Berk, A.J., 2005. Recent lessons in gene expression, cell cycle control, and cell biology from adenovirus. Oncogene 24, 7673–85.

Boyd, J.M., Loewenstein, P.M., Tang Qq, Q., Yu, L., Green, M., 2002. Adenovirus E1A N-terminal amino acid sequence requirements for repression of transcription in vitro and in vivo correlate with those required for E1A interference with TBP-TATA complex formation. J. Virol. 76, 1461–74.

Bremner, K.H., Scherer, J., Yi, J., Vershinin, M., Gross, S.P., Vallee, R.B., 2009. Adenovirus transport via direct interaction of cytoplasmic dynein with the viral capsid hexon subunit. Cell Host Microbe 6, 523–35.

Brito, A.F., Pinney, J.W., 2017. Protein-Protein Interactions in Virus-Host Systems. Front. Microbiol. 8, 1557.

Brockmann, D., Esche, H., 2003. The multifunctional role of E1A in the transcriptional regulation of CREB/CBP-dependent target genes. Curr. Top. Microbiol. Immunol. 272, 97–129.

Burgers, P.P., van der Heyden, M.A.G., Kok, B., Heck, A.J.R., Scholten, A., 2015. A Systematic Evaluation of Protein Kinase A–A-Kinase Anchoring Protein Interaction Motifs. Biochemistry 54, 11–21.

Canaves, J.M., Taylor, S.S., 2002. Classification and phylogenetic analysis of the cAMP-dependent protein kinase regulatory subunit family. J. Mol. Evol. 54, 17–29.

Carlson, C.R., Lygren, B., Berge, T., Hoshi, N., Wong, W., Taskén, K., Scott, J.D., 2006. Delineation of type I protein kinase A-selective signaling events using an RI anchoring disruptor. J. Biol. Chem. 281, 21535–45.

Challberg, M.D., Kelly, T.J., 1979. Adenovirus DNA replication in vitro: origin and direction of daughter strand synthesis. J. Mol. Biol. 135, 999–1012.

Chemes, L.B., de Prat-Gay, G., Sánchez, I.E., 2015. Convergent evolution and mimicry of protein linear motifs in host-pathogen interactions. Curr. Opin. Struct. Biol. 32, 91–101.

Chinnadurai, G., 2011. Opposing oncogenic activities of small DNA tumor virus transforming proteins. Trends Microbiol. 19, 174–83.

Cohen, M.J., King, C.R., Dikeakos, J.D., Mymryk, J.S., 2014. Functional analysis of the C-terminal region of human adenovirus E1A reveals a misidentified nuclear localization signal. Virology 468–470, 238–243.

Costanzo, V., Avvedimento, E. V, Gottesman, M.E., Gautier, J., Grieco, D., 1999. Protein kinase A is required for chromosomal DNA replication. Curr. Biol. 9, 903–6.

Daniels, C.C., Rovnak, J., Quackenbush, S.L., 2008. Walleye dermal sarcoma virus Orf B functions through receptor for activated C kinase (RACK1) and protein kinase C. Virology 375, 550–60.

Davey, N.E., Travé, G., Gibson, T.J., 2011. How viruses hijack cell regulation. Trends Biochem. Sci. 36, 159–69.

Dehghan, S., Seto, J., Liu, E.B., Walsh, M.P., Dyer, D.W., Chodosh, J., Seto, D., 2013. Computational analysis of four human adenovirus type 4 genomes reveals molecular evolution through two interspecies recombination events. Virology 443, 197–207.

Demirov, D., Gabriel, G., Schneider, C., Hohenberg, H., Ludwig, S., 2012. Interaction of influenza A virus matrix protein with RACK1 is required for virus release. Cell. Microbiol. 14, 774–89.

Dumont, D.J., Branton, P.E., 1992. Phosphorylation of adenovirus E1A proteins by the p34cdc2 protein kinase. Virology 189, 111–20.

Dumont, D.J., Marcellus, R.C., Bayley, S.T., Branton, P.E., 1993. Role of phosphorylation near the amino terminus of adenovirus type 5 early region 1A proteins. J. Gen. Virol. 74 (Pt 4), 583–95.

Eckner, R., Ewen, M.E., Newsome, D., Gerdes, M., DeCaprio, J.A., Lawrence, J.B.,

Livingston, D.M., 1994. Molecular cloning and functional analysis of the adenovirus E1A-associated 300-kD protein (p300) reveals a protein with properties of a transcriptional adaptor. Genes Dev. 8, 869–84.

Egan, C., Jelsma, T.N., Howe, J.A., Bayley, S.T., Ferguson, B., Branton, P.E., 1988. Mapping of cellular protein-binding sites on the products of early-region 1A of human adenovirus type 5. Mol. Cell. Biol. 8, 3955–9.

Engel, D.A., Hardy, S., Shenk, T., 1988. cAMP acts in synergy with E1A protein to activate transcription of the adenovirus early genes E4 and E1A. Genes Dev. 2, 1517–28.

Engel, D.A., Muller, U., Gedrich, R.W., Eubanks, J.S., Shenk, T., 1991. Induction of c-fos mRNA and AP-1 DNA-binding activity by cAMP in cooperation with either the adenovirus 243- or the adenovirus 289-amino acid E1A protein. Proc. Natl. Acad. Sci. U. S. A. 88, 3957–61.

Fax, P., Carlson, C.R., Collas, P., Taskén, K., Esche, H., Brockmann, D., 2001. Binding of PKA-RIIalpha to the Adenovirus E1A12S oncoprotein correlates with its nuclear translocation and an increase in PKA-dependent promoter activity. Virology 285, 30–41.

Fax, P., Lipinski, K.S., Esche, H., Brockmann, D., 2000. cAMP-independent activation of the adenovirus type 12 E2 promoter correlates with the recruitment of CREB-1/ATF-1, E1A(12S), and CBP to the E2-CRE. J. Biol. Chem. 275, 8911–20.

Ferrari, R., Gou, D., Jawdekar, G., Johnson, S.A., Nava, M., Su, T., Yousef, A.F., Zemke, N.R., Pellegrini, M., Kurdistani, S.K., Berk, A.J., 2014. Adenovirus small E1A employs the lysine acetylases p300/CBP and tumor suppressor Rb to repress select host genes and promote productive virus infection. Cell Host Microbe 16, 663–76.

Ferrari, R., Pellegrini, M., Horwitz, G.A., Xie, W., Berk, A.J., Kurdistani, S.K., 2008. Epigenetic reprogramming by adenovirus e1a. Science 321, 1086–8.

Fonseca, G.J., Thillainadesan, G., Yousef, A.F., Ablack, J.N., Mossman, K.L., Torchia, J., Mymryk, J.S., 2012. Adenovirus evasion of interferon-mediated innate immunity by direct antagonism of a cellular histone posttranslational modification. Cell Host Microbe 11, 597–606.

Gallina, A., Rossi, F., Milanesi, G., 2001. Rack1 binds HIV-1 Nef and can act as a Nef-protein kinase C adaptor. Virology 283, 7–18.

Gerlic, M., Faustin, B., Postigo, A., Yu, E.C.-W., Proell, M., Gombosuren, N., Krajewska, M., Flynn, R., Croft, M., Way, M., Satterthwait, A., Liddington, R.C., Salek-Ardakani, S., Matsuzawa, S., Reed, J.C., 2013. Vaccinia virus F1L protein promotes virulence by inhibiting inflammasome activation. Proc. Natl. Acad. Sci. U. S. A. 110, 7808–13.

Glenewinkel, F., Cohen, M.J., King, C.R., Kaspar, S., Bamberg-Lemper, S., Mymryk, J.S., Becker, W., 2016. The adaptor protein DCAF7 mediates the interaction of the adenovirus E1A oncoprotein with the protein kinases DYRK1A and HIPK2. Sci. Rep. 6, 28241.

Gold, M.G., Gonen, T., Scott, J.D., 2013. Local cAMP signaling in disease at a glance. J. Cell Sci. 126, 4537–4543.

Gold, M.G., Lygren, B., Dokurno, P., Hoshi, N., McConnachie, G., Taskén, K., Carlson, C.R., Scott, J.D., Barford, D., 2006. Molecular basis of AKAP specificity for PKA regulatory subunits. Mol. Cell 24, 383–95.

Gregory, S.M., Davis, B.K., West, J.A., Taxman, D.J., Matsuzawa, S., Reed, J.C., Ting, J.P.Y., Damania, B., 2011. Discovery of a viral NLR homolog that inhibits the inflammasome. Science 331, 330–4.

Hardy, S., Shenk, T., 1988. Adenoviral control regions activated by E1A and the cAMP response element bind to the same factor. Proc. Natl. Acad. Sci. U. S. A. 85, 4171–5.

Horwitz, G.A., Zhang, K., McBrian, M.A., Grunstein, M., Kurdistani, S.K., Berk, A.J., 2008. Adenovirus Small e1a Alters Global Patterns of Histone Modification. Science (80-.). 321, 1084–1085.

Howe, J.A., Bayley, S.T., 1992. Effects of Ad5 E1A mutant viruses on the cell cycle in relation to the binding of cellular proteins including the retinoblastoma protein and cyclin A. Virology 186, 15–24.

Howe, J.A., Mymryk, J.S., Egan, C., Branton, P.E., Bayley, S.T., 1990. Retinoblastoma growth suppressor and a 300-kDa protein appear to regulate cellular DNA synthesis. Proc. Natl. Acad. Sci. U. S. A. 87, 5883–7.

Huang, D.W., Sherman, B.T., Lempicki, R.A., 2008. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat. Protoc. 4, 44–57.

Huang, D.W., Sherman, B.T., Lempicki, R.A., 2009. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res. 37, 1–13.

Jelsma, T.N., Howe, J.A., Mymryk, J.S., Evelegh, C.M., Cunniff, N.F., Bayley, S.T., 1989. Sequences in E1A proteins of human adenovirus 5 required for cell transformation, repression of a transcriptional enhancer, and induction of proliferating cell nuclear antigen. Virology 171, 120–30.

Jones, M.S., Harrach, B., Ganac, R.D., Gozum, M.M.A., Dela Cruz, W.P., Riedel, B., Pan, C., Delwart, E.L., Schnurr, D.P., 2007. New adenovirus species found in a patient presenting with gastroenteritis. J. Virol. 81, 5978–84.

Kanehisa, M., Goto, S., 2000. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 28, 27–30.

Kanehisa, M., Goto, S., Kawashima, S., Nakaya, A., 2002. The KEGG databases at GenomeNet. Nucleic Acids Res. 30, 42–6.

Kannan, N., Wu, J., Anand, G.S., Yooseph, S., Neuwald, A.F., Venter, J.C., Taylor, S.S., 2007. Evolution of allostery in the cyclic nucleotide binding module. Genome Biol. 8, R264.

King, C.R., Cohen, M.J., Fonseca, G.J., Dirk, B.S., Dikeakos, J.D., Mymryk, J.S., 2016. Functional and Structural Mimicry of Cellular Protein Kinase A Anchoring Proteins by a Viral Oncoprotein. PLoS Pathog. 12, e1005621.

Komorek, J., Kuppuswamy, M., Subramanian, T., Vijayalingam, S., Lomonosova, E., Zhao, L.-J., Mymryk, J.S., Schmitt, K., Chinnadurai, G., 2010. Adenovirus type 5 E1A and E6 proteins of low-risk cutaneous beta-human papillomaviruses suppress cell transformation through interaction with FOXK1/K2 transcription factors. J. Virol. 84, 2719–31.

Komune, N., Ichinohe, T., Ito, M., Yanagi, Y., 2011. Measles virus V protein inhibits NLRP3 inflammasome-mediated interleukin-1β secretion. J. Virol. 85, 13019–26.

Kubota, T., Yokosawa, N., Yokota, S., Fujii, N., 2002. Association of mumps virus V protein with RACK1 results in dissociation of STAT-1 from the alpha interferon receptor complex. J. Virol. 76, 12676–82.

Lee, B.H., Mathews, M.B., 1997. Transcriptional coactivator cAMP response element binding protein mediates induction of the human proliferating cell nuclear antigen promoter by the adenovirus E1A oncoprotein. Proc. Natl. Acad. Sci. 94, 4481–4486.

Lee, Y.-H., Chiu, Y.-F., Wang, W.-H., Chang, L.-K., Liu, S.-T., 2008. Activation of the ERK signal transduction pathway by Epstein-Barr virus immediate-early protein Rta. J. Gen. Virol. 89, 2437–46.

Levine, A.J., van der Vliet, P.C., Rosenwirth, B., Anderson, C., Rabek, J., Levinson, A., Anderson, S., 1976. Characterization of an adenovirus early protein required for viral DNA replication: a single strand specific DNA binding proteins. Mol. Cell. Biochem. 11, 79–95.

Liu, F., Green, M.R., 1994. Promoter targeting by adenovirus E1a through interaction with different cellular DNA-binding domains. Nature 368, 520–525.

Loewenstein, P.M., Arackal, S., Green, M., 2006. Mutational and functional analysis of an essential subdomain of the adenovirus E1A N-terminal transcription repression domain. Virology 351, 312–21.

Mal, A., Piotrkowski, A., Harter, M.L., 1996. Cyclin-dependent kinases phosphorylate

the adenovirus E1A protein, enhancing its ability to bind pRb and disrupt pRb-E2F complexes. J. Virol. 70, 2911–21.

Massimi, P., Banks, L., 2005. Transformation assays for HPV oncoproteins. Methods Mol. Med. 119, 381–95.

Masterson, L.R., Cheng, C., Yu, T., Tonelli, M., Kornev, A., Taylor, S.S., Veglia, G., 2010. Dynamics connect substrate recognition to catalysis in protein kinase A. Nat. Chem. Biol. 6, 821–8.

McKenzie, A.J., Campbell, S.L., Howe, A.K., 2011. Protein kinase A activity and anchoring are required for ovarian cancer cell migration and invasion. PLoS One 6, e26552.

Mondal, A., Dawson, A.R., Potts, G.K., Freiberger, E.C., Baker, S.F., Moser, L.A., Bernard, K.A., Coon, J.J., Mehle, A., 2017. Influenza virus recruits host protein kinase C to control assembly and activity of its replication machinery. Elife 6.

Müller, U., Roberts, M.P., Engel, D.A., Doerfler, W., Shenk, T., 1989. Induction of transcription factor AP-1 by adenovirus E1A protein and cAMP. Genes Dev. 3, 1991–2002.

Palorini, R., Votta, G., Pirola, Y., De Vitto, H., De Palma, S., Airoldi, C., Vasso, M., Ricciardiello, F., Lombardi, P.P., Cirulli, C., Rizzi, R., Nicotra, F., Hiller, K., Gelfi, C., Alberghina, L., Chiaradonna, F., 2016. Protein Kinase A Activation Promotes Cancer Cell Resistance to Glucose Starvation and Anoikis. PLoS Genet. 12, e1005931.

Pattabiraman, D.R., Bierie, B., Kober, K.I., Thiru, P., Krall, J.A., Zill, C., Reinhardt, F., Tam, W.L., Weinberg, R.A., 2016. Activation of PKA leads to mesenchymal-to-epithelial transition and loss of tumor-initiating ability. Science (80-.). 351, aad3680-aad3680.

Pelka, P., Ablack, J.N.G., Fonseca, G.J., Yousef, A.F., Mymryk, J.S., 2008. Intrinsic structural disorder in adenovirus E1A: a viral molecular hub linking multiple diverse processes. J. Virol. 82, 7252–63.

Prasad, V., Suomalainen, M., Hemmi, S., Greber, U.F., 2017. Cell Cycle-Dependent Kinase Cdk9 Is a Postexposure Drug Target against Human Adenoviruses. ACS Infect. Dis. 3, 398–405.

Radko, S., Jung, R., Olanubi, O., Pelka, P., 2015. Effects of Adenovirus Type 5 E1A Isoforms on Viral Replication in Arrested Human Cells. PLoS One 10, e0140124.

Rasti, M., Grand, R.J.A., Mymryk, J.S., Gallimore, P.H., Turnell, A.S., 2005. Recruitment of CBP/p300, TATA-binding protein, and S8 to distinct regions at the N terminus of adenovirus E1A. J. Virol. 79, 5594–605.

Rozenblatt-Rosen, O., Deo, R.C., Padi, M., Adelmant, G., Calderwood, M.A., Rolland, T., Grace, M., Dricot, A., Askenazi, M., Tavares, M., Pevzner, S.J., Abderazzaq, F., Byrdsong, D., Carvunis, A.-R., Chen, A.A., Cheng, J., Correll, M., Duarte, M., Fan, C., Feltkamp, M.C., Ficarro, S.B., Franchi, R., Garg, B.K., Gulbahce, N., Hao, T., Holthaus, A.M., James, R., Korkhin, A., Litovchick, L., Mar, J.C., Pak, T.R., Rabello, S., Rubio, R., Shen, Y., Singh, S., Spangle, J.M., Tasan, M., Wanamaker, S., Webber, J.T., Roecklein-Canfield, J., Johannsen, E., Barabási, A.-L., Beroukhim, R., Kieff, E., Cusick, M.E., Hill, D.E., Münger, K., Marto, J.A., Quackenbush, J., Roth, F.P., DeCaprio, J.A., Vidal, M., 2012. Interpreting cancer genomes using systematic host network perturbations by tumour virus proteins. Nature 487, 491–5.

Sang, N., Severino, A., Russo, P., Baldi, A., Giordano, A., Mileo, A.M., Paggi, M.G., De Luca, A., 2001. RACK1 interacts with E1A and rescues E1A-induced yeast growth inhibition and mammalian cell apoptosis. J. Biol. Chem. 276, 27026–33.

Scherer, J., Yi, J., Vallee, R.B., 2014. PKA-dependent dynein switching from lysosomes to adenovirus: a novel form of host-virus competition. J. Cell Biol. 205, 163–77.

Shabb, J.B., 2001. Physiological substrates of cAMP-dependent protein kinase. Chem. Rev. 101, 2381–411.

Skalhegg, B.S., Tasken, K., 2000. Specificity in the cAMP/PKA signaling pathway. Differential expression,regulation, and subcellular localization of subunits of PKA. Front. Biosci. 5, D678-93.

Søberg, K., Jahnsen, T., Rognes, T., Skålhegg, B.S., Laerdahl, J.K., 2013. Evolutionary paths of the cAMP-dependent protein kinase (PKA) catalytic subunits. PLoS One 8, e60935.

Subramanian, T., Kuppuswamy, M., Nasr, R.J., Chinnadurai, G., 1988. An N-terminal region of adenovirus E1a essential for cell transformation and induction of an epithelial cell growth factor. Oncogene 2, 105–12.

Taylor, S.S., Ilouz, R., Zhang, P., Kornev, A.P., 2012. Assembly of allosteric macromolecular switches: lessons from PKA. Nat. Rev. Mol. Cell Biol. 13, 646–58.

Turnham, R.E., Scott, J.D., 2016. Protein kinase A catalytic subunit isoform PRKACA; History, function and physiology. Gene 577, 101–8.

van der Vliet, P.C., Levine, A.J., 1973. DNA-binding proteins specific for cells infected by adenovirus. Nat. New Biol. 246, 170–4.

Via, A., Uyar, B., Brun, C., Zanzoni, A., 2015. How pathogens use linear motifs to perturb host cell networks. Trends Biochem. Sci. 40, 36–48.

Vijayalingam, S., Subramanian, T., Zhao, L., Chinnadurai, G., 2016. The Cellular Protein Complex Associated with a Transforming Region of E1A Contains c-MYC. J. Virol. 90, 1070–1079.

Wang, H.G., Rikitake, Y., Carter, M.C., Yaciuk, P., Abraham, S.E., Zerler, B., Moran, E., 1993. Identification of specific adenovirus E1A N-terminal residues critical to the binding of cellular proteins and to the control of cell growth. J. Virol. 67, 476–88.

Welch, E.J., Jones, B.W., Scott, J.D., 2010. Networking with AKAPs: context-dependent regulation of anchored enzymes. Mol. Interv. 10, 86–97.

Whalen, S.G., Marcellus, R.C., Barbeau, D., Branton, P.E., 1996. Importance of the Ser-132 phosphorylation site in cell transformation and apoptosis induced by the adenovirus type 5 E1A protein. J. Virol. 70, 5373–83.

Whalen, S.G., Marcellus, R.C., Whalen, A., Ahn, N.G., Ricciardi, R.P., Branton, P.E., 1997. Phosphorylation within the transactivation domain of adenovirus E1A protein by mitogen-activated protein kinase regulates expression of early region 4. J. Virol. 71, 3545–53.

Whyte, P., Ruley, H.E., Harlow, E., 1988. Two regions of the adenovirus early region 1A proteins are required for transformation. J. Virol. 62, 257–65.

Wong, W., Scott, J.D., 2004. AKAP signalling complexes: focal points in space and time. Nat. Rev. Mol. Cell Biol. 5, 959–70.

Zhang, X., Odom, D.T., Koo, S.-H., Conkright, M.D., Canettieri, G., Best, J., Chen, H., Jenner, R., Herbolsheimer, E., Jacobsen, E., Kadam, S., Ecker, J.R., Emerson, B., Hogenesch, J.B., Unterman, T., Young, R.A., Montminy, M., 2005. Genome-wide analysis of cAMP-response element binding protein occupancy, phosphorylation, and target gene activation in human tissues. Proc. Natl. Acad. Sci. U. S. A. 102, 4459–64.

Appendices

Appendix A: Methods for generating high-throughput RNAseq tabular dataset.

Global cellular gene expression was examined during infections with a variety of HAdV mutants. These experiments were carried out in primary human lung fibroblasts (IMR-90) at a multiplicity of infection (MOI) of 25, 48 hours post-infection. This represents a relatively early timepoint post-infection in primary cells. Library prep from total RNA and high-throughput sequencing was performed on the Ilumina HISeq 2500 at the UCSD Genomics Centre. Sequences returned were aligned against the human hg19 reference genome (genome reference consortium human build 37) using the ELAND software. Initial data analysis, including normalization of sequencing reads and changes in gene expression, was performed using the HOMER software suite. This pilot experiment was performed only once using 6 unique viruses/conditions: (mock-infected IMR-90 cells, wild-type HAdV-5, ΔE1A HAdV, and 3 additional E1A point/deletion mutants targeting specific sites for E1A-binding partners). In total, data on 36640 unique transcripts were returned.

Appendix B: Python scripts used to analyze RNAseq and human promoter datasets.

```
"""
This script takes a tab-delimited .txt file containing the normalized RNAseq results data and extracts the data columns of interest.
"""
import sys
input = sys.argv[1]  # call script on filename of choice

handle = open(input, 'rU')
_ = handle.readline()
outfile = open('output1.csv', 'w')

# extract 3 desired data columns
for line in handle:
    items = line.strip('\n').split('\t')
    accession = items[0]
    annotation = items[7]
    annotation_edited = annotation.replace(',', '') # removes some problematic commas from within annotation field
    foldchange = items[34]
    outfile.write(','.join([accession, annotation_edited, foldchange]) + '\n') # data is now comma-delimited like other files

handle.close()

"""
This script takes the output from script1.py (output1.csv) containing our 36640 transcripts of interest and excludes all transcripts with uninteresting or negligible changes in expression
"""
handle = open('output1.csv', 'rU')
outfile = open('output2.csv', 'w')

# trim down results to genes with at least 2-fold change in expression
for line in handle:
    items = line.strip('\n').split(',')
    foldchange = items[2]
    if foldchange.startswith('0'):
        continue # ignore results that have a positive logFoldChange less than 2-fold
    elif foldchange.startswith('-0'):
        continue # ignore results that have a negative logFoldChange less than 2-fold
    outfile.write(line)

handle.close()

"""
This script takes a .csv-formatted list (CREpredictions.csv) of computationally-predicted fucntional cAMP response elements (CRE) on promoters of human RefSeq genes and extracts the data columns of interest.
"""
import csv  # easier handling of csv files
import sys

input = sys.argv[1]  # call script on filename of choice
```

```
with open(input) as handle:
    reader = csv.reader(handle)
    outfile = open('output3.csv', 'w')
    _ = next(reader)
    for line in reader:  # extract desired data columns
        accession = line[2]
        cre_prediction = line[5]
        outfile.write(','.join([accession, cre_prediction]) + '\n')

handle.close()

"""
This script takes the output from script3.py (output3.csv) containing our 20769 gene promoters of interest and
excludes all that have no predicted CRE.
"""
import csv  # easier handling of csv files

with open('output3.csv') as handle:
    reader = csv.reader(handle)
    outfile = open('output4.csv', 'w')
    for line in reader:
        accession = line[0]
        cre_prediction = line[1]
        if 'others' in cre_prediction:  # remove all genes that don't have a predicted CRE
            continue
        outfile.write(','.join([accession, cre_prediction]) + '\n')

handle.close()

"""
This script takes the data columns filtered from the two starting files and combines their contents using a
dictionary.
"""
import csv  # easier handling of csv files
outfile = open('output5.csv', 'w')

dictionary = {}

reader1 = csv.reader(open('output2.csv'))
for row in reader1:
    key = row[0]  # establish accession numbers as keys
    if key in dictionary: # avoid repeating keys, just in case
        continue
    dictionary[key] = row[1:]  # establish values as a list of other data values

reader2 = csv.reader(open('output4.csv'))
for row in reader2:
    if row[0] in dictionary:  # check for existing keys (accession numbers)
        dictionary[row[0]].append(row[1])  # if key found, append dictionary with CRE prediction!

# write out dictionary to comma-delimited format
for key, value in dictionary.items():
    data = ','.join(str(datum) for datum in value)
    outfile.write(','.join([key, data]) + '\n')
```

```
outfile.close()

"""
This script performs final edits and formatting to the output from script5.py (output5.csv).
"""
handle = open('output5.csv', 'rU')
outfile = open('rna_cre_combined_final.csv', 'w')  # final output file name

for line in handle:
    items = line.strip('\n').split(',')
    if len(items) > 3:  # get rid of entries which don't contain all 4 desired data values
        outfile.write(','.join(items[0:4]) + '\n')  # get rid of any remaining duplicates of CRE data column
    else:
        continue


handle.close()
```

Curriculum Vitae

Cason R.D. King, B.M.Sc. (Hon.)
Department of Microbiology and Immunology
Schulich School of Medicine and Dentistry
The University of Western Ontario

EDUCATION

2011 – Present, University of Western Ontario
Ph.D. Candidate – Department of Microbiology and Immunology
(Supervisor: Dr. Joe Mymryk)

2007 – 2011, University of Western Ontario
Bachelor of Medical Sciences [B.M.Sc. (Hon)] – Microbiology and Immunology

PEER-REVIEWED PUBLICATIONS

King CR, Zhang A, Tessier TM, Gameiro SF, Mymryk JS. Hacking the cell: Network intrusion and exploitation by adenovirus E1A. *mBio*. *In submission (mBio00390-18).*

King CR, Gameiro SF, Tessier TM, Zhang A, Mymryk JS. Mimicry of cellular A kinase anchoring proteins is a conserved and critical function of E1A across various human adenovirus species. *Journal of Virology*. 2018. 92(8): e01902-17.

King CR, Tessier TM, Mymryk JS. Color me infected: painting cellular chromatin with a viral histone mimic. *Trends in Microbiology*. 2016. 4(10): 774-776.

Glenwinkel F, Cohen MJ, **King CR**, Kaspar S, Bamberg-Lemper S, Mymryk JS, Becker W. The adaptor protein DCAF7 mediates the interaction of the adenovirus E1A oncoprotein with the protein kinases DYRK1A and HIPK2. *Scientific Reports*. 2016. 6: 28241.

King CR, Cohen MJ, Fonseca GJ, Dirk BS, Dikeakos JD, Mymryk JS. Functional and structural mimicry of cellular protein kinase A anchoring proteins by a viral oncoprotein. *PLoS Pathogens.* 2016. 12(5): e1005621.

King CR, Zhang A, Mymryk JS. The persistent mystery of adenovirus persistence. *Trends in Microbiology*. 2016. 24(5): 323-324.

Cohen MJ, **King CR**, Dikeakos JD, Mymryk JS. Functional analysis of the C-terminal region of human adenovirus E1A reveals a misidentified nuclear localization signal. *Virology*. 2014. 468-470: 238-243.

Marshall KS, Cohen MJ, Fonseca GJ, Todorovic B, **King CR**, Yousef AF, Zhang Z, Mymryk JS. Identification and characterization of multiple conserved nuclear localization signals within adenovirus E1A. *Virology*. 2014. 454-455: 206-214.

SCHOLARSHIPS, AWARDS, AND DISTINCTIONS

EXTERNAL AWARDS:

Ontario Graduate Scholarship (2) **May 2015**
Government of Ontario
$15'000 scholarship for excellence in graduate studies

Ontario Graduate Scholarship (1) **May 2014**
Government of Ontario
$15'000 scholarship for excellence in graduate studies

Orange Scholars Scholarship **September 2009**
The Homer Fund, Canada
$2'500 scholarship based on undergraduate grades and merit

INTERNAL AWARDS:

R.G.E. Murray Seminar Award (4) **September 2017**
Department of Microbiology and Immunology
$500 award for having the top peer-voted seminar in the department

R.G.E. Murray Seminar Award (3) **September 2016**
Department of Microbiology and Immunology
$500 award for having the top peer-voted seminar in the department

Nellie Farthing Fellowship in Medical Sciences **August 2016**
Schulich School of Medicine and Dentistry
$3'000 award for excellence in research

R.G.E. Murray Seminar Award (2) **September 2015**
Department of Microbiology and Immunology
$500 award for having the top peer-voted seminar in the department

R.G.E. Murray Seminar Award (1) **September 2014**
Department of Microbiology and Immunology
$500 award for having the top peer-voted seminar in the department

Schulich Graduate Scholarship **September 2011 – August 2016**
Schulich School of Medicine and Dentistry
$7'500 per year scholarship for a high entrance average into graduate studies

Western Scholarship of Excellence **September 2007**
University of Western Ontario
$2'000 scholarship for a high entrance average into undergraduate studies

PRESENTATION AWARDS:

Sernova Oral Presentation Award **November 2014**
Infection and Immunity Research Forum
$200 award for top oral presentation

TRAVEL AWARDS:

Graduate Student Travel Grant **June 2017**
American Society for Virology
$500 award to present at the American Society for Virology Conference

Dr. FW Luney Graduate Travel Award **June 2016**
Department of Microbiology and Immunology
$2'000 award to present at the American Society for Virology Conference

Graduate Student Travel Award **July 2013**
Division of Experimental Oncology
$1'000 award to present at the DNA Tumor Virus Meeting

DISTINCTIONS:

Transferred from M.Sc. to Ph.D. program "with distinction" **March 2013**
For passing the candidacy exam with top marks

Designated as an undergraduate "Western Scholar" **July 2011**
For meeting a minimum 80% average with no mark below 70%

Dean's Honor List **2007-2011**
All four years of undergraduate education

CONFERENCE ORAL PRESENTATIONS

*Award-winning presentation

Functional and Structural Mimicry of Cellular Protein Kinase A Anchoring Proteins by a Viral Oncoprotein. **King CR**, Cohen MJ, Mymryk JS. *American Society for Virology Meeting. University of Wisconsin-Madison, USA.* June, 2017.

Human adenovirus E1A proteins utilize functional and structural mimicry to usurp cellular protein kinase A and enhance viral infection. **King CR**, Cohen MJ, Mymryk JS. *International DNA Tumor Virus Meeting. ICGEB, Trieste, Italy.* July, 2015.

Human adenovirus E1A proteins utilize functional and structural mimicry to usurp cellular machinery and enhance viral infection. **King CR**, Cohen MJ, Mymryk JS. *American Society for Virology Meeting. London, Canada*. July, 2015.

Human adenovirus E1A proteins utilize functional and structural mimicry to usurp cellular machinery and enhance viral infection. **King CR**, Cohen MJ, Mymryk JS. *London Health Research Day. London, Canada.* April, 2015.

*An unhealthy relationship: manipulation of cellular protein kinase A by human adenovirus E1A. **King CR**, Mymryk JS. *Infection and Immunity Research Forum. University of Western Ontario, London, Canada.* November, 2014.

The E1A oncoprotein from human adenovirus uses mimicry to usurp cellular protein kinase A and enhance viral replication. **King CR**, Mymryk JS. *International DNA Tumor Virus Meeting. University of Wisconsin-Madison, USA.* July, 2014.

Characterizing the role of HAdV E1A as a viral AKAP. **King CR**, Mymryk JS. *International DNA Tumor Virus Meeting. University of Birmingham, Birmingham, United Kingdom.* July, 2013.

INVITED PRESENTATIONS

Functional and structural mimicry of cellular protein kinase A anchoring proteins by a viral oncoprotein. *Young Cancer Researchers Showcase. London Regional Cancer Program, London, Canada.* March 21, 2017. (Talk)

Functional and structural mimicry of cellular protein kinase A anchoring proteins by a viral oncoprotein. *Canadian Society for Virology Workshop at the 2016 American Society for Virology Meeting. Virginia Tech, Blacksburg, VA, USA.* June 18, 2016. (Poster)

Functional and structural mimicry of cellular protein kinase A anchoring proteins by a viral oncoprotein. *London Cancer Research Trainee Forum. London Regional Cancer Program, London, Canada.* November 5, 2015. (Talk)

TEACHING EXPERIENCE

Graduate Teaching Assistant **January 2015 – April 2016**
Microbiology and Immunology 4200B (Molecular Virology)
Duties included designing curriculum, marking exams, and administering weekly presentations

Graduate Teaching Assistant **September 2012 – December 2013**
Microbiology and Immunology 2100A (Biology of Prokaryotes)
Duties included facilitating weekly labs, proctoring tests, and grading reports

VOLUNTEER AND APPOINTED POSITIONS

Volunteer for 34th annual meeting for American Society for Virology **June 2015**
Organized and ran oral presentation workshops

Volunteer for Departmental Outreach Committee **March 2014**
Department of Microbiology and Immunology
Helped recruit and inform potential new students interested in either undergraduate or graduate studies in Microbiology and Immunology

Graduate Representative to the Canadian Society of Microbiologists **2014 – 2016**
Department of Microbiology and Immunology
Conveyed information regarding the annual general meeting of the CSM, funding opportunities, and other relevant information from the society to departmental students and faculty

Infection and Immunity Research Forum organizing committee **2011 – 2012**
Department of Microbiology and Immunology
Organized multiple annual meetings hosted by Microbiology and

Immunology graduate students at which scientists are invited
to present their research in the scope of infection and immunity

Social Committee **2011 – 2016**

Department of Microbiology and Immunology

Active member throughout my graduate studies in which organization
of departmental functions, fundraising, and social outreach were emphasized