1983

An Analysis Of The Dna Repair-recombination Functions And The Uvsx-uvsy Dna Repair Pathway Of Bacteriophage T4

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AN ANALYSIS OF THE DNA REPAIR-RECOMBINATION FUNCTIONS AND THE uvsX-uvsY DNA REPAIR PATHWAY OF BACTERIOPHAGE T4

by
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Submitted in partial fulfillment
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Doctor of Philosophy

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September, 1982

ABSTRACT

Due to the complexity of the uvsX-uvsY DNA repair pathway of bacteriophage T4, we have attempted to define the functions and repair mechanisms involved in this pathway using several different techniques. The DNA repair-recombination functions of the pathway were analyzed biochemically and genetically to aid in our understanding the role of these functions in DNA repair and other important processes in the cell.

The functions of the uvsX and uvsY gene products of the uvsX-uvsY pathway were studied by analyzing the DNA repair defects produced by mutations in these two genes. The DNA intermediates formed after uv-irradiation were analyzed by sucrose gradient centrifugation. Results of the studies suggested that the uvsX and uvsY gene products function in a "joining" reaction of DNA fragments.

The uvsX and uvsY genes are also involved in DNA maturation since DNA maturation-defective mutations in gene 49 are suppressed by uvsX and uvsY mutations. We have studied the role of the DNA repair-recombination functions in DNA maturation by analyzing the suppressors of a gene 49 mutation. In addition to mutations in the uvsX and uvsY

genes, mutations in genes 46, 47 and 59 also suppressed gene 49 mutations. From these results, we suggest that the DNA repair-recombination genes are involved in a DNA processing pathway and that the suppression of gene 49 mutations occurs because the DNA intermediates formed by these DNA repair-defective mutants bypass the requirement for the gene 49 function.

We have analyzed the interrelationships of the DNA repair-recombination functions by studying extragenic suppressors of mutations in these genes. A study of these suppressors can indicate interactions between the various gene products involved. Using this approach, we have isolated two new suppressors (sur and uvsU) of mutations in the DNA repair-recombination genes. The sur mutation is a general suppressor of these mutations suggesting the closely related nature of the repair-recombination functions. However, the uv-sensitive mutation uvsU, specifically suppresses uvsX mutations. Studies of uvsU have led us to propose a model whereby DNA replication is coupled with the DNA repair-recombination functions. In addition, an analysis of the diverse activities of the suppressors of mutations in the repair-recombination genes has allowed us to suggest a possible reaction sequence for these functions.

ACKNOWLEDGEMENTS

I wish to thank Dr. Kaney Ebisuzaki for his guidance and encouragement during the years I have worked with him. He has been both supervisor and friend and I greatly appreciate all that he has done for me.

In addition, I wish to thank the other members of Dr. Ebisuzaki's laboratory especially Cathy Zahradka for her assistance in some of the experiments and Linda Wheaton for an excellent job of typing the thesis. I thank Dr. Ian Walker and Dr. Ron Behme for being on my advisory committee.

Special thanks to my wife Judy, my two sons Michael and Jefferey, and my parents Leonard and Audrey for their constant support and understanding during the course of this work.

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INTRODUCTION

A Historical Review

It is essential for the survival of an organism to maintain the integrity of its genetic material. To achieve this, most organisms have evolved various mechanisms for the repair of their deoxyribonucleic acid (DNA) which has been damaged by different physical and chemical agents. The DNA repair process is so important that a simple organism such as bacteriophage T4 devotes approximately 5 - 10 percent of its total genetic information to DNA repair functions. This thesis describes an analysis of the DNA repair process in bacteriophage T4-infected E. coli , as well as an analysis of the relationship of DNA repair to DNA replication.

Many repair-deficient mutants of T4 have been isolated. These mutants can be segregated into several groups on the basis of similarities in their phenotypes (Table 1). Most of the mutants have also been assigned to specific DNA repair pathways using the double mutant test. This test compares the ultraviolet (uv) sensitivity of a double mutant to the sensitivity of the two single mutants (Ebisuzaki et al., 1975). Two mutants in different genes are assigned to the same DNA repair pathway if the double mutant is no more uv-sensitive than either of the single mutants. Two mutants

TABLE I

A Comparison of the Phenotypes of T4 UV-Sensitive Mutants

Mutant Gene	Sensitivities	Recombination Frequency	DNA Replication	Suppressors
denV	uv	wild type	wild type	——
uvsX	uv, alkylation, crosslinking, deamination	decreased	DNA arrest	[33], [55]
uvsY	uv, alkylation, crosslinking, deamination	decreased	DNA arrest	[33], [55]
46	uv, alkylation, crosslinking, deamination	decreased	DNA arrest	[33] [55] [das] [uvsW(dar)]
47	uv, alkylation, crosslinking, deamination	decreased	DNA arrest	[33] [55] [das] [uvsW(dar)]
59	uv, alkylation, crosslinking	decreased	DNA arrest	[33] [55] [uvsW(dar)]
uvsW(dar)	uv, alkylation, crosslinking, hydroxyurea	decreased	wild type	[uvsX] [uvsY] [59]
58	uv, alkylation	increased	DNA delay	——
mms	uv, alkylation	wild type	wild type	——
uvs58	uv, alkylation	wild type	slightly delayed	——
uvs79	uv, alkylation	wild type	slightly delayed	——

are assigned to different pathways if the uv-sensitivity of the double mutant is the sum of the sensitivities of the single mutants. The results of double mutant tests on various combinations of repair-deficient mutants has suggested that T4 codes for at least three different DNA repair pathways (Harm, 1963; Boyle and Symonds, 1969; Ebisuzaki et al.,1975; Hamlett and Berger, 1975; van Minderhout and Grimbergen, 1976).

1. The Excision Repair Pathway

The excision repair pathway consists of endonuclease V, coded for by the T4 denV gene (Yasuda and Sekiguchi, 1970; Friedberg and King, 1971) and E. coli DNA polymerase I (Maynard-Smith et al., 1970; Wallace and Melamede, 1972; Ebisuzaki et al., 1975). This pathway may also involve a T4 coded 5'-3' exonuclease specific for pyrimidine dimer containing DNA (Oshima and Sekiguchi, 1972; Friedberg et al., 1974) and T4 polynucleotide ligase (gene 30) (Maynard-Smith and Symonds, 1973).

The products of the genes involved in the excision repair pathway catalyze the removal of pyrimidine dimers from uv-irradiated DNA in the following manner. The denV gene product is responsible for the first two steps of excision repair. Recent studies have revealed that the denV

gene product is bifunctional with both pyrimidine dimer-DNA glycosylase and apyrimidinic/apurinic endonuclease activities (Demple and Linn, 1980; Radany and Friedberg, 1980; Seawell et al., 1980; Warner et al., 1980; McMillan et al., 1981; Warner et al., 1981). The pyrimidine dimer-DNA glycosylase activity cleaves the glycosyl bond of the pyrimidine on the 5' side of the dimer. The resulting apyrimidinic site is recognized by the apyrimidinic/apurinic endonuclease activity and the 3' phosphodiester bond is hydrolysed producing a nick on the 5' side of the dimer. In a subsequent step, E. coli DNA polymerase I synthesizes a DNA patch, approximately four nucleotides long (Yarosh et al., 1981), in the 5'-3' direction using the 3'end of the nick as primer. Simultaneously, a 5'-3' exonuclease activity (either the exonuclease of DNA polymerase I or the T4 dimer specific exonuclease) excises the dimer from the DNA. The final step in the process involves the joining of the DNA strands. This reaction is believed to be catalyzed by polynucleotide ligase.

The T4 excision repair pathway appears to be specific for the repair of uv-irradiated DNA. Mutants in the denV gene were found to be sensitive to uv-irradiation (Harm, 1963), but not to other DNA damaging agents (Bernstein, 1981). However, evidence has suggested that the denV gene product may be involved in the repair of heteroduplex loops

(Berger and Benz, 1975). The mechanism for the repair of heteroduplex loops may be analogous to the excision repair of pyrimidine dimers.

2. The uvsX-uvsY Repair Pathway

The second DNA repair pathway of T4 is thought to involve the products of the repair-recombination genes uvsX, uvsY, uvsW, 46, 47, 58 and 59. The isolation of two uv-sensitive mutants, x_{HARM} [uvsX] and y_{10} [uvsY], which were defective in recombination as well as DNA repair, led to the discovery of the second DNA repair pathway (Harm, 1963; Boyle and Symonds, 1969). Double mutant tests revealed that both the uvsX and uvsY genes functioned in the same DNA repair pathway. These tests also showed that this pathway was different from the denV-controlled excision repair pathway. Subsequently, new uv-sensitive mutants in genes uvsW and 58 were isolated (Hamlett and Berger, 1975). Studies of these mutants showed that genes uvsW and 58 also functioned in the uvsX-uvsY repair pathway. The DNA arrest genes 46, 47 and 59 have not been assigned to the second DNA repair pathway by double mutant tests. However, they have been grouped with genes uvsX, uvsY, uvsW and 58 since these seven genes appear to have similar functions (Table 1).

2a. Functions of the DNA repair-recombination genes

The DNA repair-recombination genes appear to have a wide variety of different functions in the infected cell. These functions include DNA repair, recombination and replication. The uvsX-uvsY repair pathway, unlike the excision repair pathway, removes a wide variety of lesions from damaged DNA. Mutants in the genes of the second repair pathway were shown to be sensitive to uv-irradiation and to treatments with various alkylating, crosslinking and deaminating agents (Bernstein, 1981).

The repair-recombination genes also affect phage growth and recombination. Mutants in all seven genes exhibited burst sizes which were substantially reduced from the wild type level. The recombination frequencies of mutants in all the genes, except 58, were reduced from the wild type level (Harm, 1963; Bernstein, 1968; Boyle and Symonds, 1969; Wu et al., 1975; Hamlett and Berger, 1975; Cunningham and Berger, 1977). The formation of concatemeric DNA by these mutants was also deficient (Shah and Berger, 1971; Wu and Yeh, 1974; Dewey and Frankel, 1975b; Cunningham and Berger, 1977). By contrast, gene 58 mutants exhibited recombination frequencies considerably elevated above the wild type level (Mufti and Bernstein, 1974; Leung et al., 1975). Furthermore, in these mutants, the formation of concatemeric DNA was delayed (Hamlett and Berger, 1975).

Mutants involved in the uvsX-uvsY repair pathway affected DNA replication to varying degrees. The [uvsW] mutant exhibited the kinetics of wild type DNA replication, while gene 58 mutants showed a delay in replication (Yegian et al., 1971; Hamlett and Berger, 1975). Mutants in genes 46, 47 and 59 initiated DNA replication normally, but shortly thereafter, DNA synthesis was arrested (Wiberg et al., 1962; Wu et al., 1972). "Minute" mutants of genes uvsX and uvsY also exhibited a DNA arrest phenotype (Dewey and Frankel, 1975a; Cunningham and Berger, 1977). However, the original isolates, x_{HARM}[uvsX] and y_{10}[uvsY], formed normal sized plaques and exhibited wild type levels of replication (Shimizu and Sekiguchi, 1974). This was later shown to be due to the presence of suppressors (Hamlett and Berger, 1975).

2b. Suppressors of DNA repair-recombination mutants

Mutations in the repair-recombination genes are suppressed by a number of different mutations. The DNA arrest and "minute" plaque phenotype of [uvsX] and [uvsY] have been shown to be suppressed by unidentified suppressor mutations (Hamlett and Berger, 1975; Cunningham and Berger, 1977). These suppressors did not affect uv-sensitivity. Hercules and Wiberg (1971) showed that the DNA arrest and

burst size phenotype of gene 46 and 47 mutations were partially suppressed by [das]. However, [das] had no effect on gene 59 mutations (Wiberg and Swanson, 1975). The [dar] and [uvsW] mutations, which may map in the same gene, suppressed the DNA arrest phenotype of gene 46; 47 and 59 mutations, but had no effect on [uvsX] or [uvsY] (Wu and Yeh, 1975; Cunningham and Berger, 1977; Wu and Yeh, 1978). However, the hydroxyurea sensitivity of [dar] and [uvsW] have been shown to be suppressed by mutations in genes 59, uvsX and uvsY (Wu and Yeh, 1975; Cunningham and Berger, 1977; Wu and Yeh, 1978). The DNA arrest phenotype of mutations in all five of the DNA arrest genes was suppressed by mutations in either gene 33 or 55 (Hosoda et al., 1971; Shah and Berger, 1971; Shalitin and Naot, 1971; Wu et al., 1972; Cunningham and Berger, 1977). Mutants in genes 33 and 55 were deficient in late gene expression (Bolle et al., 1968) suggesting that late gene product(s) are involved in the shutoff of DNA replication in DNA arrest mutants.

2c. Suppression of gene 49 by DNA repair mutations

The DNA maturation genes 16, 17 and 49 are involved in the processing of concatemeric DNA to a form which can be packaged in phage heads. Mutants in these three genes were lethal and resulted in the accumulation of empty or

partially filled phage heads (King, 1968; Simon, 1972; Luftig et al., 1971; Luftig and Ganz, 1972) and unpackaged replicative DNA. In gene 16 and 17 mutants, replicative DNA sedimenting as 200 S components accumulated, while gene 49 mutants accumulated "very fast sedimenting DNA" (>1000 S) (Frankel et al., 1971; Kemper and Janz, 1976). Gene 49 has been shown to code for an endonuclease involved in the processing of replicative DNA intermediates to a form which can be packaged (Frankel et al., 1971; Minagawa and Ryo, 1978; Nishimoto et al., 1979; Kemper and Garabett, 1981). Gene 49 mutations were suppressed by mutations in genes uvsX and uvsY. This suggested that these DNA repair genes were somehow involved in the DNA maturation process (Dewey and Frankel, 1975a; Cunningham and Berger, 1977; Shah and Delorenzo, 1977).

3. A Third Pathway of DNA Repair

Three mutants, [mms1], [uvs58] and [uvs79], are grouped together in a third DNA repair pathway. Double mutant tests showed that these three mutants did not function in either the excision or uvsX-uvsY DNA repair pathways (Ebisuzaki et al., 1975; van Minderhout and Grimbergen, 1976; van Minderhout et al., 1978). The [mms1] mutant has not been mapped but both [uvs58] and [uvs79] were shown to be mutated

in gene 41. Tests to determine if [mms1] functions in the same DNA repair pathway as [uvs58] and [uvs79] have not been performed. However, these three mutants are grouped together because they exhibit very similar phenotypes. The [mms1], [uvs58] and [uvs79] mutants were sensitive to uv and methyl methanesulfonate. These mutants have little or no effect on recombination. The [mms1] mutant exhibited normal levels of DNA replication while the [uvs58] and [uvs79] mutants showed a slight delay in DNA replication.

The mechanisms involved in this pathway are unknown. However, the third DNA repair pathway might involve replication-repair if the product of gene 41 is required. The product of gene 41 is necessary for the priming reaction of DNA replication (Liu et al., 1978).

PURPOSE OF THE THESIS

The purpose of this thesis was to analyze the uvsX-uvsY DNA repair pathway coded for by the DNA repair-recombination genes of bacteriophage T4. Since the functions of the repair-recombination gene products and the repair mechanisms involved in this pathway are only vaguely understood (see Historical Review), we have tried to define the various parameters related to the uvsX-uvsY DNA repair pathway using the following approaches:

1. The DNA intermediates in the pathway were analyzed by sucrose gradient centrifugation.

2. The involvement of the DNA repair-recombination pathway in the DNA maturation process was studied by a genetic analysis of the suppressors of a gene 49 mutation. Since the two known suppressors [uvsX] and [uvsY] were mutants in the uvsX-uvsY DNA repair pathway, we wanted to find out if all or only a part of this repair pathway was involved in the suppression of gene 49 mutations and the basis for this suppression.

3. The interactions of the DNA repair-recombination gene products and the relationship of the uvsX-uvsY DNA repair pathway to other processes in the infected cell were determined by a genetic analysis of extragenic suppressors

of mutations in the repair-recombination genes. A study of these suppressors should provide insight into the macromolecular and metabolic interactions of the DNA repair-recombination functions. For example, studies of the suppression of gene 32 mutations indicated numerous interacting components associated with the gene 32 function (Mosig et al., 1978).

MATERIALS AND METHODS

1. Bacterial and Phage Strains

The bacterial and phage strains used in this study are listed in Tables 2 and 3, respectively. Specific phage mutants will be designated in the figures and tables, but in the text an abbreviated designation is used in which the gene number or symbol is enclosed in brackets, wherever possible. For example, [das,46] is das,amN130. Where several alleles exist, specific designations will be made.

2. Media

Phage stocks were grown in the glycerol, casamino acids medium of Fraser and Jerrel (1953) and titered on EHA top and bottom agar (Steinberg and Edgar, 1962). Infected cells for burst size experiments, mapping and the construction of multiple mutants were grown in nutrient broth supplemented with 0.08 M NaCl, 1.0 mM MgCl and gelatin (0.01 g/liter). Infected cells for all other experiments were grown and labelled in either the M9 medium (Miller, 1972) or the Vogel and Bonner medium (Wu and Yeh, 1973). The latter medium is referred to as CT - (without thymine) and CT + (with thymine).

TABLE II

Bacterial Strains

Strain	Properties
E.coli B	restricts amber mutations
E.coli B40Sul	suppresses amber mutations
E.coli CR63	suppresses amber mutations
E.coli C600λ	restricts rII mutations, suppresses amber mutations
E.coli TR201	lacks thymidylate synthetase
E.coli R15	lacks DNA polymerase I, restricts amber mutations
E.coli K803-Rif^R-2	restricts wild type T4 but not βgt at 27°, suppresses amber mutations
E.coli NF58[illegible]if^R-2	restricts wild type T4 but not βgt at 27°, restricts amber mutations

TABLE III

Bacteriophage Strains

Strain	Mutant Gene(s)	Properties
$T4D^+$		wild type T4
amC42	[1]	lacks deoxyribonucleotide kinase
amN66	[16]	DNA maturation defective
amN56	[17]	DNA maturation defective
amE605	[30]	DNA arrest, lacks DNA ligase
tsL84	[40]	abnormal head assembly
amN81	[41]	deficient in DNA primase
amN122	[42]	lacks deoxycytidylate hydroxymethylase
amN130	[46]	DNA arrest, DNA repair deficient
amNG163 x 3	[47]	DNA arrest, DNA repair deficient
amS78	[47]	DNA arrest, DNA repair deficient
amE727 x 1	[49]	DNA maturation defective, lacks endonuclease VII
tsC9	[49]	DNA maturation defective, lacks endonuclease VII
amBL292	[55]	lacks late protein synthesis
amE51	[56]	lacks deoxycytidine triphosphatase
amE219	[58]	DNA delay, DNA repair deficient, deficient in DNA primase

TABLE III (continued)

Strain	Mutant Gene(s)	Properties
amC5 x 3	[59]	DNA arrest, DNA repair deficient
amHL628	[59]	DNA arrest, DNA repair deficient
βgt	[βgt]	lacks β-glucosyl transferase
das13	[das]	suppresses gene 46 and 47 mutations
v	[denV]	uv-sensitive, lacks endonuclease V
FlD3	[rIIA]	lacks rIIA protein
rIIb50	[rIIB]	lacks rIIB protein
mms1	[mms]	DNA repair deficient
r10	[sur]	suppresses gene 46, 47, 59, uvsX and uvsY mutations
C69	[uvsU]	DNA repair deficient
amS17	[uvsX]	DNA arrest, DNA repair deficient
amC16	[uvsX]	DNA arrest, DNA repair deficient
x_{HARM}	[uvsX]	DNA repair deficient
amS52	[uvsY]	DNA arrest, DNA repair deficient
y_{10}	[uvsY]	DNA repair deficient
m22	[uvsW]	DNA repair deficient
amS17-r10	[sur,uvsX]	pseudorevertant of amS17
amS76	[uvsU,uvsX,49]	pseudorevertant of amE727 x 1

3. Reagents

[Methyl-^{3}H] and [2-^{14}C] thymidine were obtained from New England Nuclear Corporation. Deoxyadenosine and mitomycin C were received from Sigma Chemical Co. and thymidine from Calbiochem.

4. Preparation of ^{3}H-labelled T7

Escherichia coli TR201 or B40SU1 (10^{9} cells/ml) were infected with T7 at a multiplicity of infection (moi) = 0.1 and labelled with [methyl-^{3}H] thymidine (10 μC/1.25 μg/ml). After complete lysis, the phage were purified by a repeated cycle of differential centrifugation (Richardson, 1966). This was followed by CsCl density gradient centrifugation (density = 1.476) in a SW50.1 rotor at 23,000 rpm for 40 hours. Phage-containing fractions were dialyzed against 0.5 M NaCl containing 10^{-3} M MgCl (Davison and Freifelder, 1962).

5. Preparation of ^{14}C-labelled T4

T4 phage were labelled under conditions similar to those described above, except for the use of moi = 5 and [2-^{14}C]thymidine (0.4 μC/10μg/ml). The labelled phage were

purified by differential centrifugation as described below.

6. Preparation of High Titer Phage Stocks

E. coli B40Sul or CR63 (1×10^8 cells/ml) were infected with phage (moi = 0.01) and aerated at 37°C until the cells were lysed (6-8 hours). Deoxyribonuclease was added to the lysate and incubation was continued for 30 minutes at room temperature. This was followed by the addition of a few drops of chloroform to complete the lysis of the cells. The lysate was centrifuged at 5,000 x g for 30 minutes to remove cell debris and the supernatant was centrifuged at 20,000 x g for 60 minutes. The pellet was resuspended in 0.05 M potassium phosphate buffer (pH 6.8) and further purified by a second cycle of differential centrifugation. Phage stocks were stored in 0.05 M potassium phosphate buffer at 5°C.

7. Phage Crosses and Burst Size Determination

Phage crosses were performed as described by Chase and Doerman (1958). Log-phase cells (3×10^9 cells/ml) were infected at a moi = 10. T4 antiserum was added at 5 minutes postinfection to inactivate unadsorbed phage and the culture was diluted (1×10^{-6}) into warm medium (37°C) at 10

minutes. Incubation with aeration was continued for 60 minutes at which time chloroform was added to lyse the cells.

8. UV-Irradiation

UV-irradiation of phage was performed as previously described (Behme and Ebisuzaki, 1975). Phage (1×10^{10} plaque forming units/ml) in 0.05 M phosphate (pH 6.8) were irradiated at a distance of 59 cm with a General Electric germicidal lamp (G8T5). The irradiated phage were immediately diluted into nutrient broth and plated to determine the surviving fraction.

Infected cells (1×10^{9} cells/ml) were irradiated in ice cold CT^{-} or M9 medium lacking casamino acids, at 59 cm from the source for 1.5 minutes (0.8 $J/m^2/sec$).

9. Kinetics of DNA Synthesis

E. coli R15 (5×10^{8} cells/ml) was infected at a moi = 10 and labelled at 5 minutes postinfection with [methyl-^{3}H]thymidine ($5\mu C/3\mu g$/ml) in the presence of deoxyadenosine (150 μ g/ml). Samples taken at various times were precipitated with 5% trichloroacetic acid (TCA) and

bovine serum albumin was added as carrier. The samples were filtered on GF/A glass fiber filters, washed with 5% TCA, dried and counted for radioactivity.

10. A Complementation Test for Distinguishing DNA Repair Mutants

E. coli R15 was infected in the presence or absence of 5-10 μg/ml mitomycin C at a moi = 10 for single infections or at a moi = 5 per phage strain for mixed infections. At 5 minutes postinfection, [methyl-^{3}H]thymidine (5μC/3μg/ml) and deoxyadenosine (150 μg/ml) were added. Samples were taken at various times and precipitated in cold 5% TCA. The samples were filtered, washed, dried and counted for radioactivity.

11. Degradation of Host DNA

The degradation of host DNA was measured using a procedure described by Hercules and Wiberg (1971). E. coli B was diluted fiftyfold into fresh medium and grown to a concentration of 5 x 10^8 cells/ml in the presence of [methyl-^{3}H]thymidine (5 μC/3μg/ml) and deoxyadenosine (150 μg/ml). The labelled cells were centrifuged, washed and resuspended in fresh medium. After 15 minutes incubation,

the cells were infected with various phage strains (moi = 10) and samples were removed at various times. The samples were precipitated with 5% TCA and prepared for counting as previously described.

12. Analysis of DNA Repair by Sucrose Gradient Centrifugation

The analysis of DNA repair by sucrose gradient centrifugation is a modification of a procedure described by Wu and Yeh (1973). E. coli TR201 or R15 (1×10^9 cells/ml) was infected (25°C) in CT or M9 medium at moi = 10. Three minutes postinfection, [methyl-^{3}H]thymidine (10 μC/3μg/ml) was added and the incubation continued for 30 minutes. The infected cells were chilled, centrifuged and resuspended in medium lacking casamino acids and uv-irradiated as previously described. A non-irradiated sample was used as a control. After irradiation, the infected cells were diluted twofold into medium containing 2 mg/ml thymidine plus twice the usual concentration of casamino acids. Samples were incubated (33°C) for various times, then diluted fourfold into ice cold Tris buffer (0.15 M Tris•HCl (pH 8.0), 0.05 M NaCl, 0.05 M EDTA). Samples for alkaline sucrose gradients were centrifuged and resuspended in 0.4 ml Tris buffer (pH 8.0). Lysozyme (100 μg/ml) was

added and the samples incubated for 2.5 minutes at 5°C. This was followed by the addition of Triton X-100 (1%) and NaOH (0.4 N). The lysates were left on ice for 10 minutes. Labelled phage was added at the first lysis step as a reference. Lysates were poured on top of 5 to 20% alkaline sucrose gradients containing 0.05 M Na_3PO_4 (pH 12.2), 0.05 M EDTA and 0.01% Triton X-100 and centrifuged in a SW40 rotor at 40,000 rpm for 270 minutes.

Samples for neutral sucrose gradients were diluted twofold into a solution containing 0.1 M EDTA (pH 8.5), 0.1 M NaCN and 200 μg/ml lysozyme and kept at 65 C for 1 minute. Sarkosyl (0.1%) was added and after a 10 minute incubation, the samples were cooled on ice. The lysates were poured on top of 5 to 20% neutral sucrose gradients containing 0.15 M NaCl, 0.015 M Na citrate and 0.015 M EDTA (pH 8.0) and centrifuged in a SW40 rotor at 40,000 rpm for 210 minutes. Fractions collected from the top of the gradients were precipitated by the addition of 5% TCA and filtered on GF/A glass fiber filters. The filters were washed with 5% TCA, dried and counted for radioactivity.

13. Analysis of DNA Intermediates by Sucrose Gradient Centrifugation

DNA intermediates were labelled and analyzed as described by Shah and Berger (1971). E. coli TR201 infected with various phage strains (moi = 10) were pulse labelled at 5 minutes postinfection with [methyl-^{3}H] thymidine (10 μC/ml) in the presence of deoxyadenosine (150 μg/ml). At 7 minutes postinfection, unlabelled thymidine (2 mg/ml) was added and the incubation continued for various times. Samples were lysed in an equal volume of lysis mixture containing 0.1 M Tris·HCl (pH 8.0), 0.1 M EDTA, 0.01 M KCl and 100 μg/ml lysozyme and incubated for 10 minutes at 37°C. This was followed by a 3 minute incubation at 65°C and the addition of Sarkosyl (2%). Incubation was continued for 20 minutes. Lysates were poured on top of 5 to 20% neutral sucrose gradients containing 0.02 M Tris (pH 8.0), 0.01 M EDTA and 0.1% Sarkosyl. Gradients were centrifuged in a SW40 rotor at 40,000 rpm for 120 minutes, then fractionated. The fractions were prepared for counting as previously described.

RESULTS

1. Sedimentation Analysis of Intracellular DNA in Repair-Defective Mutants.

The purpose of these studies is to gain further insight into the pathways of DNA repair by sucrose gradient centrifugation analysis of the DNA "intermediates" formed after uv-irradiation. In these studies we have compared the repair reactions in cells infected with wild-type T4 and the uv-sensitive mutant v[denV] (Harm, 1963) to assess the totality of repair reactions and the performance of the denV gene-controlled excision repair pathway. In other experiments, we have used single and double mutants to channel the repair reactions into selected pathways and have studied especially the defects introduced by the x_{HARM} [uvsX] and y_{10} [uvsY] mutations.

1a. The excision repair pathway

By comparing the fate of uv-irradiated intracellular DNA in cells infected by wild type T4 and the uv-sensitive mutant [denV], it is possible to assess the contribution of the denV gene-controlled excision repair pathway. This repair reaction was studied by alkaline sucrose gradient

centrifugation of ^{3}H-labeled DNA which was extracted at various times after uv-irradiation.

The role of the denV gene in excision repair is graphically illustrated in Figures 1 and 2. After uv-irradiation of cells infected by wild type T4, the DNA is rapidly nicked, resulting in a broad distribution in size (Figure 1b; average number of nicks, approximately two to three per single strand: See Figure 2.). This nicking is rapidly followed by ligation reaction(s) which were noted as early as 2 minutes after uv-irradiation (Figure 1c). However, completion of repair to reconstitute mature size DNA molecules in wild type T4 infection requires up to 30 minutes after infection. In contrast, in [denV]-infected cells, the endonucleolytic activity is slower, fewer strand breaks appear (Figure 1b and 2), and the appearance of mature size repaired DNA molecules is delayed from 30 to 50 minutes after irradiation (Figure 1i-k). These experiments indicate that the denV gene-controlled excision repair pathway has an important role in repair but in its absence other pathways of repair operate, slowly introducing nicks into DNA and eventually "joining" or reforming mature size DNA molecules.

FIGURE 1

Alkaline sucrose gradient sedimentation of ^{3}H-labelled DNA extracted at various times after uv-irradiation from cells infected with wild type T4 and v[denV]. See Materials and Methods for further details. The time given in each panel denotes the incubation time (33°C) after uv-irradiation. In panel a, N signifies DNA from nonirradiated cells. Solid and dashed arrows indicate the sedimentation position of mature T4 and T7 DNA, respectively. The direction of sedimentation is from left to right. Symbols: wild type T4 (●), v[denV] (○).

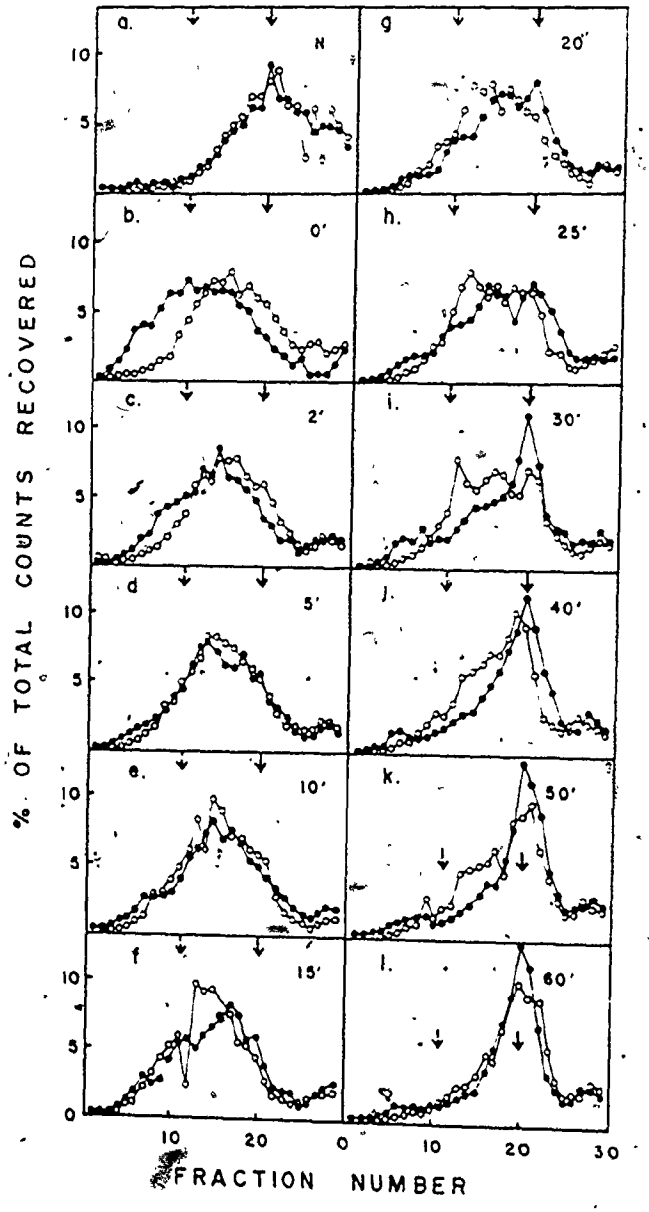
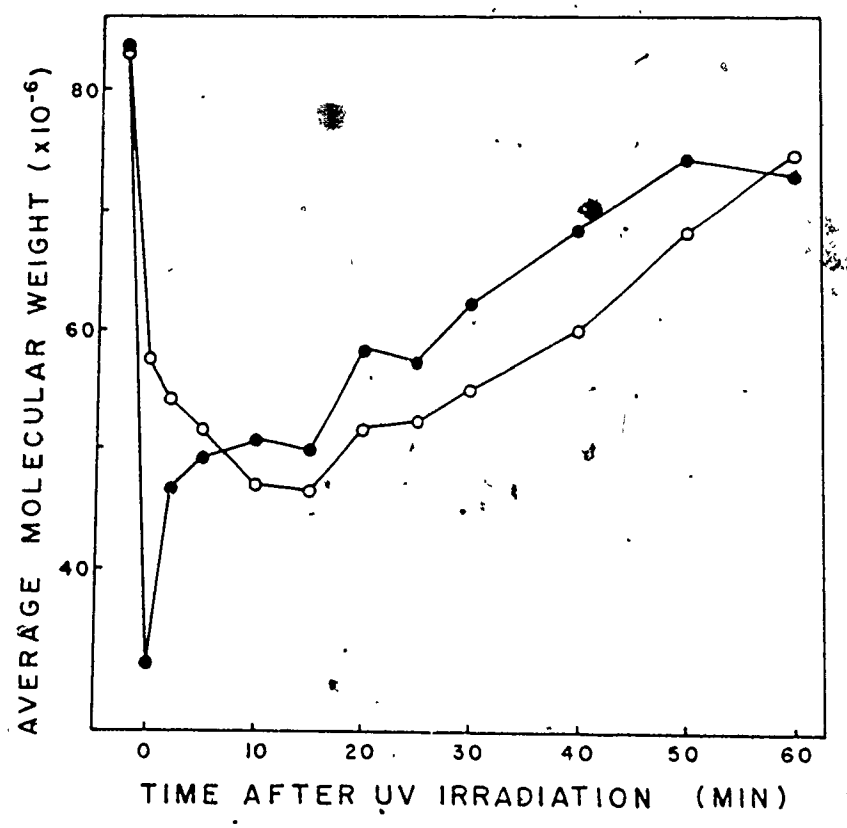

a.
N
g
20'
b.
0'
h.
25'
c.
2'
i.
30'
d
5'
j.
40'
e.
10'
k.
50'
f
15'
l.
60'
% OF TOTAL COUNTS RECOVERED
FRACTION NUMBER

FIGURE 2

Average molecular weight of single strand DNA extracted at various times after uv-irradiation from cells infected with wild type T4 and v[denV]. This figure depicts data shown in Figure 1. The average molecular weight was calculated using the formula $S°_{20,w} = 0.0528\ M^{0.4}$, with $S°_{20,w} = 37.2$ S for T7 DNA under alkaline conditions (Studier, 1965). The first point indicates the molecular weight of DNA from nonirradiated, infected cells. Symbols as in Figure 1.


AVERAGE MOLECULAR WEIGHT (x10⁻⁶)
80
60
40
0
10
20
30
40
50
60
TIME AFTER UV IRRADIATION (MIN)

1b. mms pathway

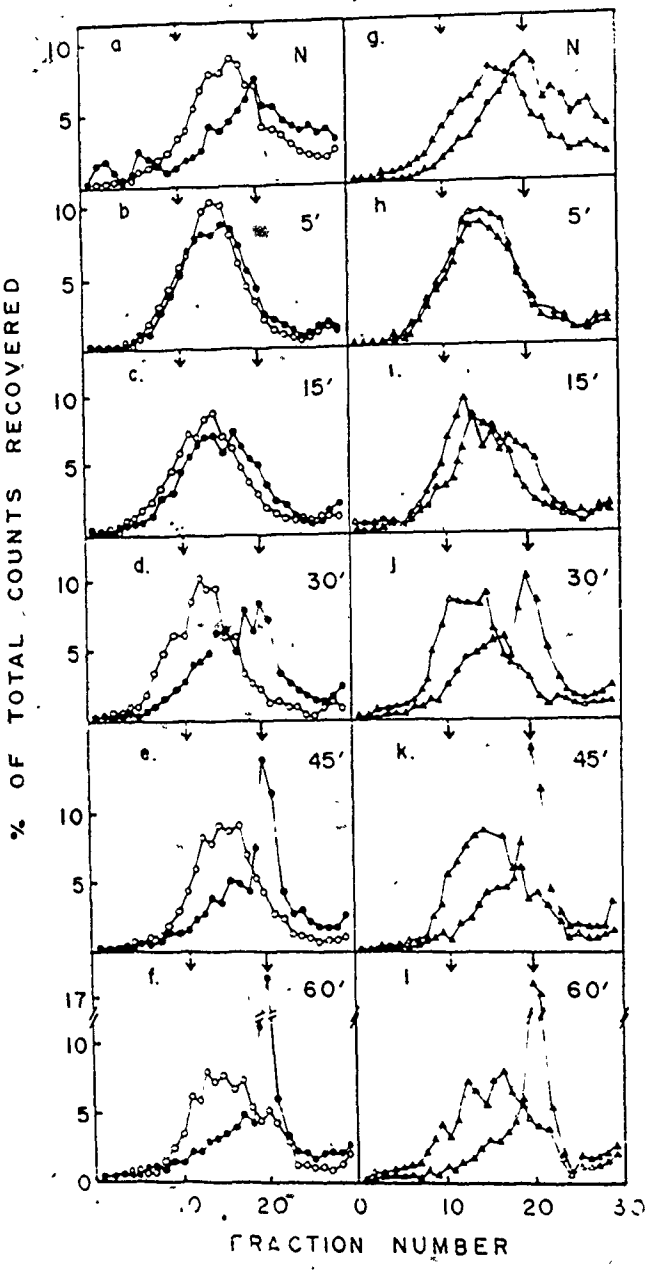
In a previous communication (Ebisuzaki et al., 1975), it was shown that the mms gene defined a separate pathway of repair. It is possible to study the contribution of the mms pathway by comparing cells infected with [denV,mms] and [denV] in experiments similar to those described in Figure 1. Such experiments indicated that the mms gene has little or no effect on the repair reactions as assayed by the procedures used here (Figure 3). This failure to detect any differences might be due to the lack of sensitivity in the analysis since the mms gene makes only minor contributions to uv-sensitivity (Ebisuzaki et al., 1975) or due to the possibility that the mms pathway involves mechanisms not measurable by the analysis used here.

1c. uvsX-uvsY pathway

In experiments similar to those described in Figures 1 and 2, we have studied the role of the uvsX and uvsY genes in DNA repair. In order to avoid contributions by the excision repair pathway, we have again used [denV] as the control (Figure 3) and compared this mutant with [denV,uvsX] and [denV,uvsY]. As indicated in Figure 3a and g, single strand DNA from cells infected with [denV,uvsX] and [denV,uvsY] is fragmented (approximately one nick per single

FIGURE 3

Alkaline sucrose gradient sedimentation of ^{3}H-labelled DNA extracted at various times after uv-irradiation from cells infected with v[denV], v,mms1[denV,mms], v,x_{HARM} [denV,uvsX] and v,y_{10} [denV,uvsY]. Conditions were the same as described in Figure 1. Symbols: v[denV] (●), v,x_{HARM} [denV,uvsX] (○), v,mms1 [denV,mms] (▲), v,y_{10} [denV,uvsY] (Δ).

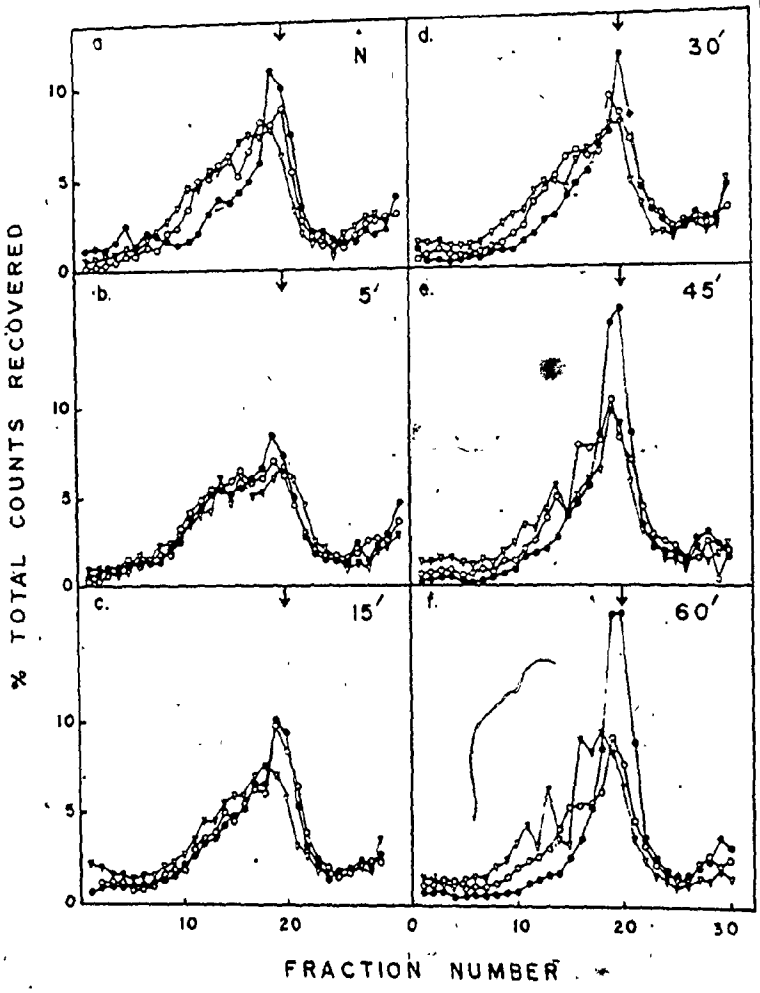

a
N
g.
N
b
5'
h
5'
c.
15'
i.
15'
d.
30'
j
30'
e.
45'
k.
45'
f.
60'
l
60'
% OF TOTAL COUNTS RECOVERED
FRACTION NUMBER

strand) and there are few concatemers, even before uv-irradiation. After uv-irradiation, endonucleolytic cleavage takes place in cells infected with [denV,uvsX] and [denV,uvsY] at rates comparable to the controls involving [denV] (Figure 3b, h). However, cells infected with [denV,uvsX] and [denV,uvsY] were defective in subsequent reactions which involve "joining" of strands to make mature size DNA (Figure 3f, l).

As a means of providing a more complete analysis of the repair reactions, neutral sucrose gradients were performed (Figure 4). These studies indicated that with cells infected with [denV], [denV,uvsX] or [denV,uvsY] uv-irradiation introduced very few double-strand breaks (Figure 4b). However, in cells infected with [denV,uvsX] or [denV,uvsY], a sizeable fraction of the DNA duplexes was shorter than mature size, before uv-irradiation. These studies together with the alkaline sucrose gradients mentioned earlier indicate that after uv-irradiation of infected cells, single-strand breaks are introduced into DNA duplex molecules and that these breaks are subsequently sealed to make intact duplex molecules, if the uvsX and uvsY products are present.

FIGURE 4

Neutral sucrose gradient sedimentation of ^{3}H-labelled DNA extracted at various times after uv-irradiation from cells infected with v[denV], v,x_{HARM} [denV,uvsX] and v,y_{10} [denV,uvsY]. Conditions were the same as described in Figure 1. Symbols: v[denV] (●), v,x_{HARM} [denV,uvsX] (○), v,y_{10} [denV,uvsY] (▽).

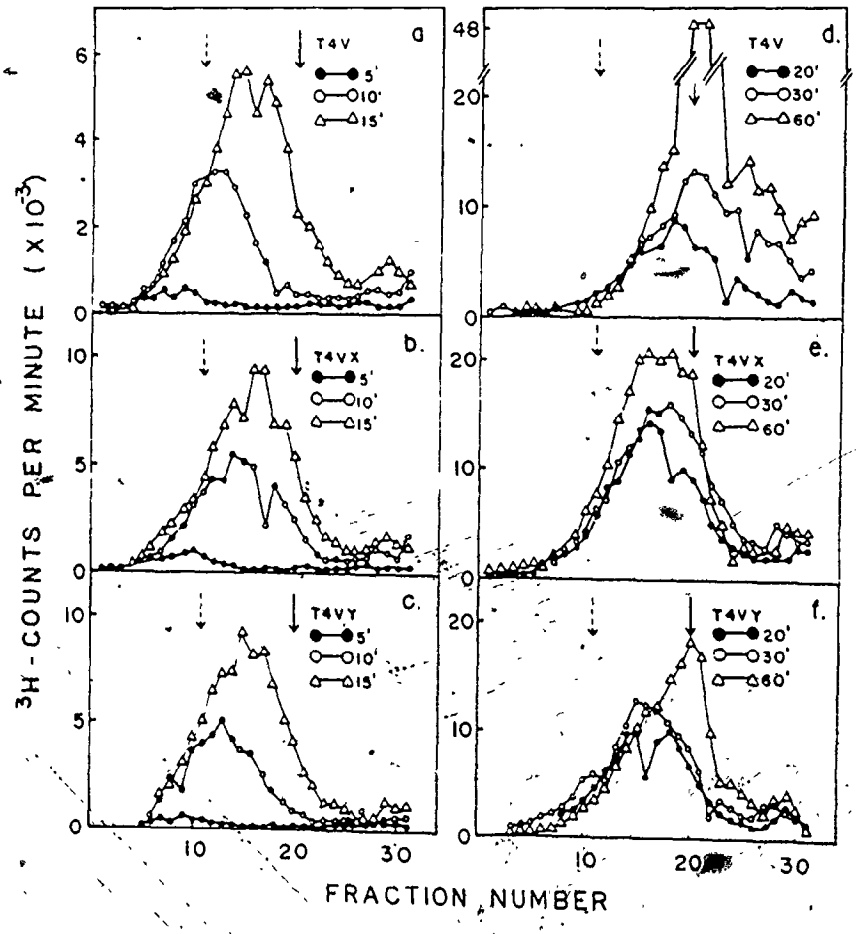

a
N
d.
30′
b.
5′
e.
45′
c.
15′
f.
60′
% TOTAL COUNTS RECOVERED
FRACTION NUMBER

1d. Intracellular DNA prior to uv-irradiation

As noted previously, in cells infected with [denV,uvsX] and [denV,uvsY] most of the single-strand DNA was smaller than mature size. Is this decreased size a function of a slower rate of replication or of assimilation of fragments? To test this possibility, DNA was extracted and analyzed by alkaline sucrose gradients at various times after infection. Until about 15 minutes after infection, the intracellular DNA in cells infected with [denV], [denV,uvsX] and [denV,uvsY] was generally of similar size (Figure 5a-c). In infection with [denV], the single strands continue to be extended and at 30 minutes postinfection, the single strands are of mature size and concatemers are also found. In contrast, in cells infected with [denV,uvsX] and [denV,uvsY], there is very little increase in the size of the single strands between 15 and 30 minutes (Figures 5e, f). In infections with [denV,uvsX], there was little increase in the length of the DNA strands even 60 minutes postinfection. DNA extracted from cells infected with [denV,uvsY] is generally similar in size to that from [denV,uvsX] infected cells but at 60 minutes, the single strands reach mature size. We have no explanation for this difference in behavior of the two double mutants. It should also be noted that in cells infected with [denv,uvsX] and [denV,uvsY], the amount of concatemers is greatly reduced as

FIGURE 5

Alkaline sucrose gradient sedimentation of ^{3}H-labelled DNA extracted at various times postinfection from cells infected (25° C) with v[*denV*], v,x$_{HARM}$[*denV*,*uvsX*] and v,y$_{10}$[*denV*,*uvsY*].


a
T4V
5'
10'
15'
b.
T4VX
5'
10'
15'
c.
T4VY
5'
10'
15'
d.
T4V
20'
30'
60'
e.
T4VX
20'
30'
60'
f.
T4VY
20'
30'
60'
^{3}H-COUNTS PER MINUTE (X 10^{-3})
FRACTION NUMBER

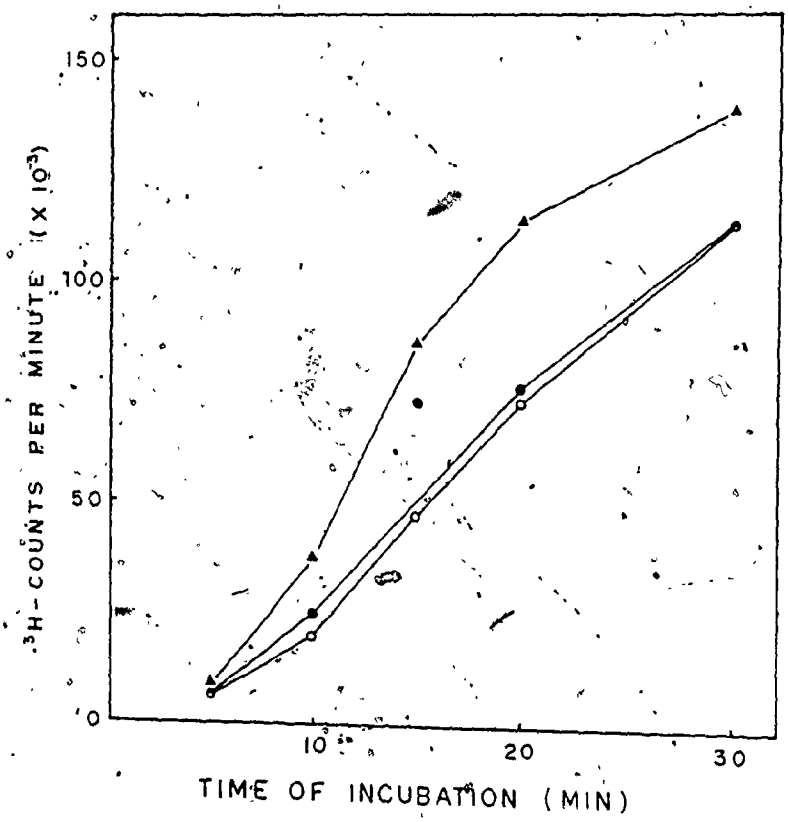
compared to control infections with [denV]. The observations that the rate of replication of the single strand is similar in infections with [denV], [denV,uvsX] and [denV,uvsY] during the early phase after infection suggests that there is no gross abnormality in replication or in the joining of Okazaki fragments. The accumulation of DNA fragments during the later period after infection, suggests that there might be a defect in the "joining" of long DNA fragments or in some sort of uncontrolled endonuclease activity.

1e. DNA synthesis

In order to ascertain whether changes in the rate of DNA synthesis might account for the defect observed in infection with [denV,uvsX] and [denV,uvsY], we assayed for incorporation of [methyl-^{3}H]thymidine in cells infected with [denV], [denV,uvsX] and [denV,uvs Y]. Generally, the total incorporation of [methyl-^{3}H]thymidine is similar in cells infected with the three mutants (Figure 6). In data not shown here, at 60 minutes after infection there is somewhat greater (about 20-30%) total incorporation of thymidine in cells infected with [denV] as compared to cells infected with [denV,uvsX] and [denV,uvsY]. These results indicate that [uvsX] and [uvsY] mutations do not introduce a gross

FIGURE 6

The DNA synthesis of cells infected with v[denV], v,x_{HARM} [denV,uvsX] and v,y_{10} [denV,uvsY] at 25°C. The incorporation of [^{3}H]thymidine into acid-insoluble material was measured at various times postinfection. See Materials and Methods for further details. Symbols: v[denV] (⊙), v,x_{HARM} [denV,uvsX] (▲), v,y_{10} [denV,uvsY] (●).


150
100
50
0
10
20
30
^{3}H-COUNTS PER MINUTE (X 10^{-3})
TIME OF INCUBATION (MIN)

defect in the overall DNA polymerization reaction(s).

1f. DNA synthesis after uv-irradiation

In order to test the role of the denV, uvsX and uvsY genes in DNA synthesis after uv-irradiation, the experimental protocol was altered so that unlabelled thymidine was used prior to uv-irradiation and [methyl-^{3}H]thymidine was introduced after uv-irradiation. The total incorporation of [methyl-^{3}H]thymidine after uv-irradiation paralleled the capacity for repair, with wild type showing the greatest incorporation, followed by [denV], and with [denV,uvsX] and [denV,uvsY] showing the least incorporation (Table 4). Alkaline sucrose gradient analysis indicated that the DNA synthesized after uv-irradiation ("postlabeled ") in cells infected with T4 was approximately of the same size as the DNA fragments formed from DNA labeled prior to uv-irradiation ("prelabeled") (Figure 7g-i and Figure 1d, f, j). This coincidence in size and the large amount of DNA synthesized after uv-irradiation suggest that the bulk of the "postlabeled" DNA is not associated with a gap filling reaction in DNA repair. In contrast, soon after uv-irradiation in cells infected with [denV], [denV,uvsX] or [denV,uvsY], the "postlabeled" DNA strands are very heterogeneous and considerably smaller in size than

TABLE IV

Incorporation of [Methyl-^{3}H] Thymidine by UV-Irradiated and Nonirradiated T4-Infected Cells [a]

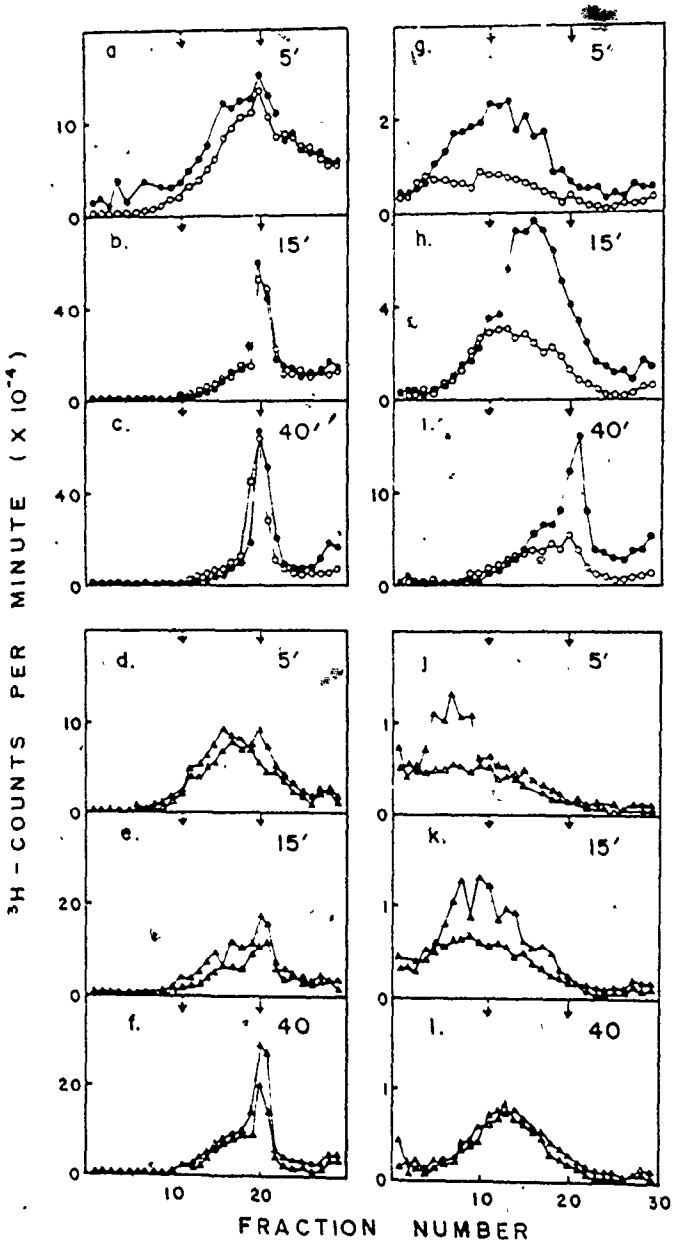
	Wild Type	v[denV]	v,x HARM [denV,uvsX]	v,y$_{10}$ [denV,uvsY]
5' Label				
+uv	278 [b]	182	123	114
-uv	1720	2080	1140	772
15' Label				
+uv	1250	542	267	165
-uv	5260	5000	2040	2330
40' Label				
+uv	2270	1420	339	254
-uv	5000	6670	2380	2940

[a] Infected cells were grown (25°C) in media containing 20µg/ml thymidine, uv-irradiated, then labelled with [methyl-^{3}H] thymidine (10µC/3µg/ml) at 33°C for the times indicated. Control samples were not uv-irradiated.

[b] Counts per minute ($\times 10^{-3}$).

FIGURE 7

Alkaline sucrose gradient sedimentation of ^{3}H-labelled DNA extracted from nonirradiated and uv-irradiated cells infected with wild type T4, v[denV], v,x$_{HARM}$[denV,uvsX] and v,y$_{10}$ [denV,uvsY]. Samples of infected cells were taken from the experiment described in Table 4. The times given in each panel indicate the time of incorporation of [^{3}H]thymidine at 33°C. Panels (a)-(f) show the sedimentation of DNA from nonirradiated, infected cells while panels (g)-(l) show the sedimentation of DNA from uv-irradiated, infected cells. Symbols: wild type T4 (●), v[denV] (o), v,x$_{HARM}$[denV, uvsX] (▲), v,y$_{10}$[denV,uvsY] (Δ).


a
5′
b.
15′
c.
40′
d.
5′
e.
15′
f.
40
g.
5′
h.
15′
i.
40′
j
5′
k.
15′
l.
40
^{3}H - COUNTS PER MINUTE (X 10^{-4})
FRACTION NUMBER

the fragments derived from "prelabeled" DNA (Figure 7g-l). This type of synthetic pattern may result if replication is aborted at the sites of thymine dimers which may persist because of the [denV] mutation. At later times after uv-irradiation, the newly synthesized single-strand DNA reaches mature size only in wild type- and [denV]-infected cells (Figure 7i). In contrast to the results obtained in uv-irradiated cells, in the nonirradiated control cells, the DNA is either mature size in wild type- and [denV]-infected cells or is approaching mature size in [denV,uvsX]- and [denV,uvsY]-infected cells (Figure 7a-f). These experiments indicate that varying amounts of DNA replication occur after uv irradiation but it is not known whether this replication is directly related to repair.

2. DNA Repair-Recombination Functions in the DNA Processing Pathway of T4

In the following experiments, we study the interactions of certain DNA repair-recombination genes on the DNA maturation process of T4. We have found that gene 49 mutations were suppressed by mutations in genes 46, 47 and 59 as well as uvsX and uvsY. We suggest that these five genes constitute a contiguous part of a DNA repair pathway, whose primary function involves the processing of DNA.

These functions are also necessary for DNA replication.

2a. Amber mutants of uvsX and uvsY

Amber mutants of uv-sensitive genes uvsX and uvsY were isolated as part of a separate study to identify the corresponding gene products. Since previous observations indicated that [uvsX] and [uvsY] mutants were found among the suppressors of gene 49 mutations (Dewey and Frankel, 1975a; Cunningham and Gerger, 1977; Shah and Delorenzo, 1977), we plated [49] on E. coli B and tested the resulting plaques for uv-sensitivity on E. coli B and on the amber suppressor strain, B40Sul. Amber uv-sensitive mutants were tested to see if they were [uvsX] or [uvsY] mutants by a complementation test requiring DNA synthesis in the presence of mitomycin C. As noted previously, mitomycin C inhibited DNA replication in cells infected with [uvsX] or [uvsY] (Shimizu and Sekiguchi, 1974; Melamede and Wallace, 1977) but elevated levels of DNA replication were observed on coinfection with [uvsX] and [uvsY] (Figure 8). Although this complementation was performed with x_{HARM} (Harm, 1963) and y_{10} (Boyle and Symonds, 1969) similar complementation results were obtained with the newly isolated amber[uvsX] and [uvsY] mutants (Figure 9). Furthermore, the complementations were specific when these mutants were

FIGURE 8

The effect of mitomycin C (5μg/ml) on the DNA synthesis of T4 phage-infected E. coli R15. The incorporation of [^{3}H]thymidine was measured in the absence (open symbols) and presence (closed symbols) of mitomycin C. See Materials and Methods for further details. Symbols: wild type T4 (o,●), x_{HARM} [uvsX] (Δ,▲), y_{10} [uvsY] (□,■), x_{HARM} [uvsX] + y_{10} [uvsY] in the presence of mitomycin C (x).

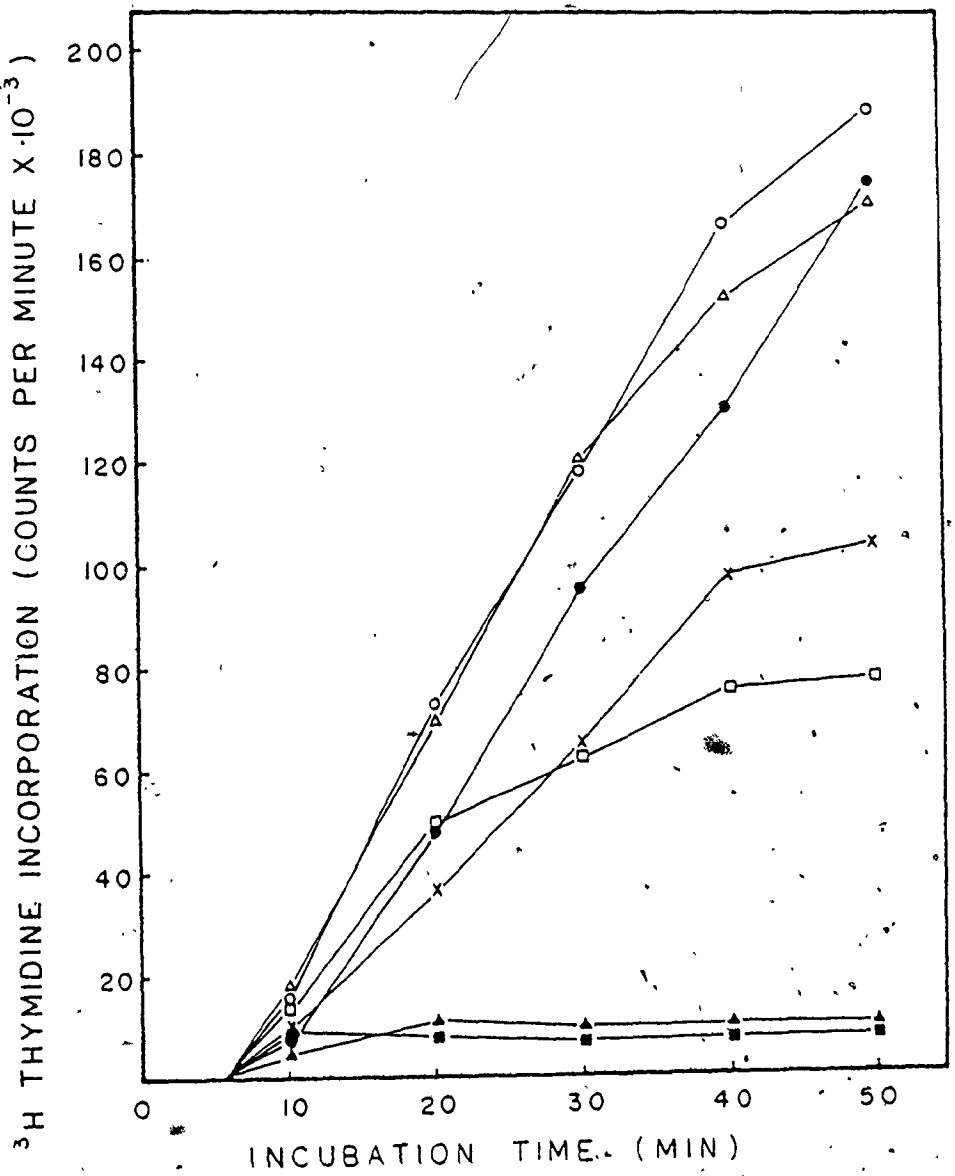
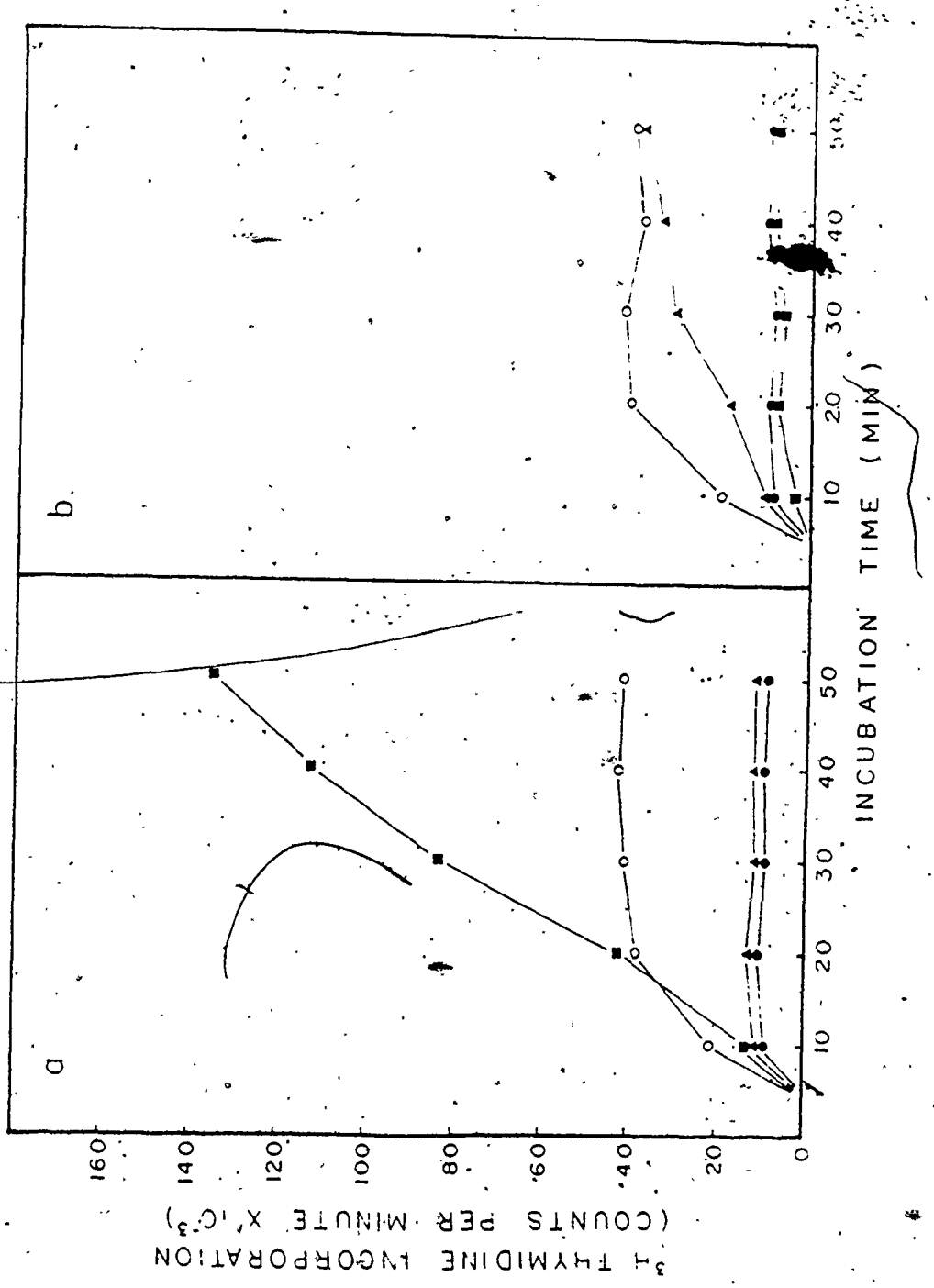

^{3}H THYMIDINE INCORPORATION (COUNTS PER MINUTE X·10^{-3})
200
180
160
140
120
100
80
60
40
20
0
10
20
30
40
50
INCUBATION TIME (MIN)

FIGURE 9

A complementation test for distinguishing [uvsX] and [uvsY] mutants. Pseudorevertants of amE727xl[49] were mixedly infected with x_{HARM}[uvsX] or y_{10}[uvsY] and tested for the incorporation of [^{3}H]thymidine in the absence (open symbols) and presence (closed symbols) of mitomycin C (5μg/ml). See Materials and Methods for further details. Symbols: (a) amS17,amE727xl[uvsX,49] (o,●), amS17,amE727 xl[uvsX,49] + x_{HARM}[uvsX] (▲), amS17,amE727xl[uvsX,49] + y_{10}[uvsY] (■); (b) amS52,amE727xl[uvsY,49] (o,●), amS52, amE727xl[uvsY,49] + x_{HARM}[uvsX] (▲), amS52,amE727xl[uvsY,49] + y_{10}[uvsY] (■).


a
b
0
20
40
60
80
100
120
140
160
10
20
30
40
50
INCUBATION TIME (MIN)
^{3}H THYMIDINE INCORPORATION
(COUNTS PER MINUTE X10^3)

tested against each other and with other uv-sensitive mutants, including [30], [46], [47], [58] and [59]. We observed am[uvsX] and [uvsY] mutants that gave levels of DNA-replication approaching wild-type levels as well as others that gave lower levels similar to the xm and ym mutants described by Cunningham and Berger (1977) and the fdsA and fdsB mutants of Dewey and Frankel (1975a). For all our subsequent experiments we have used amS17[uvsX] and amS52[uvsY] which give low levels of DNA replication and show a DNA arrest phenotype.

2b. New suppressors of gene 49 mutants

Of the 10 amber uv-sensitive mutants isolated as suppressors of [49], one was identified as a gene 47 mutant (Figure 10). We have observed that the DNA synthesis of cells infected with gene 47 mutants is inhibited by mitomycin C to a similar extent as the DNA synthesis of [uvsX] and [uvsY] mutants. The suppression of [49] by amS78[47] as well as by [uvsX] and [uvsY] suggested that a DNA repair pathway might be involved in the suppression of gene 49 mutations. Consequently, a series of multiple mutants were constructed and tested for suppression by determining their burst size and plaque forming ability (Table 5). These studies indicated that [46] and

FIGURE 10

The suppression of a gene 49 mutation by a gene 47 mutation. The pseudorevertant amS78,amE727x1[47,49] complemented with mutations in genes uvsX, uvsY, 58, 59 and 46 but did not complement a gene 47 mutation. Complementation was tested as described in Figure 9. Symbols: no mitomycin C (open symbols), 5µg/ml mitomycin C (closed symbols), amS78,amE727x1[47,49] (○,●), amNG163x3[47] (△,▲), amS78,amE727x1[47,49] + x_{HARM} [uvsX] (■), amS78,amE727x1[47,49] + y_{10} [uvsY] (▼), amS78,amE727x1[47,49] + amNG163x3[47] in the presence of mitomycin C (x).

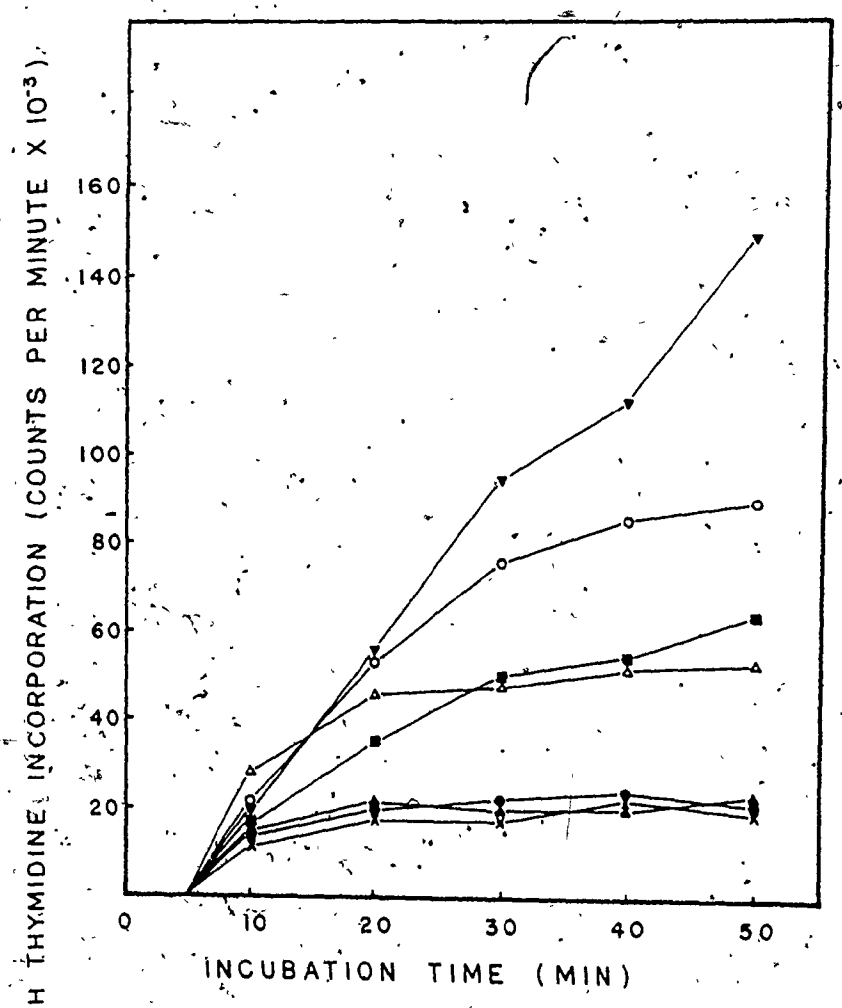

^{3}H THYMIDINE INCORPORATION (COUNTS PER MINUTE X 10^{-3})
160
140
120
100
80
60
40
20
0
10
20
30
40
50
INCUBATION TIME (MIN)

TABLE V

Burst Size Analysis of T4 Mutants

Infecting Phage	Mutant Gene(s)	Burst Size[a]
T4D$^+$	——	152 ± 48
amE605	[30]	6 ± 2
amN130	[46]	19 ± 2
amNG163 x 3	[47]	8 ± 2
amE727 x 1	[49]	2 ± 1
amE219	[58]	54 ± 19
amHL628	[59]	34 ± 5
das13	[das]	150 ± 58
amS17	[uvsX]	23 ± 9
amS52	[uvsY]	35 ± 35
m22	[uvsW]	67 ± 7
am605,amE727 x 1	[30,49]	1 ± 1
amN130,amE727 x 1	[46,49]	14 ± 4
amNG163 x 3,amE727 x 1	[47,49]	9 ± 2
amE219,amE727 x 1	[58,49]	1 ± 1
amHL628,amE727 x 1	[59,49]	29 ± 4
amS17,amE727 x 1	[uvsX,49]	38 ± 6
amS52,amE727 x 1	[uvsY,49]	26 ± 16
m22,amE727 x 1	[uvsW,49]	4 ± 2
das13,amN130	[das,46]	51 ± 6
das13,amNG163 x 3	[das,47]	39 ± 4
das13,amE727 x 1	[das,49]	1 ± 1
das13,amN130,amE727 x 1	[das,46,49]	30 ± 12
das13,amNG163 x 3,amE727 x 1	[das,47,49]	25 ± 3

[a]The average of three experiments.

amNG163x3[47] did suppress [49] as indicated by the elevated burst size but failed to produce plaques. The triple mutants [das,46,49] and [das,47,49] gave an increased burst size as well as plaque formation indicating more effective suppression. [Das] which was previously isolated as a suppressor of mutations in genes 46 and 47 (Hercules and Wiberg, 1971), does not suppress [49] by itself. Although [59] also suppressed [49], the mutants [uvsW] and [58] did not, despite evidence that genes uvsW and 58 function in the same DNA repair pathway as uvsX and uvsY. Similarly, [30] did not suppress [49] although gene 30 has been classified together with genes 46 and 47 as a DNA arrest phenotype (Bolle et al., 1968; Wood and Revel, 1976).

2c. Pathway analysis

The suppression of gene 49 mutations by mutations in genes uvsX, uvsY, 59, 46 and 47 suggest that these five genes are involved in a common function, possibly a single DNA repair pathway. Two genes are assigned to the same pathway, based on the argument that double mutants on a single pathway have a uv-sensitivity similar to that of the single mutants, whereas double mutants on separate pathways have additive effects on uv-sensitivity (Ebisuzaki et al., 1975). Previous studies indicated that genes uvsX, uvsY,

uvsW, and 58 are involved in the same DNA repair pathway (Harm, 1963; Boyle and Symonds, 1969; Symonds et al., 1973; Hamlett and Berger, 1975). Gene 59 can also be assigned to this pathway since the double mutant [uvsX,59] was no more uv-sensitive than the single mutants (Figure 11).

Similar tests were not possible with genes 46 and 47 because the recombinants [uvsX,46], [uvsX,47], [46,59], [uvsX,das, 46] and [uvsX,das,47], where uvsX = amS17, were all lethal. Separately [das,46], [das,47] and [uvsX] plated on E. coli B. The reason for the lethality is unknown; and the situation is further complicated by the observation that compared to [46] and [das,46], [uvsX,46]. and [uvsX,das,46] have respectively lower levels of DNA replication (Figure 12).

The difficulties encountered with genes 46 and 47 led to an alternative approach to studying the relationship of these genes in the DNA repair pathway. If we assume an uncomplicated situation, then the metabolic intermediate(s) or product(s) from the first mutational block in the pathway could accumulate and should not be influenced by subsequent blocks in the pathway. On the other hand, if the genes functioned in two unrelated pathways, intermediates or products from both blocks could accumulate. Shah and Berger (1971) noted that pulse labeled T4 DNA from cells infected

FIGURE 11

The uv-sensitivity of various T4 phage strains plated on E.coli B. Symbols: wild type T4 (x), amS17[uvsX] (▼), amHL628 [59] (▲), amS17,amHL628[uvsX,59] (●).

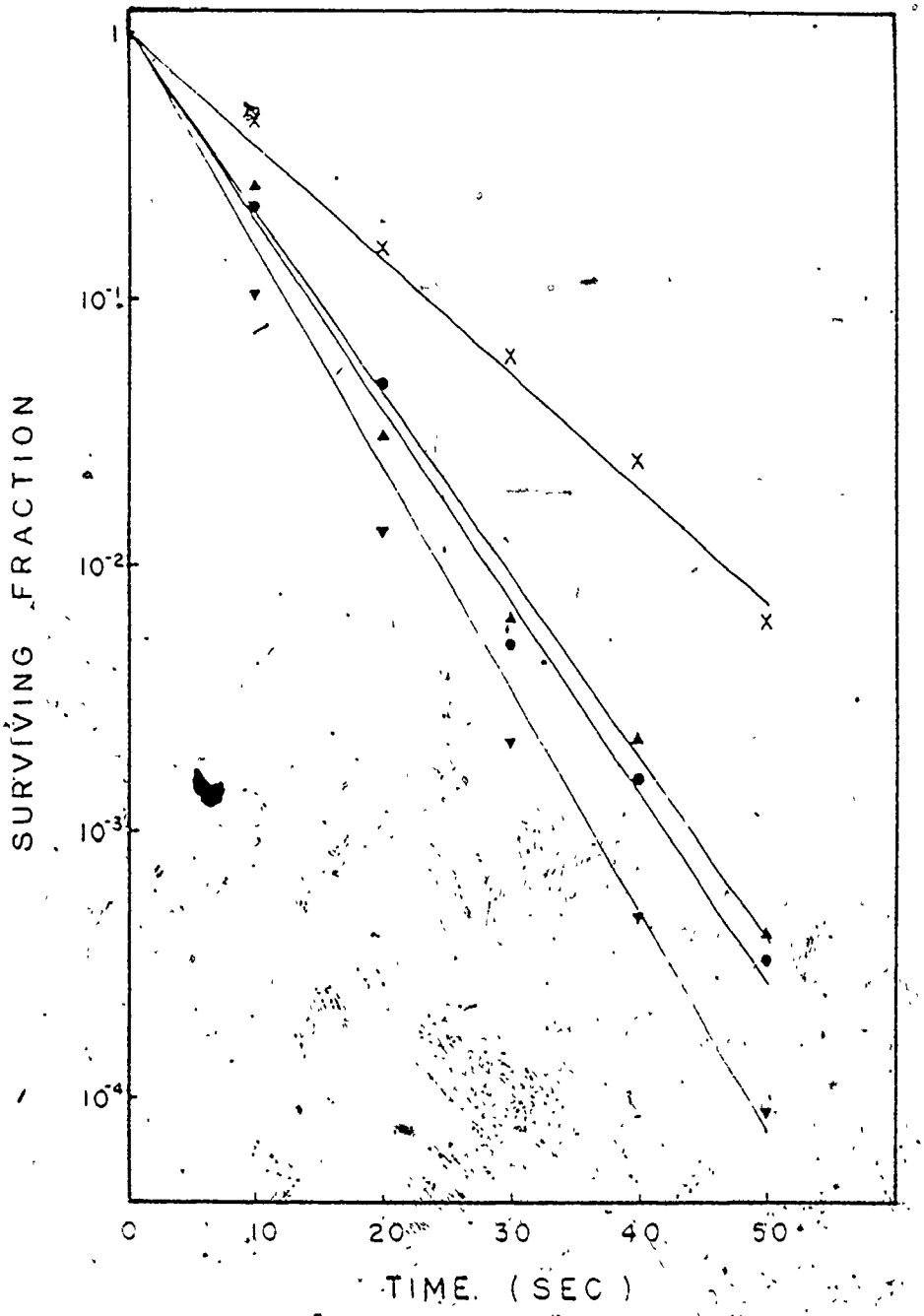
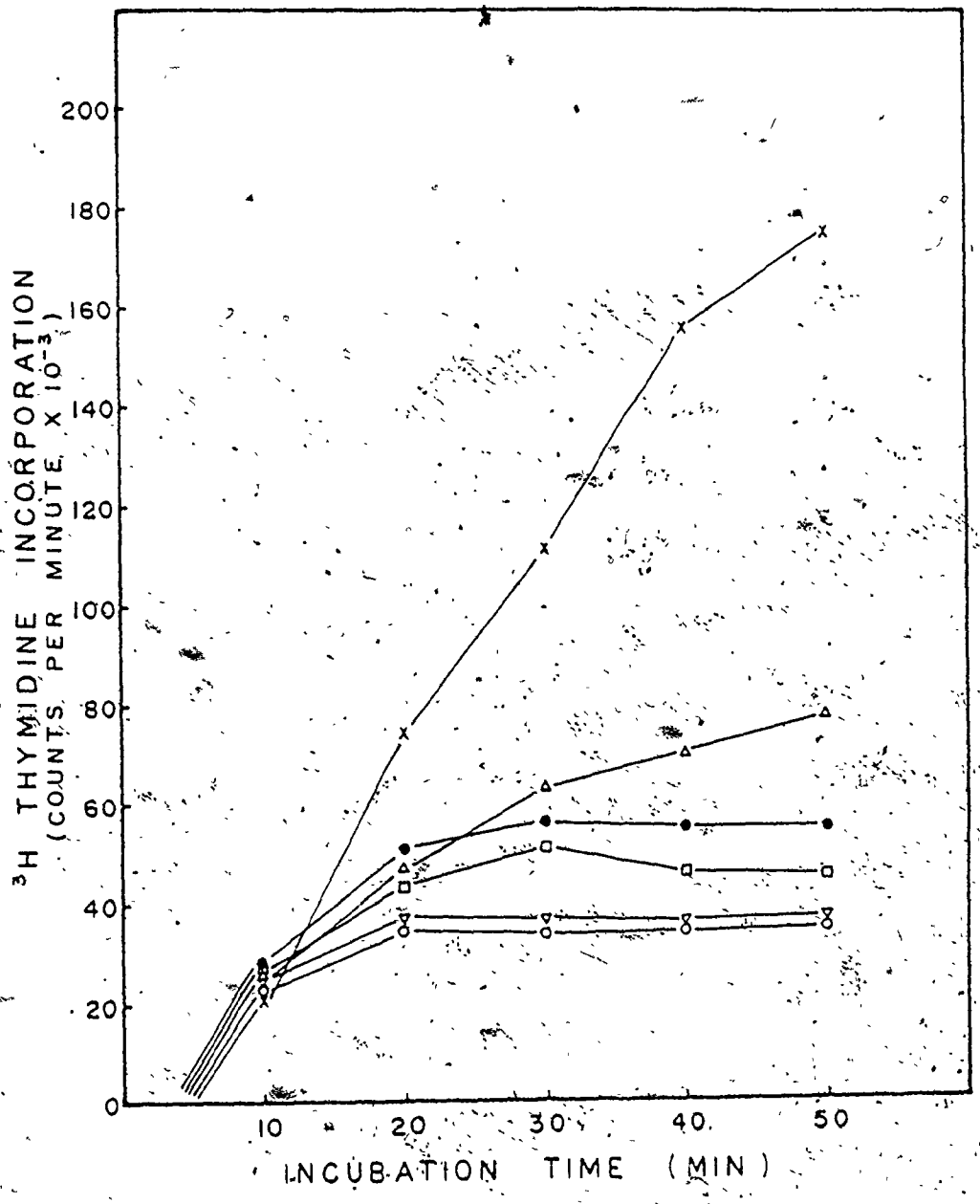

SURVIVING FRACTION
1
10^{-1}
10^{-2}
10^{-3}
10^{-4}
0
10
20
30
40
50
TIME. (SEC)

FIGURE 12

The DNA synthesis of E.coli B cells infected with various T4 phage strains. The incorporation of [^{3}H]thymidine into acid-insoluble material was measured at various times postinfection. See Materials and Methods for further details. Symbols: das13[das] (×), amN130[46] (●), amS17[uvsX] (○), amS17,amN130[uvsX,46] (▽), das13,amN130[das,46] (△), das13,amS17,amN130[das, uvsX,46] (□).


^{3}H THYMIDINE INCORPORATION
(COUNTS PER MINUTE, X 10^{-3})
200
180
160
140
120
100
80
60
40
20
0
10
20
30
40
50
INCUBATION TIME (MIN)

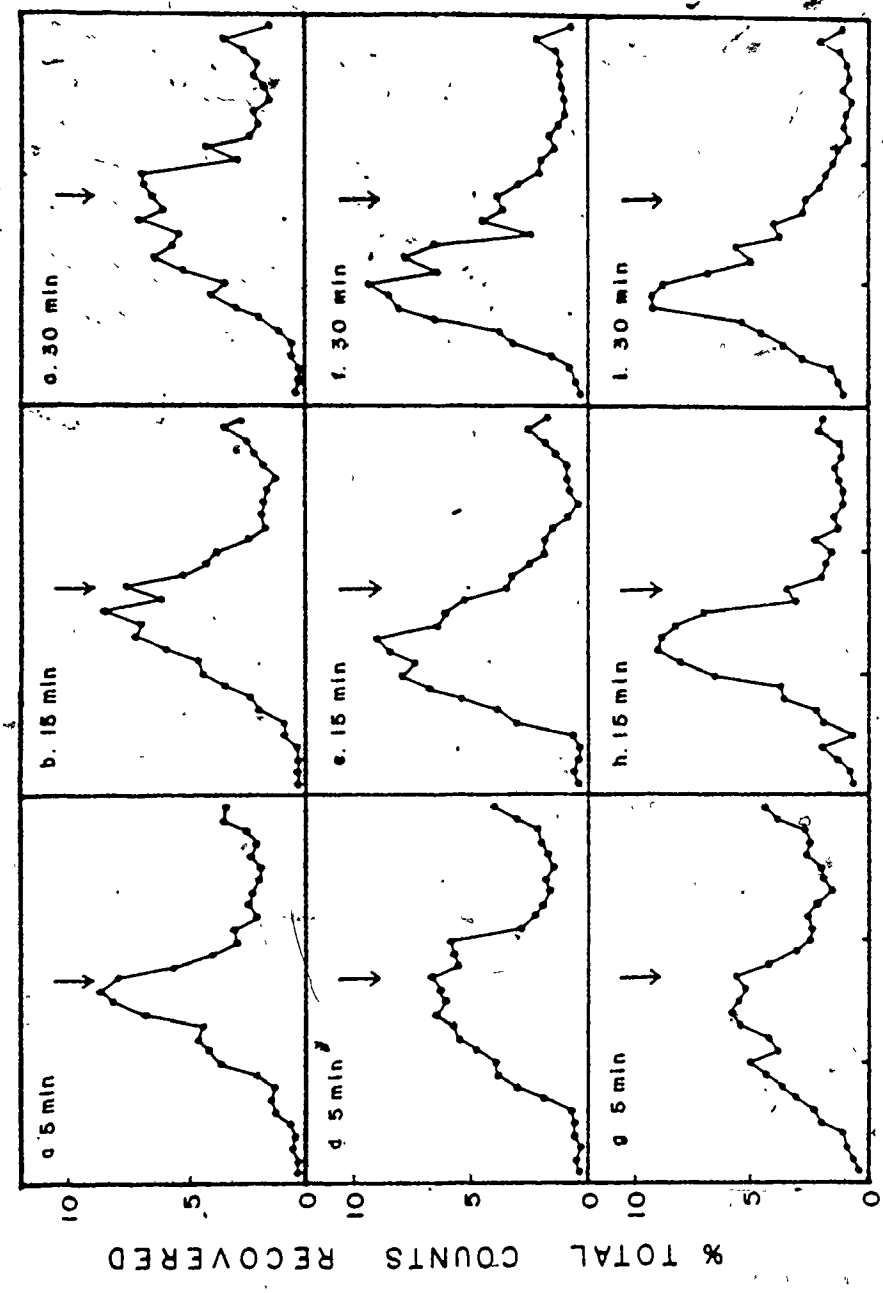
with a 46-47 double mutant sedimented in neutral sucrose with S values smaller than the T4 marker DNA, after long chase times. In contrast, DNA from [uvsX]- or [uvsY]-infected cells sedimented with T4 marker DNA under similar conditions (Wakem and Ebisuzaki, 1976). We note here that DNA from cells infected with [uvsX,46] resembled that of [46]-infected cells (Figure 13). Results similar to these were obtained with [uvsX,47]-infected cells (data not shown). One interpretation of these results is that the gene 46-47 function precedes that of uvsX and is on the same pathway.

2d. Relationship of genes 16 and 17

Although mutants in genes 16 and 17 resemble gene 49 mutants in being defective in DNA maturation and packaging, [16] and [17] were not suppressed by the gene 49 suppressors. Revertants of [16] and [17] appeared at much lower frequency than revertants of [49] and were not uv-sensitive. Furthermore, the recombinants [uvsX,16,49] and [uvsX,17,49] were lethal. These relationships will be considered in the Discussion.

FIGURE 13

Neutral sucrose gradient sedimentation of DNA extracted from T4 phage-infected E.coli TR201 pulse labelled for 2 minutes then chased for the times indicated. Panels (a-c) show the sedimentation of DNA from amS17[uvsX]-infected cells, (d-f) from amN130[46]-infected cells, (g-l) from amS17, amN130[uvsX,46]-infected cells.


a. 5 min
b. 15 min
c. 30 min
d. 5 min
e. 15 min
f. 30 min
g. 5 min
h. 15 min
i. 30 min
% TOTAL COUNTS RECOVERED
FRACTION NUMBER

3. An Analysis of the DNA Repair-Recombination Functions by Means of Suppressors: The Role of Das.

Mutations in the DNA repair-recombination genes uvsX, uvsY, 46, 47 and 59, have an array of suppressors (see Table 6). One of these suppressors, [das] has been previously described as a specific suppressor for the gene 46 and 47 mutations (Hercules and Wiberg, 1971). [Das] alleviates the DNA arrest phenotypes and the depressed burst size of the gene 46-47 mutations. The other previously described suppressors, [dar] and [uvsW], have similar properties and may be identical (Hamlett and Berger, 1975; Wu and Yeh, 1975; Cunningham and Berger, 1977). They are involved in the suppression of the DNA arrest phenotypes of the gene 59, 46 and 47 mutations. In later sections, we describe two new suppressors, [sur] which suppresses mutations in the above mentioned five DNA repair-recombination genes (Results, section 4) and [uvsU] which appears to specifically suppress the uvsX mutation (Results, section 5).

In this section we describe additional facets concerning the role of [das], together with a proposal concerning the construction of the DNA repair pathway as analyzed by the study of various suppressors. Obviously, a meaningful analysis of the DNA repair pathway requires a biochemical study of the relevant gene products and their functions, but this objective has been difficult to attain.

TABLE VI

The Effect of Various Suppressors on Mutations in the DNA Repair-Recombination Genes of T4.

SUPPRESSORS	GENE MUTATIONS RECOGNIZED BY SUPPRESSORS
uvsU	uvsX
uvsW(dar)	59 | 46 | 47
das	a: 46 | 47 b: 46 | 47 | uvsX | uvsY
sur	59 | 46 | 47 | uvsX | uvsY 59 | 46 | 47 | uvsX | uvsY
PROPOSED REACTION SEQUENCE	59 → (46 - 47) → (uvsX - uvsY)

a Suppression of a DNA replication defect is indicated by ————————.

b Suppression of a DNA repair defect is indicated by — — — — .

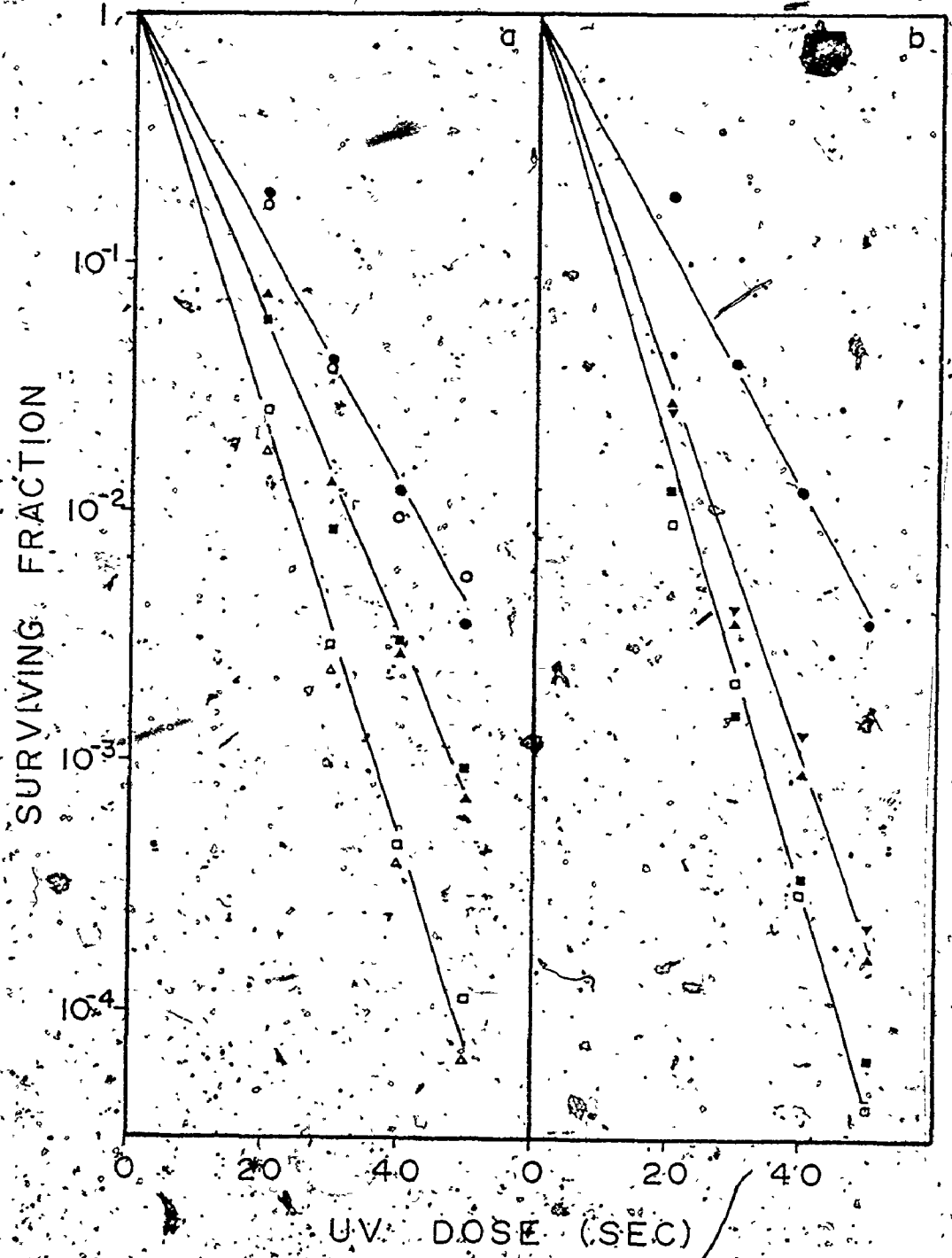
For this reason, we have felt that a study of the suppressors might not only aid in the biochemical analysis but also provide a view of the complex web of interactions of the DNA repair-recombination functions.

3a. UV-Sensitivity

The [das] mutation specifically suppressed both the DNA arrest phenotypes and burst size defects of the gene 46 and 47 mutations (Hercules and Wiberg, 1971). We felt that the question of the specificity of the das function should be studied further since previous studies indicated the DNA arrest genes 46, 47 and 59 and the uvs genes X and Y functioned together to form part of the DNA processing pathway (Results, section 2; Wakem and Ebisuzaki, 1981). In fact, the close relationship of these genes was also supported by the observation that [das] partially suppressed the uv-sensitivity of [uvsX] and [uvsY] (Figure 14a). The double mutants [das,uvsX] and [das,uvsY] were less sensitive to uv-irradiation than the corresponding single mutants [uvsX] and [uvsY]. The [das] mutant was not uv-sensitive. In a similar test [das] did not suppress the uv-sensitivity of a gene 59 mutation (Figure 14b). The double mutants [das,47] and [das,46] were uv-sensitive (Figure 14b) but the degree of suppression, if any, could not be directly

FIGURE 14

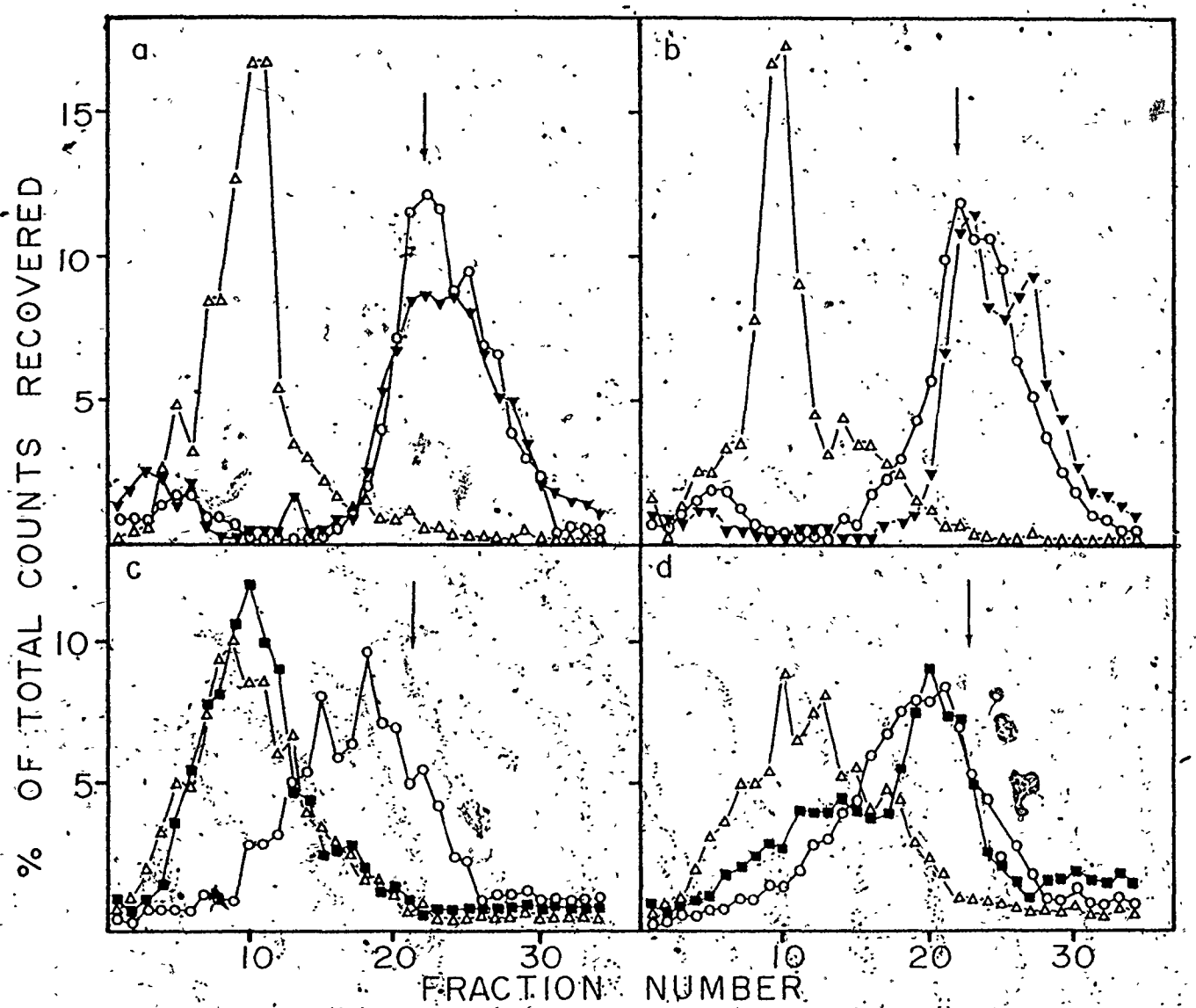
The effect of das13[das] on the uv-sensitivity of mutations in the DNA repair-recombination genes. The phage were uv-irradiated, then plated on E.coli B. Symbols: (a) wild type T4 (o), das13[das] (●), amS17 [uvsX] (Δ), amS52[uvsY] (□), das13,amS17[das,uvsX] (▲), das13,amS52[das,uvsY] (■); (b) das13,[das] (●), amC5x3[59] (□), das13,amC5x3[das,59] (■), das13,amN130[das,46] (▲), das13,amNG163x3[das,47] (▼).


a
b
1
10^{-1}
10^{-2}
10^{-3}
10^{-4}
SURVIVING FRACTION
0
20
40
0
20
40
UV DOSE (SEC)

compared because of the low efficiency of plating of the gene 46 and 47 mutants on E. coli B (Epstein et al., 1963). For this reason, we used an alternative procedure (Wakem and Ebisuzaki, 1976) to study the possible suppression of uv-sensitivity (DNA repair) by [das]. DNA repair was assayed by labelling infected cells with [^{3}H]thymidine, uv-irradiating them and chasing with medium containing unlabelled thymidine for various periods of time. The infected cells were analyzed for DNA breakage and rejoining by alkaline sucrose gradient centrifugation (Figure 15). The pol I mutant of E. coli (R15) was used in these studies to block the excision repair pathway (Ebisuzaki et al., 1975). In wild type T4 and [das]-infected cells, phage DNA was degraded into smaller fragments almost immediately after uv-irradiation and subsequently rejoined by 60 minutes postinfection (Figure 15). In contrast, DNA intermediates were nicked but not rejoined even after 90 minutes postinfection in cells infected with the gene 47 mutant. In the double mutants, [das,46] (data not shown) and [das,47] (Figure 15), DNA was similarly nicked and rejoining occurred but at a slower rate and was not completed by 90 minutes postinfection. We conclude that [das] partially suppressed the defect in DNA repair in the gene 46 and 47 mutations.

FIGURE 15

Alkaline sucrose gradient sedimentation of ^{3}H-labelled DNA extracted at various times after uv-irradiation from cells infected with wild type T4, das13[das], amNG163x3[47], or das13,amNG163x3[das,47]. The panels show the sedimentation of DNA extracted from (a) wild type T4-, (b) das13[das]-, (c) amNG163x3[47]- and (d) das13,amNG163x3[das,47]-infected cells. Symbols: DNA extracted from nonirradiated, infected cells (o), DNA extracted from uv-irradiated, infected cells after a 0 minute (Δ), 60 minute (▼) or 90 minute (■) incubation in fresh medium (33°C).


a
b
c
d
15
10
5
10
5
10
20
30
10
20
30
% OF TOTAL COUNTS RECOVERED
FRACTION NUMBER

3b. DNA Replication

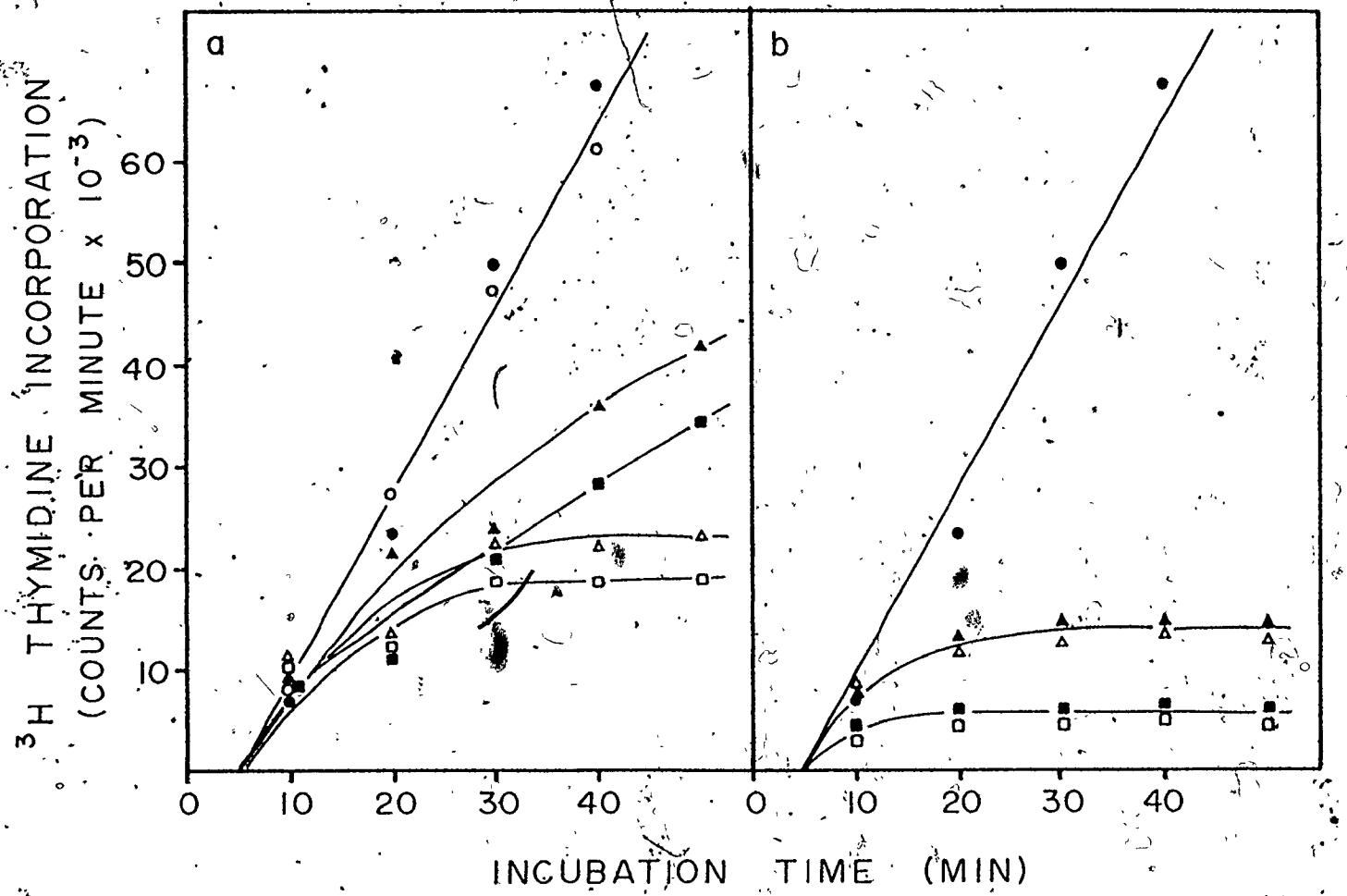
It was previously noted by Hercules and Wiberg (1971) that [das] partially suppressed the DNA arrest phenotypes of the gene 46 and 47 mutations. However, [das] did not suppress the DNA replication defects of the [uvsX] and [uvsY] mutations (Figure 16). Under similar conditions, [das] partially suppressed the DNA arrest phenotype of gene 46 and 47 mutations. We feel that this distinction is important because it serves to separately categorize 46-47 from uvsX-uvsY.

4. A New Suppressor of Mutations in the DNA Repair-Recombination Genes of Bacteriophage T4: sur

The diversity of suppressors that affect the mutations of the DNA repair-recombination genes seems remarkable. Inevitably this diversity must reflect the complexities of the DNA repair-recombination functions and of the multiple interactions that are involved, particularly with DNA replication and DNA processing. We have studied these suppressors with the view that they would aid in the understanding of the role of the DNA repair-recombination functions.

FIGURE 16

The effect of das13[das] on the DNA synthesis of mutations in the DNA repair-recombination genes. The incorporation of [^{3}H]thymidine into acid-insoluble material was measured at various times postinfection. Symbols: (a) wild type T4 (○), das13[das] (●), amN130[46] (△), amNG163x3[47] (□), das13,amN130[das,46] (▲), das13,amNG163x3[das,47] (■); (b) das13[das] (●), amS17[uvsX] (△), amS52[uvsY] (□), das13,amS17[das,uvsX] (▲), das13,amS52[das,uvsY] (■).


a
b
^{3}H THYMIDINE INCORPORATION
(COUNTS PER MINUTE x 10^{-3})
60
50
40
30
20
10
0
10
20
30
40
INCUBATION TIME (MIN)

In this section, we describe a new suppressor, [sur] (suppressor of repair) which suppresses the mutations of all five DNA repair-recombination genes that have been ascribed as part of the DNA processing pathway (Wakem and Ebisuzaki, 1981). Although we have studied some of the general properties of [sur], in main, the investigations have been concerned with the specificity and range of the suppressor function and some considerations of the possible mechanism of the suppressor.

Amber [uvsX] and [uvsY] mutations isolated as suppressors of a mutation in gene 49 were equally sensitive to uv-irradiation but differed widely in the kinetics of DNA replication (Wakem and Ebisuzaki, 1981). One of these [uvsX] mutants which exhibited an elevated level of DNA replication was found to be a double mutant consisting of a typical [uvsX] mutation with a DNA arrest phenotype and another uv-sensitive mutation [uvsU], which suppressed the [uvsX] mutation to give a wild type level of DNA replication (Results, section 5). However, [uvsU] could not be considered a typical suppressor of [uvsX] because of its complex origin. We looked for a "typical" suppressor with an uncomplicated origin and [sur] was the result of this search.

4a. Isolation of [sur]

When amS17[uvsX] was plated on E. coli B, the majority of plaques were tiny. We plated amS17[uvsX] on E. coli B and picked a number of larger plaques with the expectation that they were either true revertants or suppressors. Out of thirty such isolates, only six were stable and of this latter group only one was uv-sensitive. E. coli B infected with this uv-sensitive derivative, amS17-r10, yielded a level of DNA replication intermediate between that of a wild type T4 and a [uvsX] mutant infection (Figure 17). When amS17-r10 was crossed with wild type T4, amS17[uvsX] and r10[sur] were obtained. The [sur] mutant was identified by its ability to produce recombinants with amS17[uvsX], which were uv-sensitive and produced near normal-sized plaques on E. coli B. These recombinants were identical to amS17-r10 with respect to uv-sensitivity and kinetics of DNA replication.

4b. Properties of [sur]

The [sur] mutant resembled wild type phage with respect to the following parameters: plaque size, kinetics of phage formation (Figure 18), DNA replication (Figure 17), uv-sensitivity (Figure 19a) and mitomycin C sensitivity (data not shown). These characteristics distinguish sur

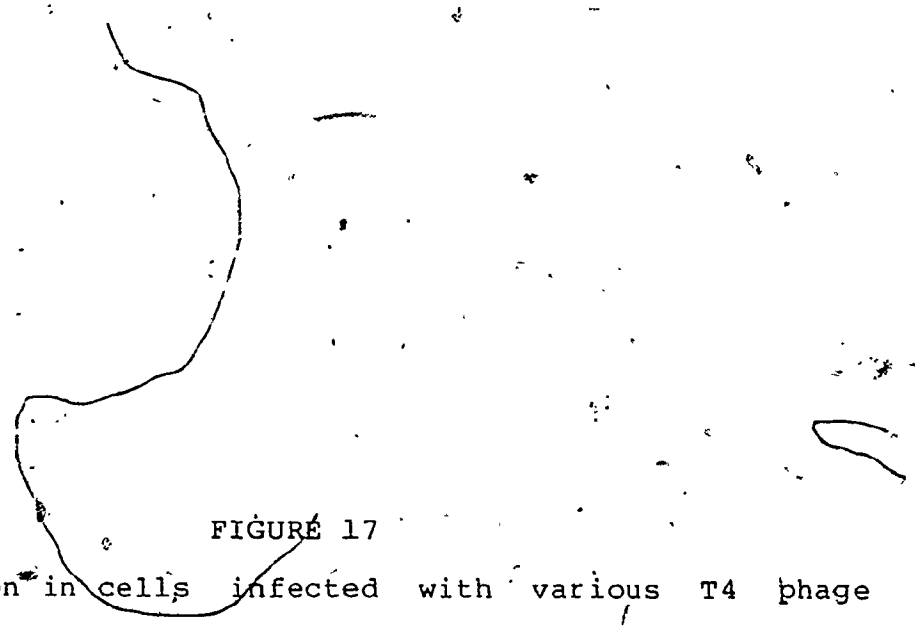
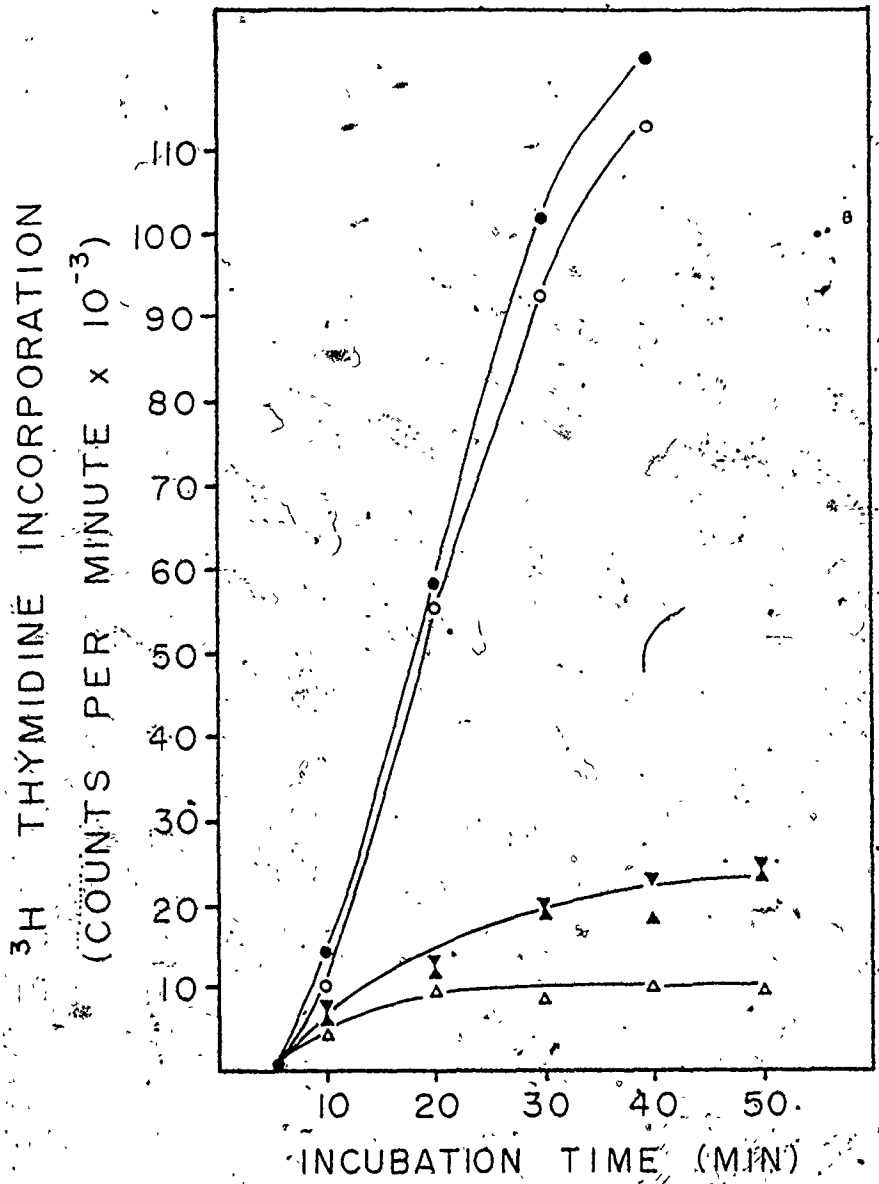


FIGURE 17

DNA replication in cells infected with various T4 phage strains. The incorporation of [^{3}H]thymidine into acid-insoluble material was measured at various times postinfection. Symbols: wild type T4 (o), r10[sur] (●), amS17[uvsX] (Δ), r10,amS17[sur,uvsX] (▲) and amS17-r10 [sur,uvsX] (▼).


^{3}H THYMIDINE INCORPORATION
(COUNTS PER MINUTE x 10^{-3})
110
100
90
80
70
60
50
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30
40
50
INCUBATION TIME (MIN)

FIGURE 18

One step growth curves of wild type T4 and r10[sur] in E.coli B40Sul. Infected cells were plated for infective centres at various times postinfection. Symbols: wild type T4 (o) and r10[sur] (Δ).

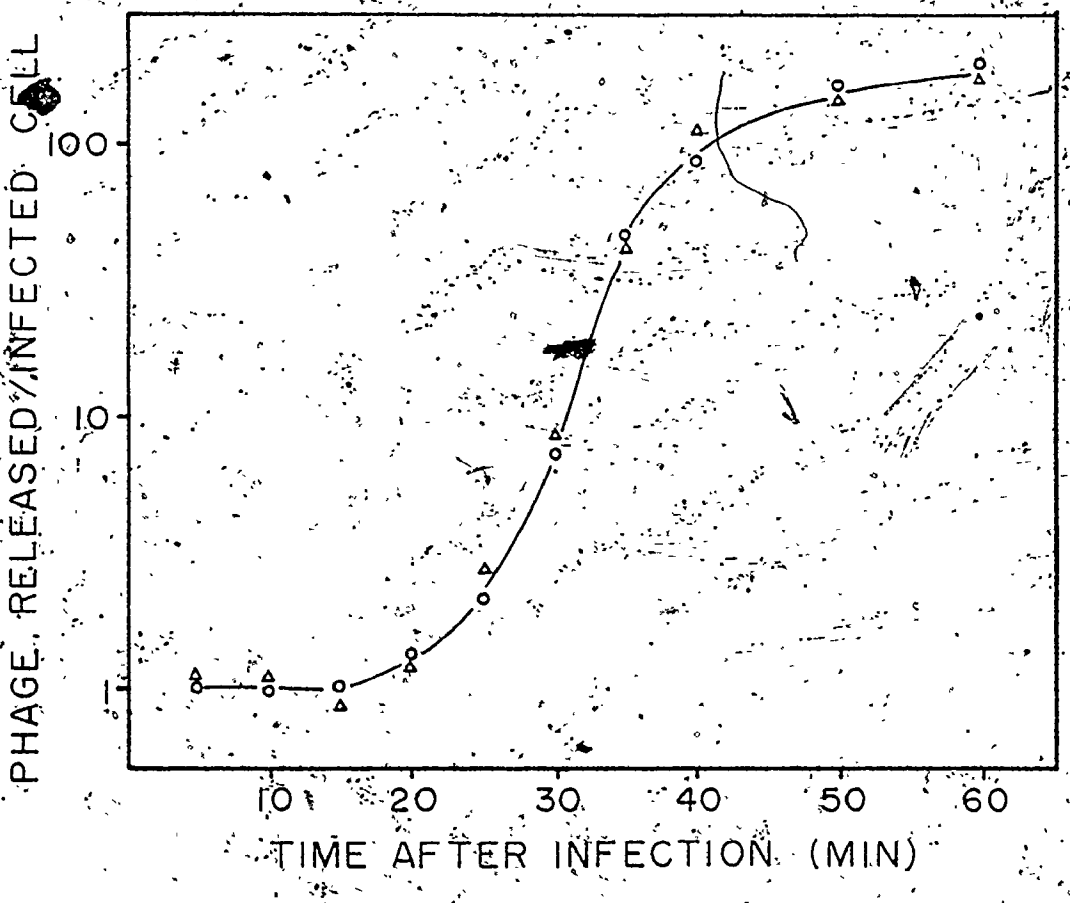
PHAGE RELEASED/INFECTED CELL
100
10
1
10
20
30
40
50
60
TIME AFTER INFECTION (MIN)

FIGURE 19

The partial suppression of uv-sensitivity by r10[sur]. Various r10[sur]-containing DNA repair-recombination mutants were uv-irradiated and plated on E.coli B. Symbols: wild type T4 (o), r10[sur] (●), amS17[uvsX] (Δ), r10,amS17[sur,uvsX] (▲), amS52[uvsY] (▽), r10,amS52 [sur,uvsY] (▼), amHL628[59] (□) and r10,amHL628[sur,59] (■).

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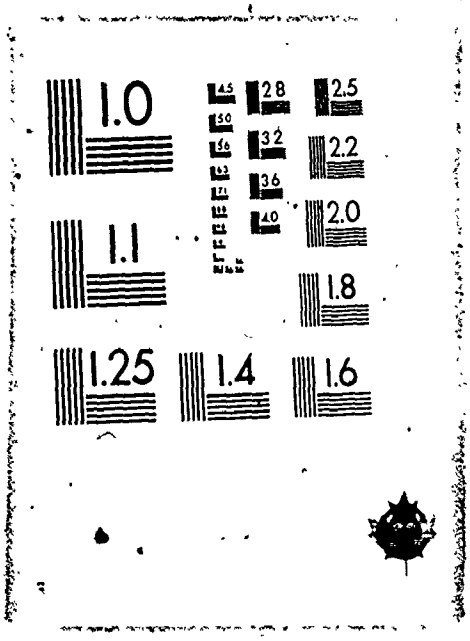

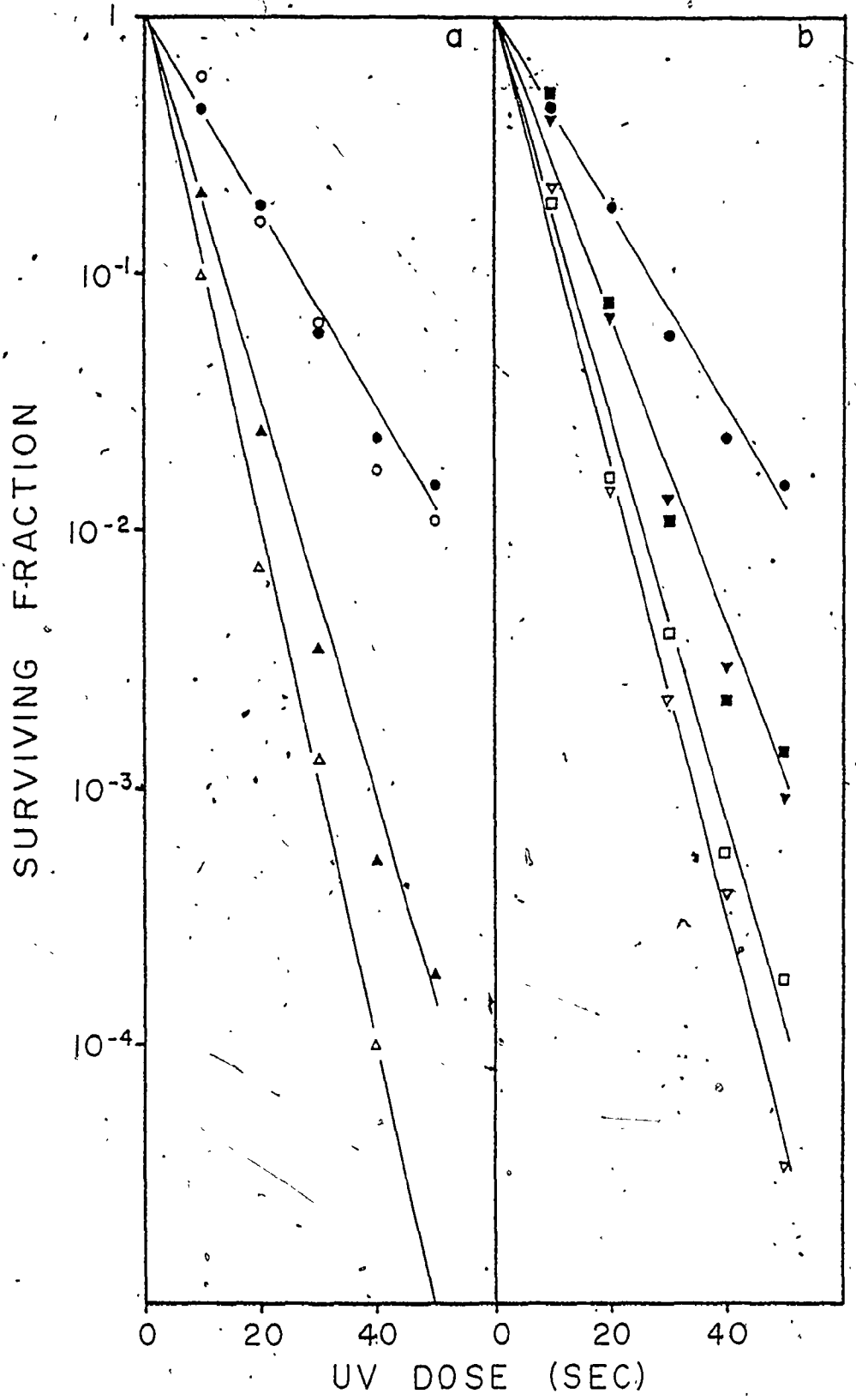
a
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SURVIVING FRACTION
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20
40
UV DOSE (SEC)

from the suppressors, [uvsW] and [uvsU], both of which are uv-sensitive (Hamlett and Berger, 1975; Results, section 5) and also indicate general similarities to the suppressor [das] which also resembles wild type T4 (Hercules and Wiberg, 1971).

The [sur] mutation appears to be a general suppressor of mutations in the genes involved in DNA repair-recombination functions (uvsX-uvsY DNA repair pathway). The [sur] mutation partially suppressed the burst size defects (Table 7) and DNA arrest phenotypes (Figures 17 and 20) of mutations in genes 46, 47, 59, uvsX and uvsY. The uv-sensitivity of mutations in genes 59, uvsX and uvsY was also partially suppressed by [sur] (Figure 19). UV-sensitivity tests were not possible with mutants [46] and [47] because of their low plating efficiency. Therefore an alternative assay for DNA repair was used, in which infected cells were labelled with ^{3}H-thymidine, uv-irradiated, chased for various times with cold thymidine and analyzed by alkaline sucrose gradient centrifugation (Wakem and Ebisuzaki, 1976). For these experiments the polI mutant E. coli R15 was used to inhibit the excision repair pathway (Ebisuzaki et al., 1975). In infections with wild type T4 and [sur], the phage DNA was nicked immediately after uv-irradiation and slowly rejoined to mature size by 60 minutes postinfection (Figure 21). Infections with the [47]

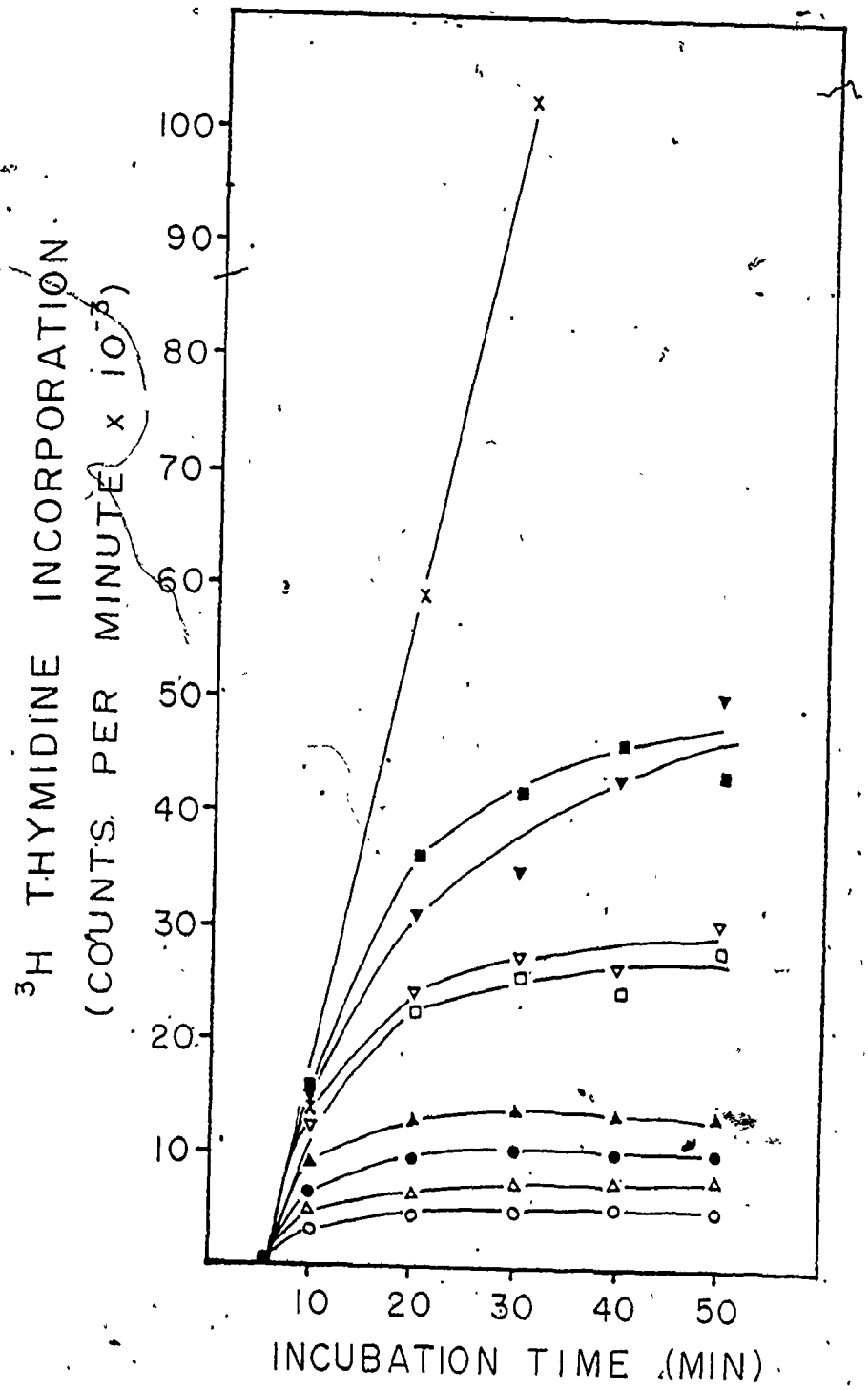
TABLE VII

The Effect of r10[sur] on the Burst Size of Various T4 Mutations

Infecting Phage	Mutant Gene	Burst Size
$T4D^+$	____	151 ± 59
r10	[sur]	168 ± 41
amN130	[46]	12 ± 1
r10,amN130	[sur,46]	16 ± 1
amNG163x3	[47]	7 ± 2
r10,amNG163x3	[sur,47]	18 ± 2
amHL628	[59]	18 ± 4
r10,amHL628	[sur,59]	30 ± 4
amS17	[uvsX]	15 ± 3
r10,amS17	[sur,uvsX]	27 ± 2
amS52	[uvsY]	21 ± 3
r10,amS52	[sur,uvsY]	35 ± 2

FIGURE 20

The partial suppression of DNA replication defects by r10[sur]. Mutants in DNA repair-recombination genes were recombined with r10[sur] and tested for DNA replication as in Figure 1. Symbols: r10[sur] (x), amS52[uvsY] (o), r10,amS52[sur,uvsY] (●), amHL628[59] (△), r10,amHL628[sur,59] (▲), amN130[46] (▽), r10,amN130[sur,46] (▼), amNG163x3[47] (□) and r10,amNG163x3[sur,47] (■).


^{3}H THYMIDINE INCORPORATION
(COUNTS PER MINUTE) x 10^{-3}
100
90
80
70
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30
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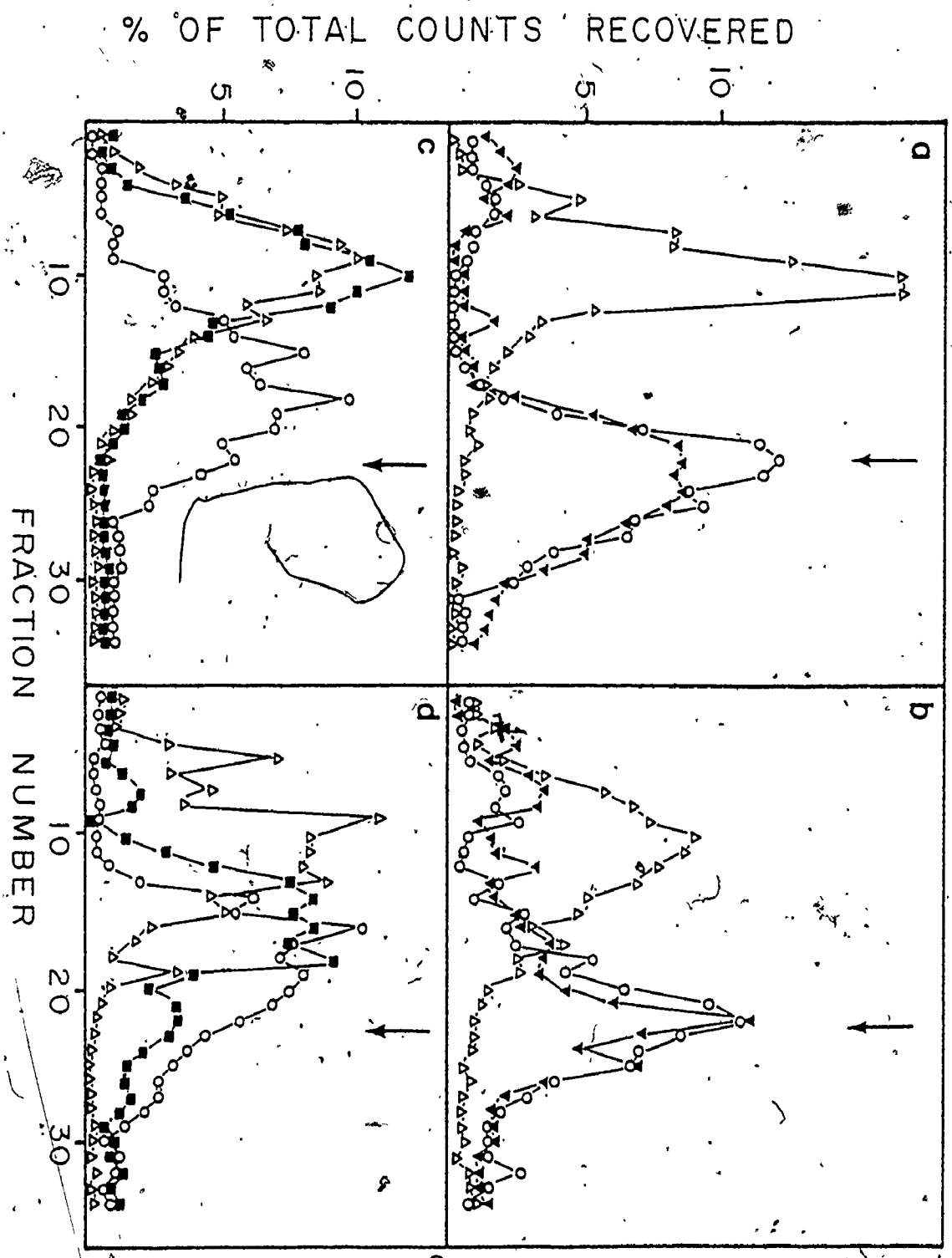
50
INCUBATION TIME (MIN)

FIGURE 21

The partial suppression of a gene 47 DNA repair defect by r10[sur]. ^{3}H-labelled DNA extracted at various times after uv-irradiation from cells infected with different phage mutants was subjected to alkaline sucrose gradient centrifugation analysis. The panels show the sedimentation of DNA extracted from (a) wild type T4-, (b) r10[sur]-, (c) amNG163x3[47]- and (d) r10,amNG163x3[sur,47]-infected cells. Symbols: DNA extracted from nonirradiated, infected cells (o), DNA extracted from uv-irradiated, infected cells after a 0 minute (Δ), 60 minute (▼) or 90 minute (■) incubation in fresh medium (33°C).


% OF TOTAL COUNTS RECOVERED
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FRACTION NUMBER

mutant indicated normal nicking of DNA but little or no rejoining even after 90 minutes postinfection. However, with the [sur,47] mutant, the normal nicking was followed with a slow rejoining of the DNA strands. Similar observations were made with the [46] and [sur,46] mutants (data not shown). These results indicated that [sur] suppressed the DNA repair defects of the gene 46-47 mutations.

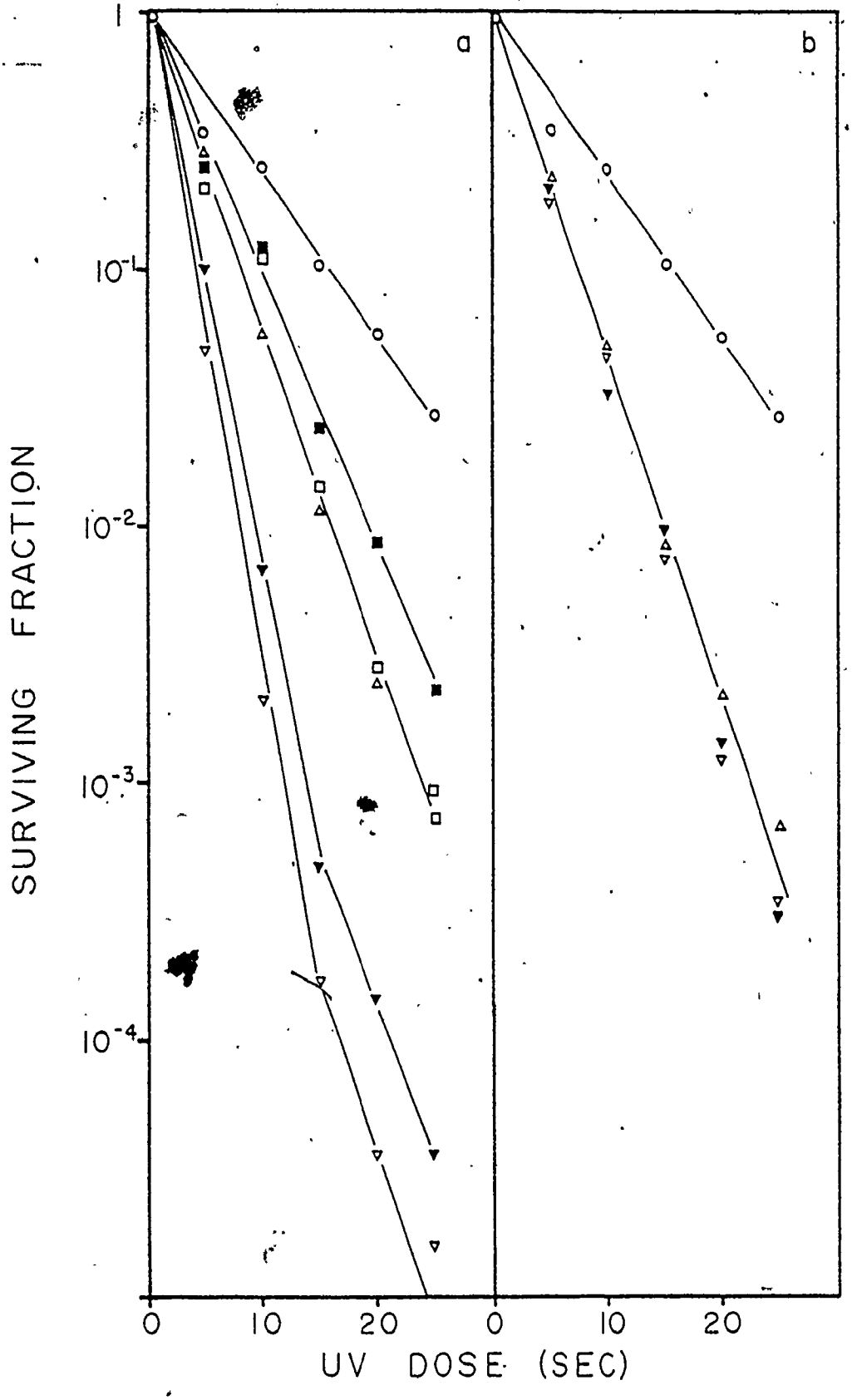
These results distinguish [sur] from the other suppressors. By contrast [das] was unable to suppress the DNA repair and DNA replication defects of the gene 59 mutation (Wiberg and Swanson, 1975; Results, section 3) and [das] was also ineffective in suppressing the DNA replication defects of [uvsX] and [uvsY] (Results, section 3). The other two suppressors, [uvsW] and [uvsU], differ from [sur] in that they only suppress DNA replication defects.

4c. Effect of [sur] on the excision repair pathway

Since [sur] suppresses mutations involved in the uvsX-uvsY DNA repair pathway, we tested the effect of [sur] on the denV-controlled excision repair pathway. As shown in Figure 22, the uv-sensitivity of [denV] was not affected by [sur]. The [sur,denV,uvsX] and [denV,uvsX] strains

FIGURE 22

The effect of r10[sur] on the uv-sensitivity of various phage strains containing the v[denV] mutation. Phage strains were uv-irradiated and plated on (a) E.coli B or (b) E.coli B40Sul. Symbols: wild type T4 (o), v[denV] (Δ), amS17[uvsX] (□), r10,amS17[sur,uvsX] (■), v,amS17[denV,uvsX] (▽) and r10,v,amS17[sur,denV,uvsX] (▼).


a
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1
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10^{-4}
SURVIVING FRACTION
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20
UV DOSE (SEC)

exhibited a similar uv-sensitivity on E. coli B40Sul. On this host the amber,uvsX mutation is suppressed and only the [denV] and [sur] mutations are expressed. However, on the restrictive host E. coli B all three mutations are expressed and a comparison of the uv-sensitivities of [sur,denV,uvsX] and [denV,uvsX] showed that the [denV] mutation did not affect the suppression of [uvsX] by [sur]. The difference in uv-sensitivity between [sur,denV,uvsX] and [denV,uvsX] was similar to the difference in uv-sensitivity between [sur,uvsX] and [uvsX].

These results suggest that [sur] does not suppress all DNA repair defects and that its activity might be specific for the uvsX-uvsY DNA repair pathway.

4d. Effect of [sur] on host DNA degradation

One proposed mechanism for the suppression of the [46,47] mutations by das involves a compensatory increase in nuclease activity, which was detected both in vitro and in vivo (Hercules and Wiberg, 1971; Mickelson and Wiberg, 1981). Since [sur] is similar to [das] in many of its general properties, we tested the effect of [sur] on host DNA degradation, following the experimental protocol described by Hercules and Wiberg (1971). In these experiments, a mutant in gene 42 (dCMP hydroxymethylase) was

used to prevent re-incorporation of the solubilized nucleotides in DNA. As indicated in Figure 23, there was an increase in the release of acid soluble radioactivity in infections involving [sur,42] compared to [42]. However, [sur] had no effect on the release of labelled nucleotides in the [42,46] infection. These results clearly distinguish [sur] from [das], where the combination of [das] with [42,46] leads to a clear increase in host DNA degradation (Hercules and Wiberg, 1971). One interpretation of these results is that sur functions in host DNA degradation but at a step(s) preceding the genes 46-47 function and at a step(s) different from that of das.

4e. Dominance test for [sur]

Whether a mutation is dominant or recessive with respect to the wild type allele provides clues on the nature of the function involved. We compared the kinetics of DNA replication in mixed infections of [sur,46] and [46] to determine if the [sur] mutant was dominant or recessive to the wild type allele. These experiments, which involved different ratios of [sur,46]:[46], indicated that [sur] was co-dominant with respect to the wild type allele (Figure 24).

FIGURE 23.

The degradation of labelled host DNA by various phage mutants. DNA degradation was determined by measuring the acid-insoluble material remaining at various times postinfection. Symbols: amN122[42] (o), r10,amN122[sur,42] (Δ), amN122,amN130[42,46] (□) and r10,amN122,amN130[sur,42, 46] (▽).

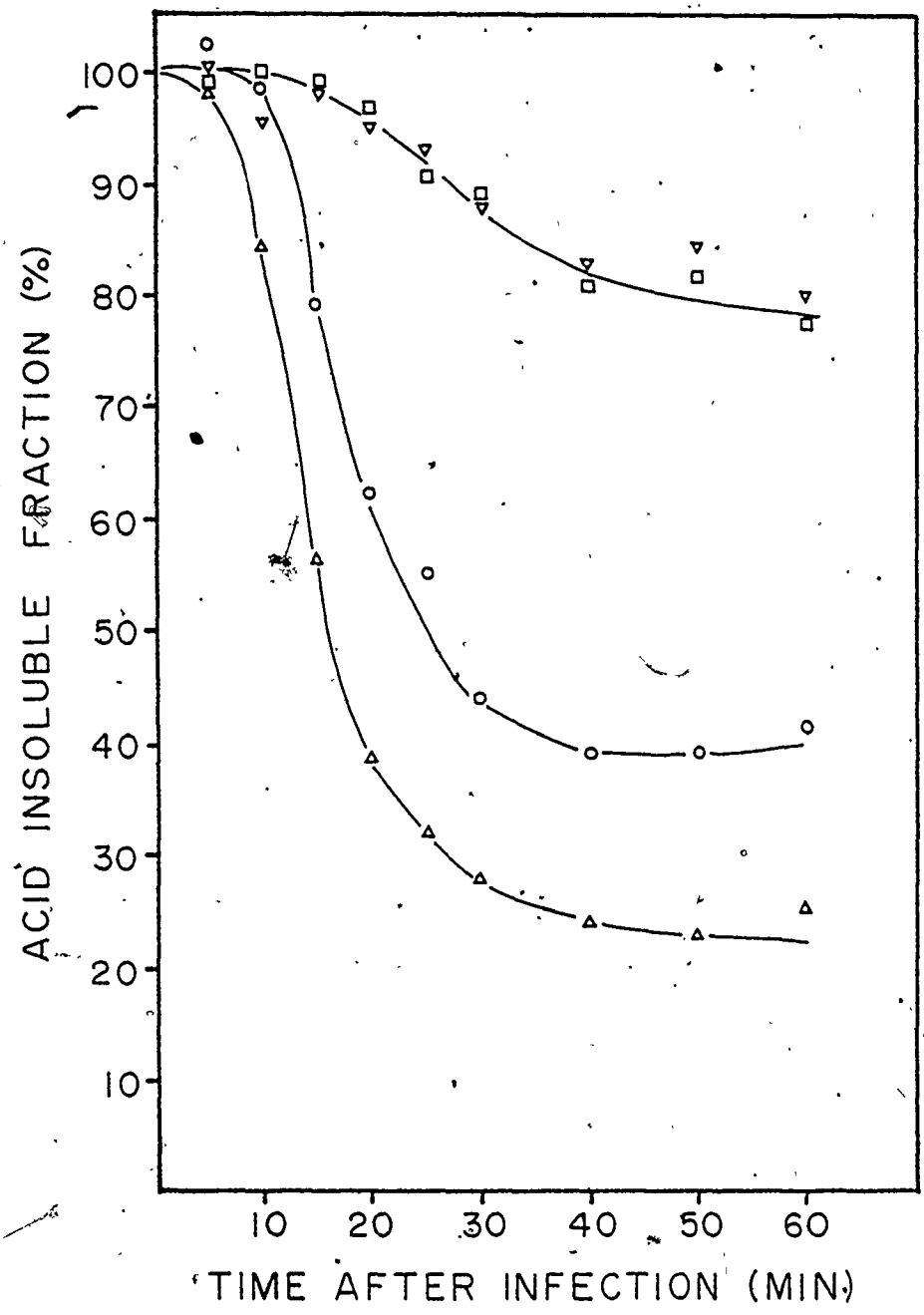
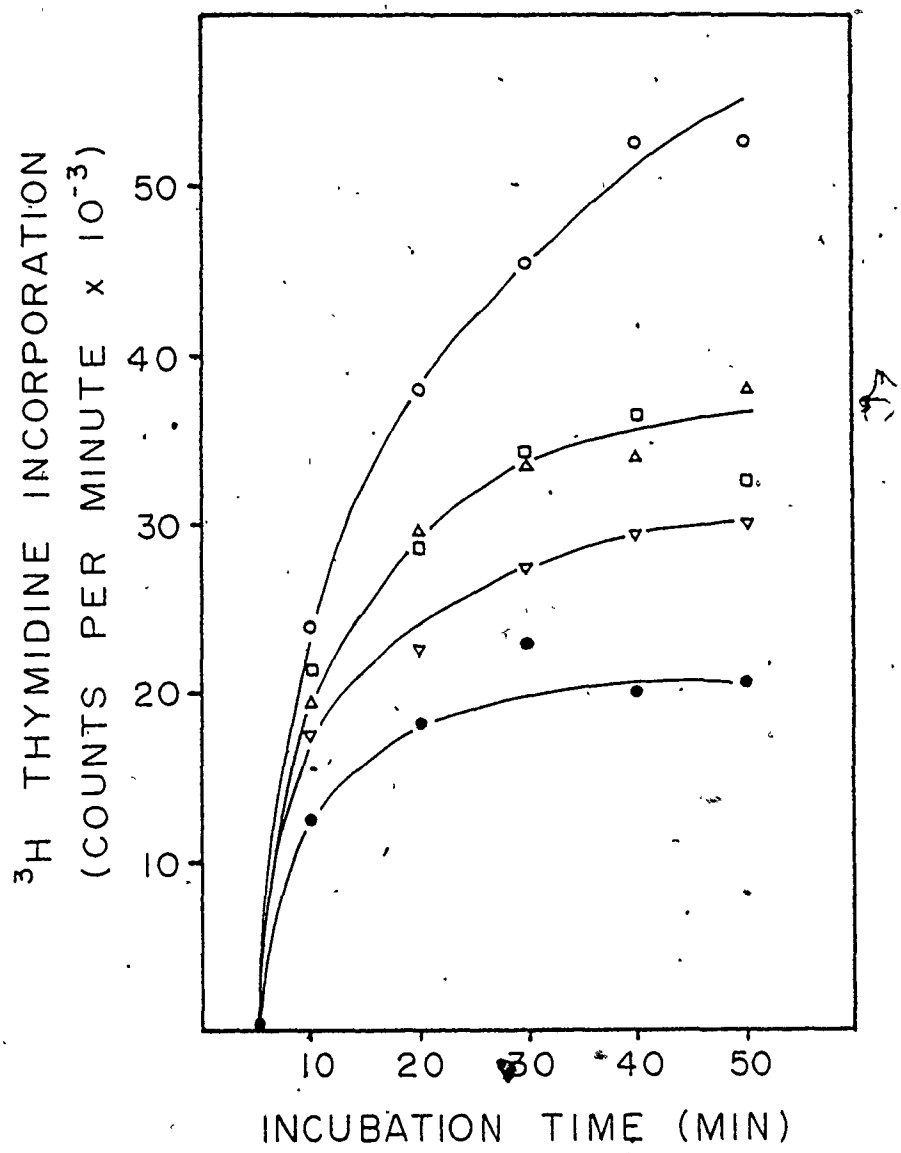

ACID INSOLUBLE FRACTION (%)
100
90
80
70
60
50
40
30
20
10
10
20
30
40
50
60
TIME AFTER INFECTION (MIN.)

FIGURE 24

A dominance test for r10[sur]. Dominance was determined by measuring the DNA replication of cells mixedly infected with r10,amN130[sur,46] plus amN130[46]. The incorporation of [^{3}H]thymidine into acid-insoluble material was measured at various times postinfection. Symbols: r10,amN130[sur,46] (o), amN130[46] (●), r10,amN130[sur,46] + amN130[46] in the following ratios: 1[sur,46]:1[46] (□), 1[sur,46]:5[46] (▽), 5[sur,46]:1[46] (△).


^{3}H THYMIDINE INCORPORATION
(COUNTS PER MINUTE x 10^{-3})
10
20
30
40
50
10
20
30
40
50
INCUBATION TIME (MIN)

5. The Coupling of DNA Repair-Recombination Functions with DNA Replication: uvsU and the "Switch" Model

5a. Isolation of an unusual uv-sensitive mutant

In our previous studies of the pseudorevertants of a gene 49 mutation, we noted two types of amber [uvsX] and [uvsY] mutants (Results, section 2; Wakem and Ebisuzaki, 1981). Both types of mutants had identical levels of uv-sensitivity but one was "wild" type in its DNA replication properties while the other had a "DNA arrest" phenotype. Since previous observations (Hamlett and Berger, 1975; Results, section 4) indicated that the [uvsX] mutants with an elevated level of DNA replication had a suppressor, we crossed one of these mutants (amS76) with wild type T4 to separate the presumptive suppressor. Two uv-sensitive mutants emerged from this cross, one was a small plaque, typical amber [uvsX] mutant (amC16) and the other a large plaque, double mutant containing the amE727x1[49] mutation and a non-amber, uv-sensitive mutation, C69[uvsU]. The [49] mutation segregated at a frequency of less than 1% (4/500) from the double mutant. We have attempted to isolate [uvsU] from the [49] mutation by crossing the double mutant [uvsU,49] with wild type T4 and testing the progeny for uv-sensitivity and failure to dissociate the [49] mutation in crosses with wild type T4. However, several difficulties

should be noted:

(1) [uvsU,49] has plating properties indistinguishable from wild type T4;

(2) the [49] mutation dissociates from the double mutant at a low frequency;

(3) the number of progeny that could be tested was restricted because of the number of manipulations required;

(4) since the characterization depended on a negative result (i.e. the failure to dissociate the [49] mutation), there was always an uncertainty as to whether the uv-sensitive isolate was really dissociated from the [49] mutation. For these reasons, we have used the double mutant, [uvsU,49] and when it was necessary to study the [uvsU] mutation exclusively, appropriate controls were introduced. We have crossed the double mutant [uvsU,49] with the double mutant, amC16,amE727x1[uvsX,49], to reconstruct a triple mutant amC16,C69,amE727x1[uvsX,uvsU,49] with properties identical to those of the original amS76 mutant.

5b. Complementation tests for [uvsU]

Previously we had developed a complementation assay in which E. coli R15 was multiply-infected with two DNA repair-defective mutants and assayed for DNA replication in

the presence of mitomycin C (Results, section 2; Wakem and Ebisuzaki, 1981). By use of this test, mitomycin C sensitive mutants, such as [uvsU] (Figure 25), could be given a gene assignment. The [uvsU] mutant complemented mutants in genes uvsY (Figure 25), 32, 46, 47, 58 and 59 (data not shown), but did not complement a [uvsX] mutant (Figure 25). These results suggested that [uvsU] was an allele of [uvsX].

5c. Mapping of uvsX and uvsU

The positioning of uvsX on the T4 map has undergone revisions. Initially uvsX was placed between genes 42 and 43 (Wood et al., 1968). Subsequently, Dewey and Frankel (1975) and Shah and DeLorenzo (1977) placed uvsX between genes 41 and 42 and Childs (1980) indicated that the gene order was 41, uvsX, βgt, 42. Autoradiograms of SDS polyacrylamide gels have shown that the am[uvsX] mutants reported here were not in gene 41 (data not shown). In addition, the amS17[uvsX] mutant was mapped and we found the following order for the genes: 41, 40, uvsX, βgt, 42 (Figure 26).

Although complementation experiments indicated that [uvsU] was a mutant in the uvsX gene, crosses involving [uvsU,49] with [uvsX] yielded a high frequency of uvs$^+$

FIGURE 25

A complementation test for C69[uvsU,49]. Cells were mixedly infected with C69[uvsU,49] and either [uvsX], [uvsY], [32], [46], [47], [58] or [59] in the presence of mitomycin C (10μg/ml) and tested for the incorporation of [^{3}H]thymidine into acid-insoluble material (Wakem and Ebisuzaki, 1981). The C69[uvsU] mutant complemented all the mutants tested except amS17[uvsX]. Symbols: in the absence of mitomycin C, wild type T4 (○), C69[uvsU,49] (△); in the presence of mitomycin C, wild type T4 (●), C69[uvsU,49] (▲), amS17,amE727x1[uvsX,49] (▽), amS52,amE727x1[uvsY,49] (□), C69[uvsU,49] + amS17,amE727x1[uvsX,49] (▼), C69[uvsU,49] + amS52,amE727x1[uvsY,49] (■).

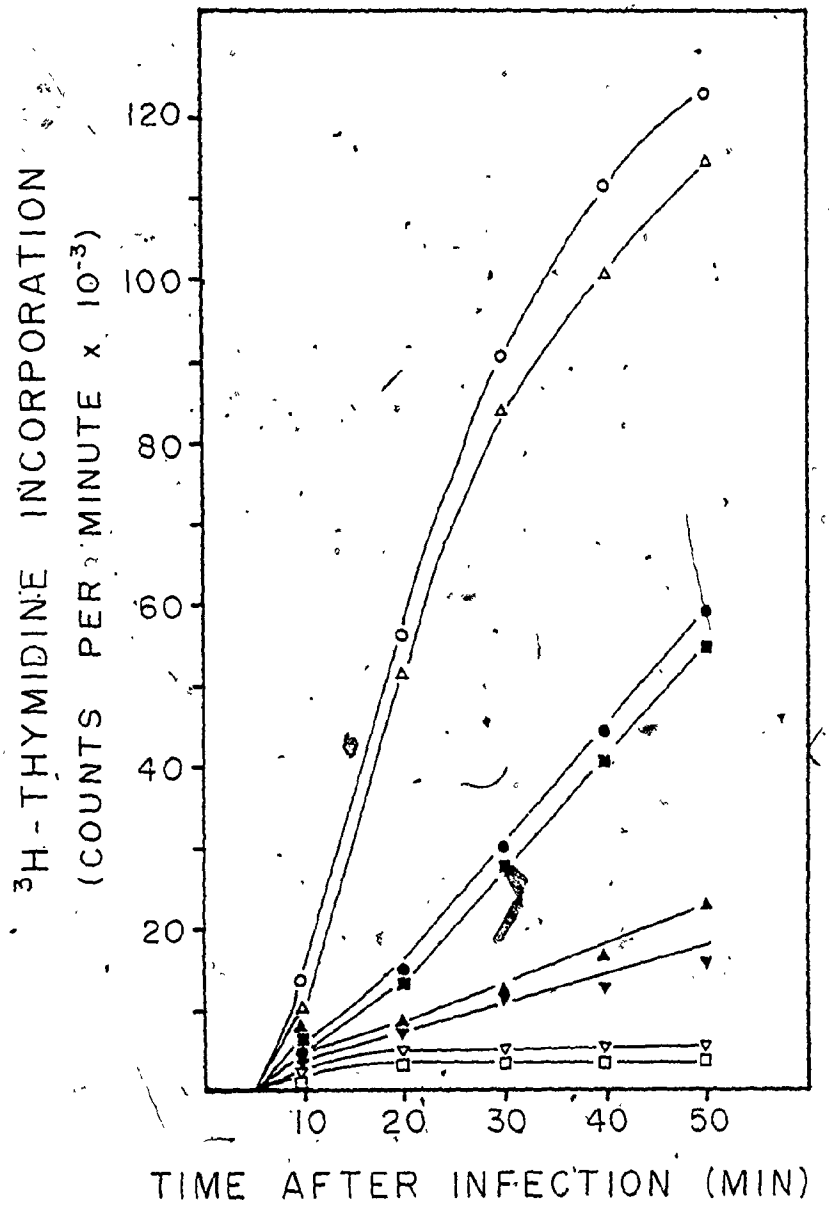
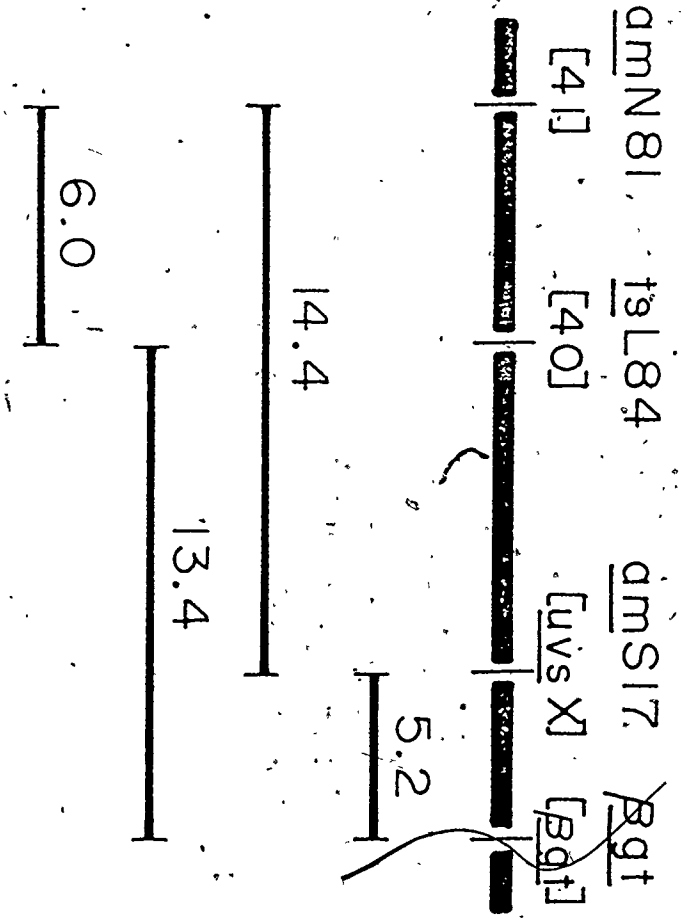

^{3}H-THYMIDINE INCORPORATION
(COUNTS PER MINUTE x 10^{-3})
120
100
80
60
40
20
10
20
30
40
50
TIME AFTER INFECTION (MIN)

FIGURE 26

Genetic recombination map of amS17[uvsX] in relation to genes 40, 41 and βgt. The numbers above the lines indicate the map in percent recombination frequency (2 x percent wild type or βgt). The results represent the average of two experiments.


amN81
[4]
tsL84
[40]
amS17
[uvs X]
Rgt
[Rgt]
6.0
13.4
14.4
5.2

recombinants suggesting that uvsX and uvsU were separate genes (Table 8). This was confirmed by two factor crosses involving [am,uvsU,49] with [+,+,49], where am stands for an amber mutation in a second gene in the cross. These crosses indicated a linkage of uvsU and amBL292[55] (Table 8). The results of a cross involving [55,uvsU,49] and [47] (Figure 27) suggested that [uvsU] maps to the right of [55]. In another cross involving [55,uvsU,am49] and [ts49], the recombinants [55,am49] and [am49] were not detected (0/239) and the recombinants in this cross represented by (b) and (c) were asymmetrically represented (Figure 28). Also, we had previously noted that [49] segregated from [uvsU,49] at a low frequency. These observations suggest that [uvsU] is located between [55] and [49] and that there may be some unusual complications. The asymmetry noted above was not due to excessive heterozygosity in the gene 55 and 49 markers. Furthermore, in a similar cross involving [55,am49] x [ts49], both recombinants, [55,ts49] and [am49] were recovered in approximately equivalent amounts, suggesting that the asymmetry might be a consequence of the C69[uvsU] mutation. Although the mapping data suggested that [uvsU] might be close to gene 49, the functional studies indicated below, suggest that they are two different genes.

TABLE VIII

Two Factor Crosses Involving C69[uvs]

Cross	Recombinant	Frequency (%)
1. amS17 x C69,amE727x1 [uvsX x uvsU,49]	uvs+	12.8
2. amE51,C69,amE727x1 x amE727x1 [56,uvsU,49 x 49]	C69,amE727x1 [uvsU,49]	20.0
3. amN81,C69,amE727x1 x amE727x1 [41,uvsU,49 x 49]	C69,amE727x1 [uvsU,49]	23.0
4. amC42,C69,amE727x1 x amE727x1 [1,uvsU,49 x 49]	C69,amE727x1 [uvsU,49]	23.0
5. amBL292,C69,amE727x1 x amE727x1 [55,uvsU,49 x 49]	C69,amE727x1 [uvsU,49]	11.2

FIGURE 27

The map position of C69[uvsU,49] in relation to genes 47 and 55. Recombinants from a cross involving amBL292, C69[55,uvsU,49] and amNG163x3[47] are indicated as percentage of the frequency at which each recombinant was detected.

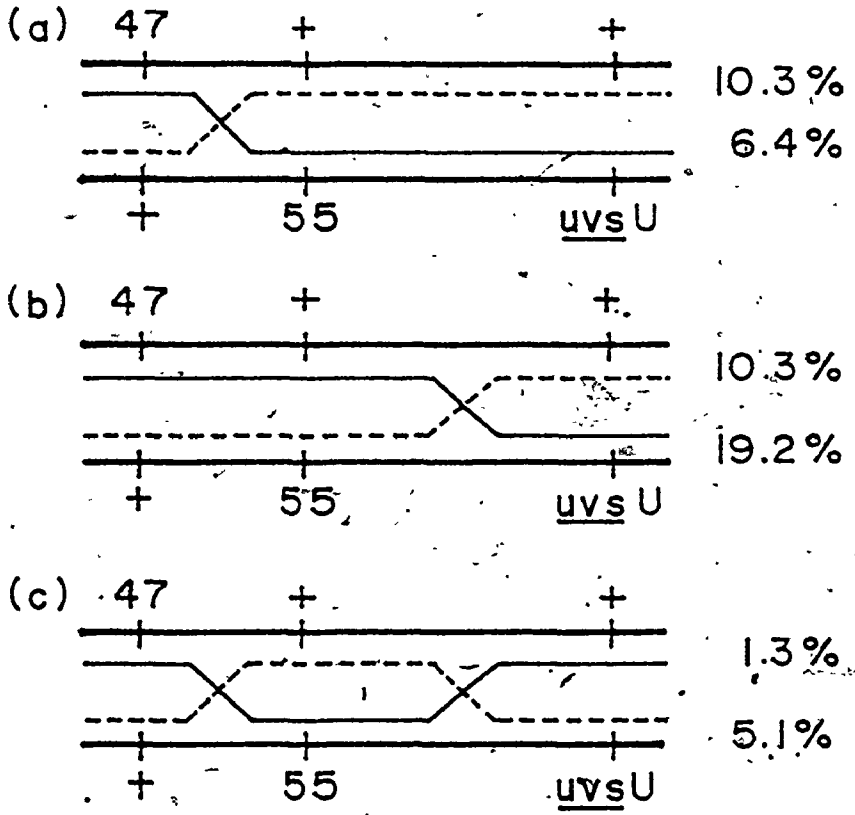
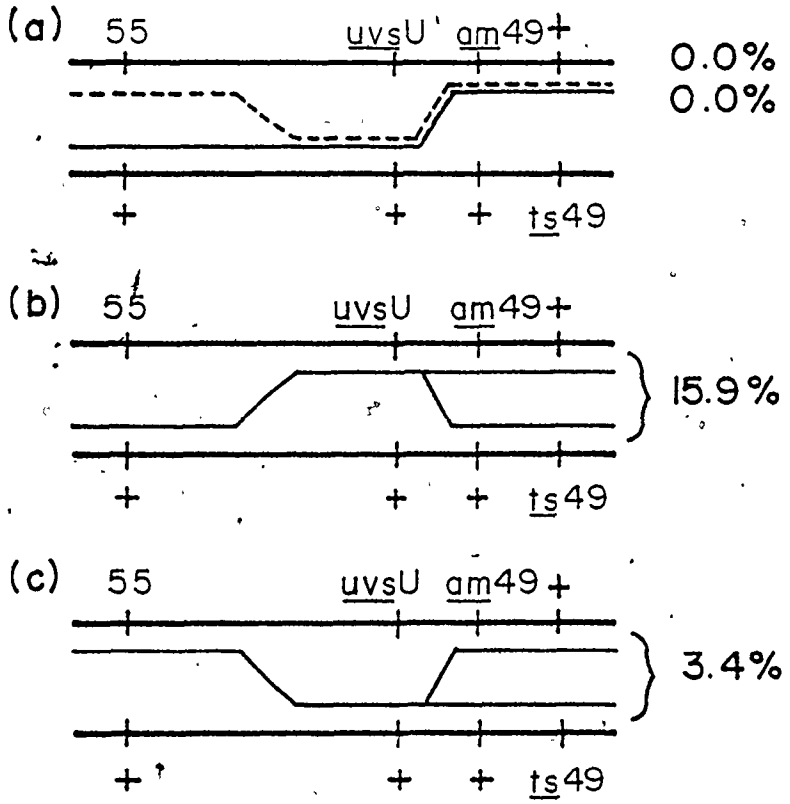

(a)
47
55
uvsU
10.3%
6.4%
(b)
47
55
uvsU
10.3%
19.2%
(c)
47
55
uvsU
1.3%
5.1%

FIGURE 28

The map position of C69[uvsU,49] in relation to genes 55 and 49. Recombinants from a cross involving amBL292, C69,amE727x1[55,uvsU,49] and tsC9[49] are indicated as the frequency (%) at which each recombinant was detected. The order for the mutants [55], [am49] and [ts49] was previously determined in a three factor cross. The recombination frequency between [am49] and [ts49] was approximately 2% and between [55] and [am49] was approximately 18% (data not shown).


(a)
55
uvsU
am49+
0.0%
0.0%
+
+
+
ts49
(b)
55
uvsU
am49+
15.9%
+
+
+
ts49
(c)
55
uvsU
am49+
3.4%
+
+
+
ts49

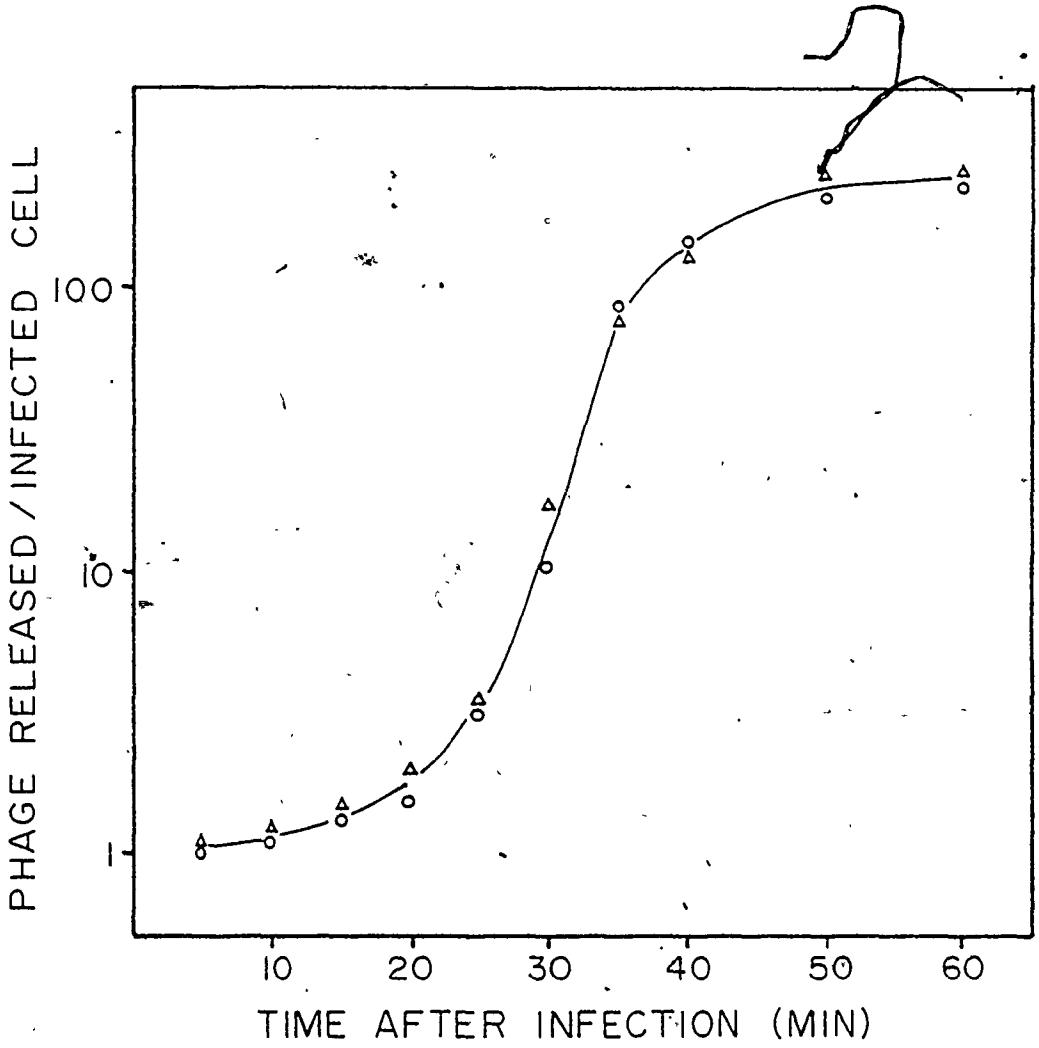
5d. Properties of [uvsU]

The gene 49 mutant, amE727x1 produced very few phage in E.coli B (Table 5) but the double mutant [uvsU,49] appeared to be almost indistinguishable from wild type T4 in its kinetics of DNA replication (Figure 25) and its growth properties on E.coli B40Sul (Figure 29). Also, it should be noted that there was no delay in the appearance of phage in infections involving this double mutant, as might be expected if the [uvsU] mutant were defective in a maturation gene. The [uvsU] mutant is probably not an amber suppressor because it did not suppress any of the other amber mutants with which it had been recombined (e.g. mutants in genes 42, 44, 46, 47, 55, 56, 59, uvsY and lys). Why [uvsU] is so effective in the suppression of the [49] mutant is not known but it should be noted that amS17,amE727x1[uvsX,49] had a fairly large burst size (38 phage/cell) and that a mechanism for this suppression has been proposed (Discussion, section 2). Possibly, the higher phage yield with [uvsU,49] might be the result of a higher level of DNA replication (Figure 31).

The [uvsU,49] mutant had a level of uv-sensitivity that was intermediate between that of wild type T4 and the [uvsX] mutant (Figure 28) and as noted previously, [uvsU] was also sensitive to mitomycin C. The [uvsU] mutant was equally sensitive to uv-irradiation when it was tested on E.coli B

FIGURE 29

One step growth curves of wild type T4 and C69[uvsU,49] in E.coli B40Sul. Infected cells were plated for phage at various times postinfection. Symbols: wild type T4 (O), C69[uvsU,49] (Δ).


PHAGE RELEASED / INFECTED CELL
100
10
1
10
20
30
40
50
60
TIME AFTER INFECTION (MIN)

or B40Sul, indicating that [uvsU] was not an amber mutant. Since [uvsX,uvsU,49] was no more sensitive to uv-irradiation than [uvsX,49] (Figure 30), we concluded that uvsU and uvsX were on the same DNA repair pathway (Ebisuzaki et al., 1974).

The [uvsU] mutation suppressed the DNA arrest phenotype of the [uvs X] mutation (Figure 31) but had no effect on the uv-sensitivity of [uvsX] (Figure 30). The suppression by [uvsU] was specific for the [uvsX] mutant since [uvsU] failed to suppress the DNA arrest phenotype of [uvsY], [46], [47] and [59] (data not shown). If the bypass mode of suppression is correct (Wakem and Ebisuzaki, 1981), these observations would be difficult to reconcile if [uvsU] were a gene 49 mutant.

The [uvsU] and [uvsW] mutants have many similar properties but they also differ in some ways. The [uvsW] mutants are sensitive to hydroxyurea (Hamlett and Berger, 1975) but [uvsU] mutants are not (data not shown). Also the [uvsU] mutants suppress the gene 49 mutation whereas the [uvsW] mutant does not (Results, section 2; Wakem and Ebisuzaki, 1981). We believe that these observations are related for the following reasons. Since the [uvsW] mutant does not suppress the [49] mutation, the DNA intermediates in a [uvsW] infection requires the gene 49 function (Results, section 2; Wakem and Ebisuzaki, 1981). Since the

FIGURE 30

The uv-sensitivity of various phage strains plated on E.coli B and B40Sul. Symbols: plated on E.coli B, wild type T4 (o), C69[uvsU,49] (□), amS17,amE727x1[uvsX,49] (Δ), amS76[uvsX,uvsU,49] (▽); plated on E.coli B40Sul, C69[uvsU,49] (■).

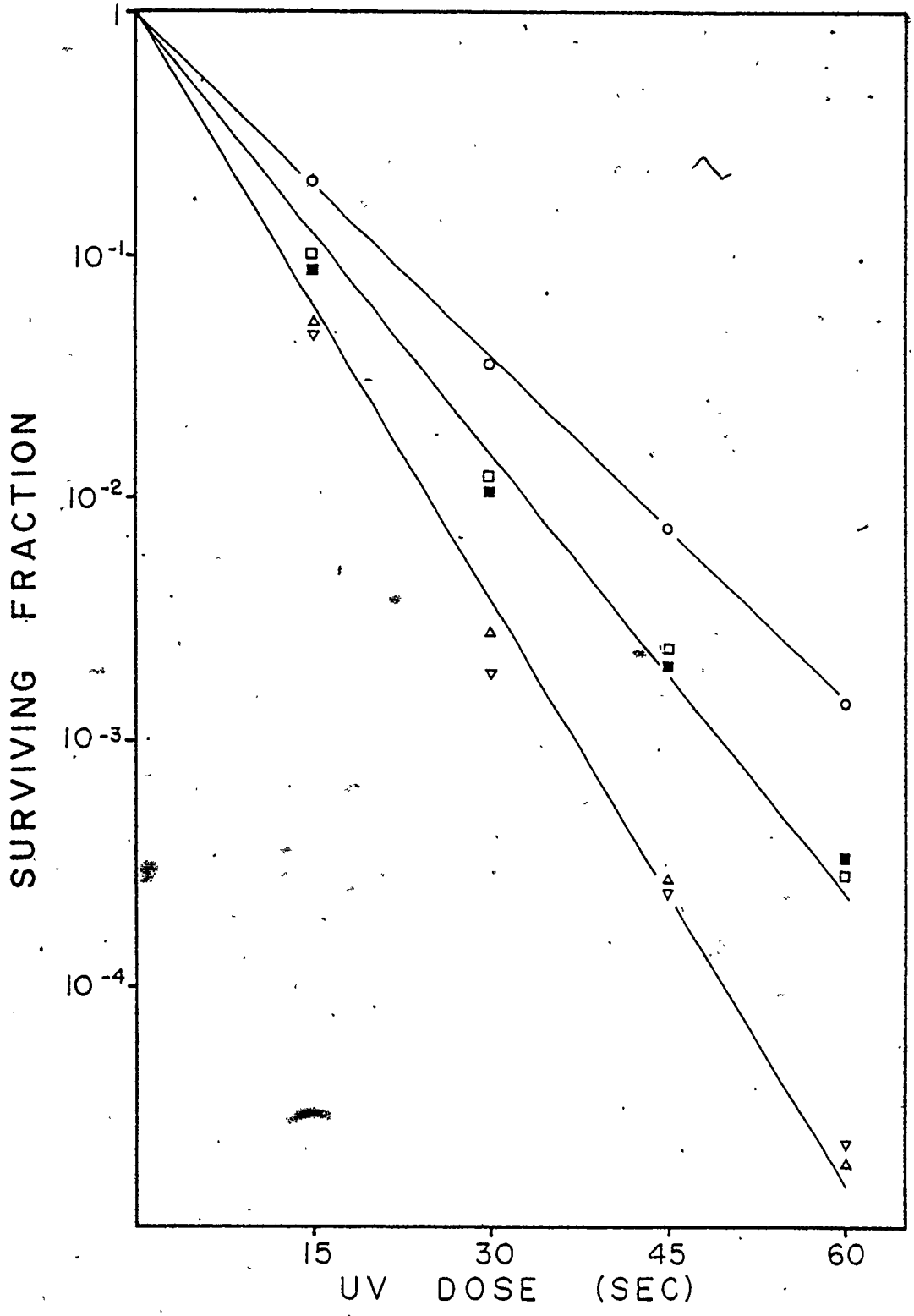
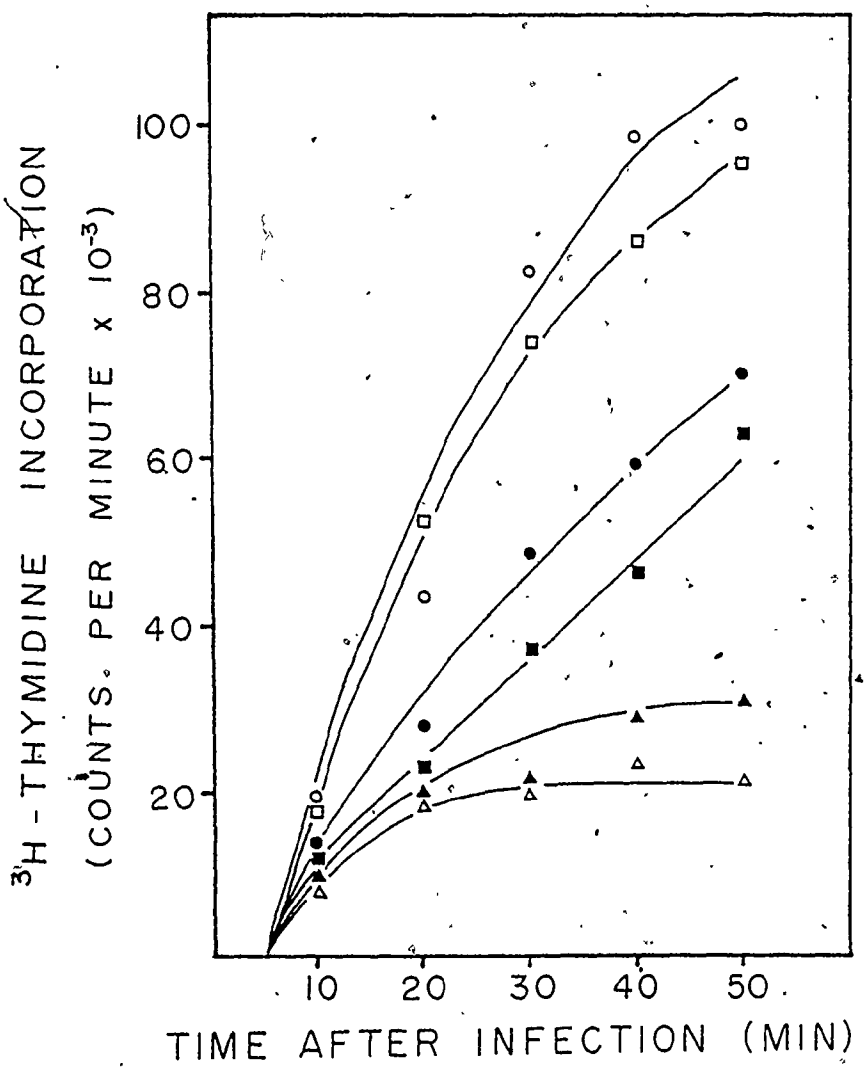

1
10^{-1}
10^{-2}
10^{-3}
10^{-4}
SURVIVING FRACTION
15
30
45
60
UV DOSE (SEC)

FIGURE 31

A dominance test for C69[uvsU,49]. Dominance was determined by measuring [^{3}H]thymidine incorporation into acid-insoluble material in cells mixedly infected with amS76[uvsX,uvsU,49] plus amS17,amE727xl[uvsX,49] at various times postinfection. Symbols: C69[uvsU,49] (○), amS76[uvsX,uvsU,49] (□), amS17,amE727xl[uvsX,49] (△), amS76[uvsX,uvsU,49] + amS17,amE727xl[uvsX,49] in the following ratios: 1[uvsX,uvsU,49]:1[uvsX,49] (■), 1[uvsX,uvsU,49]:5[uvsX,49] (▲), 5[uvsX,uvsU,49]:1[uvsX,49] (●).


^{3}H - THYMIDINE INCORPORATION
(COUNTS. PER MINUTE x 10^{-3})
100
80
60
40
20
10
20
30
40
50
TIME AFTER INFECTION (MIN)

gene 49 function is hydroxyurea-sensitive (Goscin and Hall, 1972), a [uvsW] mutant should also be hydroxyurea-sensitive and those mutants such as [uvsU] which do not require the gene 49 function should be (and are) hydroxyurea-insensitive. Cunningham and Berger (1977) have also noted that the hydroxyurea sensitivity of the [uvsW] mutant was suppressed by [uvsX] and [uvsY] mutations. This observation is consistent with the placement of the uvsW gene product at the beginning of the uvsX-uvsY pathway and the bypass model proposed earlier (Results, section 2; Wakem and Ebisuzaki, 1981).

5e. Dominance

We tested the [uvsU] mutant for dominance by measuring the DNA replication of cells mixedly infected with [uvsU,uvsX,49] and [uvsX,49]. As shown in Figure 31, [uvsU] was codominant with respect to the wild type allele. Other experiments measuring the DNA replication of cells mixedly infected with [uvsU,49] and [49] in the presence of mitomycin C confirm these results (data not shown).

5f. The effect of [uvsU] on recombination

The effect of [uvsU] on genetic recombination was tested by determining the frequency of rII^+ recombinants in crosses involving different combinations of [rII_1,49], [rII_2,49], [uvsU,rII_1,49] and [uvsU,rII_2, 49]. The [uvsU] mutant had little or no effect on recombination (Table 9) but these results should be viewed with some reservations because the [uvsU] mutant was also not very sensitive to uv-irradiation (Figure 30). Since we have studied only a single [uvsU] mutant, we do not know whether the mutant studied here was leaky.

TABLE IX

The Effect of C69[uvsU] on Genetic Recombination

CROSS	Frequency of rII^+ Recombination (%)
1. F1D3,amE727x1 x rIIb50,amE727x1 [rIIA,49 x rIIB,49]	2.6
2. C69,F1D3,amE727x1 x rIIb50,amE727x1 [uvsU,rIIA,49 x rIIB,49]	2.8
3. F1D3,amE727x1 x C69,rIIb50,amE727x1 [rIIA,49 x uvsU,rIIB,49]	2.7
4. C69,F1D3,amE727x1 x C69,rIIb50,amE727x1 [uvsU,rII,49 x uvsU,rIIB,49]	2.9

DISCUSSION

1. Sedimentation Analysis of Intracellular DNA in Repair-Defective Mutants

Following uv-irradiation of cells infected with wild type T4 phage, the denV gene-controlled excision repair pathway assumes an important role by rapidly nicking DNA and initiating a "joining" reaction. Presumably this endonuclease activity is followed by the removal of thymine dimers by the 5'-3' exonuclease described by Ohshima and Sekiguchi (1972) and/or DNA polymerase I (Cozzarrelli et al., 1969). The gaps created by the above nucleolytic activities are believed to be sealed by DNA polymerase I and polynucleotide ligase. In the experiments cited here, the overall ligation step may involve two or more types of reactions since the initial "joining" occurs very rapidly whereas the complete "joining" to restore mature size DNA molecules takes much longer.

If the excision repair pathway is inhibited by mutation of the denV gene, there is a slow endonucleolytic activity, which may be due to a slightly leaky [denV] mutant (Ohshima and Sekiguchi, 1975) or due to other host or phage-coded endonucleases. This endonucleolytic activity and subsequent "joining" may be going on simultaneously since there is

little change in the size of the single-strand fragments for a considerable period of time. In cells infected by [denV], the appearance of mature size, repaired DNA was delayed approximately 10-20 minutes.

In cells infected by [denV,uvsX] or [denV,uvsY], where both the denV gene-controlled excision repair and uvsX- uvsY pathways are inhibited, the endonucleolytic activity is still present but the single-strand DNA fragments are not "joined" after irradiation. A similar failure in "joining" was observed even in the absence of uv-irradiation. These observations suggest the uvsX and uvsY gene functions are involved in a "joining" function which is only incidentally related to DNA repair. This fragmentation of DNA and the decreased amount of concatemers observed during normal infection with [denV,uvsX] or [denV,uvsY], probably accounts for the decreased phage yields previously noted in cells infected with [uvsX] and [uvsY] (Harm, 1964; Boyle and Symonds, 1969).

Finally, what is the "joining" reaction in the context of the [uvsX] and [uvsY] mutations? It is not known whether "joining" involves the two original strands from the same DNA molecule, strands from two different molecules, or if the "joining" involves replacement of one of the fragments by a synthetic route. Likewise, the nature of the DNA intermediate(s) in the "joining" reaction is unknown, but it

is probably more complex than a duplex molecule with a nick or a simple gap, since such structures should be repaired by DNA polymerase I and DNA ligase (Anraku et al., 1969). The defects in the uvsX and uvsY genes are not obvious defects in DNA synthesis or of a slower rate of strand growth (or joining of Okazaki fragments). We suggest that since the [uvsX] and [uvsY] mutants are deficient in genetic recombination and in multiplicity reactivation (Harm, 1964; Boyle and Symonds, 1969), the defect in "joining" may be a failure in the formation or processing of "pre-recombinant" DNA molecules. As a final note, where the denV gene is defective, "joining" might be considered as "repair" by the sucrose gradient centrifugation analysis but it might not be "repair" in a functional sense.

2. DNA Repair-Recombination Functions in the DNA Processing Pathway of T4

2a. DNA repair pathway and the suppression of gene 49 mutations

The finding that mutations in genes uvsX, uvsY, 59, 46 and 47 suppress gene 49 mutations suggests related functions for these genes. While the properties of the five mutants differ in detail, the mutants are similar in many respects including uv-sensitivity and recombination (Harm, 1963; Harm, 1964; Baldy, 1968; Bernstein, 1968; Boyle and Symonds, 1969; Wu et al., 1975; Cunningham and Berger, 1977), concatemer formation, and DNA replication (Wiberg et al., 1962; Shah and Berger, 1971; Wu et al., 1972; Wu and Yeh, 1974; Wakem and Ebisuzaki, 1976; Cunningham and Berger, 1977). The common critical defect of these mutants, may be in their inability to join DNA fragments after DNA damage and to form concatemers. These common features suggest that these five genes should be classified as a single group.

Could these related functions be involved in a common DNA repair pathway? It was previously shown that uvsX and uvsY were in the same pathway (Harm, 1963; Boyle and Symonds, 1969) and by similar tests, we show gene 59 to be in this pathway. The evidence that genes 46 and 47 function

in this pathway is less certain, but the putative role of genes 46 and 47 as an exonuclease(s) (Wiberg, 1966; Kutter and Wiberg, 1968) is readily accommodated in a repair pathway. Furthermore, the suppression of gene 49 mutations by these five mutations could be most easily accounted for if these genes functioned as contiguous components in a common pathway. On the other hand, genes 58 and uvsW have been assigned to the same DNA repair pathway (Hamlett and Berger, 1975) but differ greatly in the kinetics of the DNA replication. The five mutants involved in the suppression of gene 49 mutations have mainly a DNA arrest phenotype, whereas the gene 58 mutants have a DNA delay phenotype and gene uvsW mutants resemble wild-type T4 DNA replication (Yegian et al., 1971; Hamlett and Berger, 1975). If, in fact, genes uvsW, 58 and the other five genes function in the same pathway, possibly the suppression of gene 49 mutations only involves part of the pathway.

2b. Mechanism of the suppression of the DNA processing pathway

Previously, it was observed that gene 49 mutant infections resulted in the accumulation of fast sedimenting DNA (Dewey and Frankel, 1975b; Kemper and Janz, 1976) and that mutations in uvsX or uvsY prevented this accumulation

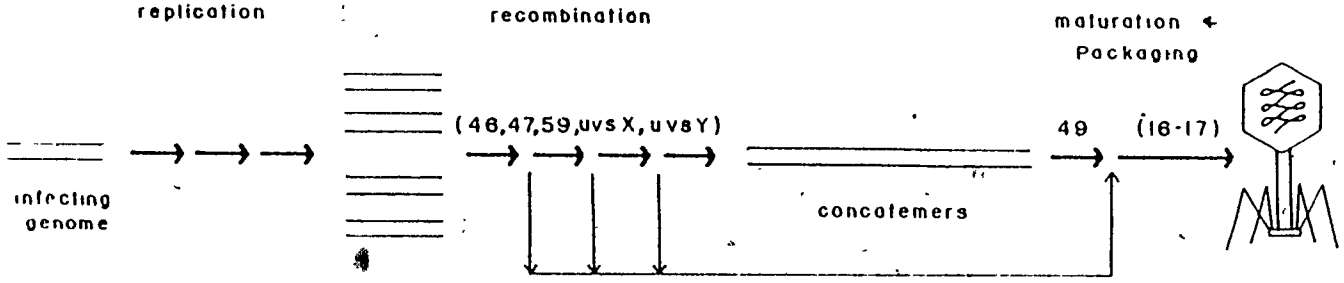
(Dewey and Frankel, 1975b). These observations suggest that the uvsX and uvsY functions are involved in the synthesis of the substrate (concatemers) for the gene 49 endonuclease. Our findings that mutations in genes 46, 47 and 59 also suppress gene 49 mutations suggest that genes 46, 47 and 59 together with uvsX and uvsY are involved in the DNA processing pathway (Figure 32). Thus, the DNA repair pathway becomes part of a DNA processing pathway and this may be one of the primary functions of the DNA repair genes. We propose that the suppression of the gene 49 mutation results from the failure of the DNA repair mutants in a joining reaction to form concatemers and this serves as a crude equivalent of a gene 49 endonuclease. In this way, the DNA intermediates resulting from mutations in the DNA repair genes bypass the gene 49 function. We suggest that these DNA intermediates reenter the DNA processing pathway prior to the genes 16-17 function(s) because if the gene 16-17 function(s) preceded the gene 49 function, then the recombinants [uvsX,16,49] and [uvsX,17,49] should not be lethal. Since these recombinants are lethal, the genes 16-17 function(s) follow the gene 49 function and are necessary for the processing of the DNA intermediates formed by the DNA repair mutants.

We have proposed that a primary role of the DNA repair pathway is in DNA processing. However, this view does not

FIGURE 32

Proposed mechanism for the suppression of gene 49 mutants by secondary mutations in genes 46, 47, 59, uvsX or uvsY. The heavy arrows denote the usual pathway for the processing of DNA in wild type T4 infection. The lighter arrows show that the DNA intermediates which accumulate in gene 46, 47, 59, uvsX or uvsY mutant infections bypass the requirement for the gene 49 endonuclease and funnel into the DNA processing pathway between gene 49 and genes 16-17.

DNA PROCESSING PATHWAY



exclude other roles for these genes such as in DNA replication.

3. An Analysis of the DNA Repair-Recombination Functions by Means of Suppressors: The Role of Das

It seems most probable that the high specificity of the suppressors of mutations in the DNA repair-recombination genes have as their basis a corresponding specificity in the gene products that are being involved. This suppression could occur via protein-protein interactions as suggested for the suppression of gene 32 mutations (Mosig et al., 1978) or through interactions at the metabolic level as indicated in the suppression of the recB-C (nuclease) mutation by the sbcB (exonuclease I) mutation in E. coli (Kushner et al., 1971). Although the mechanisms of the suppression of the T4 DNA repair- recombination mutations are unknown, we suggest that the study of these suppressors provides information on the relationships of the repair-recombination functions within the DNA repair pathway.

3a. Dissection of the DNA repair pathway

In a previous section, we presented evidence that the genes 46, 47, 59, uvsX and uvsY were involved as part of the DNA processing pathway of T4 phage (Results, section 2; Wakem and Ebisuzaki, 1981). That these same five genes are involved in a unitary function was also supported by the finding that a single suppressor [sur], which appears to be specific for mutations in the uvsX-uvsY DNA repair pathway, suppressed all five mutations (Results, section 4). This group of five genes could be further subdivided by two suppressors [uvsW,(dar)] and [das]. The [uvsW,(dar)] mutations suppressed mutations in genes 59, 46 and 47 but did not suppress mutations in genes uvsX or uvsY (Wu and Yeh, 1975; Cunningham and Berger, 1977; Wu and Yeh, 1978). The [das] mutation suppressed the DNA arrest and DNA repair defects of mutations in genes 46 and 47 and the DNA repair defects in gene uvsX and uvsY mutations (Figure 14 and 16). On the other hand, [das] neither suppressed the DNA arrest phenotype (Wiberg and Swanson, 1975) nor the DNA repair defect (Figure 14b) of the gene 59 mutation. The cross reactivity in the suppressor functions as summarized in Table 6, suggested that the repair-recombination gene functions or products might be arranged as follows: 59→(46-47)→(uvsX-uvsY). We propose that this arrangement suggested mainly because of the overlapping suppression by

[das] and [uvsW(dar)], might be the sequence for the pathway. We have placed the 59, 46-47 functions in the leading position in the pathway because of the expectation that the (46-47) exonuclease function (Prashad and Hosoda, 1972; Mickelson and Wiberg, 1981) would be required at an early step in recombination or DNA repair. An analysis of the DNA intermediates also supported the view that the (46-47) function(s) preceded the uvsX function (Results, section 2; Wakem and Ebisuzaki, 1981). The specific suppression of [uvsX] by [uvsU] suggests that genes uvsX and uvsY control different functions but the order within the uvsX and uvsY group is not known.

3b. Mechanism of suppression by [das].

Since [das] mutants have an elevated nuclease activity, the proposed bypass for the genes 46-47 (nuclease) defect seems reasonable (Hercules and Wiberg, 1971; Mickelson and Wiberg, 1981), but the underlying basis for the increased nuclease activity is not known. The dominance of the [das] mutation over the wild type allele and the alteration in lysozyme activity in the [das] mutant (Hercules and Wiberg, 1971), suggests the possibility of an alteration of a control function. However, we have not found any indications of altered patterns of protein synthesis in

autoradiograms of SDS polyacrylamide gels of labelled proteins prepared from cells infected with wild type T4 and the [das] mutant at 6, 9, 12 and 15 minutes postinfection (data not included). To summarize, the bypass proposal is appealing because of the elevated nuclease activity in cells infected with [das] mutants but the mechanism of the das function and its relationship to the suppression of [uvsX] and [uvsY] is unknown.

4. A New Suppressor of Mutations in the DNA Repair-Recombination Genes of Bacteriophage T4: sur

There seems to be little doubt that [sur] is a different kind of suppressor from those already reported (Results, sections 3 and 5). [Sur] most closely resembles [das] but differs from [das] in its wider range of activity and in its effect on host DNA degradation. Although mapping data would be helpful to clearly distinguish [sur], this objective has been difficult to attain because [sur] not only lacks an easily identifiable phenotype but also the mapping strategy used for [das] (Hercules and Wiberg, 1971) was inapplicable. This method involved crosses between [das,am,46,47] with [46,47] to measure the recombination frequency between [das] and [am]. This method also depends on the ability of [das,46,47] to form clear plaques and on

the inability of [46,47] to form plaques under restrictive conditions. In a comparable situation the [sur,46] plaques were too small to be clearly distinguished from the [46] mutant. The other mutants [59], [uvsX] or [uvsY] could not be used in place of [46] because they formed small plaques.

4a. The mechanism of suppression by [sur]

While [sur] suppressed all five amber mutants of the DNA repair-recombination pathway, it seems unlikely that [sur] is an amber suppressor since other amber mutants in genes unrelated to DNA repair-recombination were not suppressed. These amber mutants included mutants in genes 24, 25, 30, 42, 49 and 55.

Since [sur] suppressed mutations in five DNA repair-recombination genes of the uvsX-uvsY DNA repair pathway, it would seem that suppression might require, for example, a compensatory increase in another pathway or a general alteration in cellular functions. However, we have not observed any obvious indications of an altered regulation of protein synthesis, as judged by the appearance of autoradiograms of SDS polyacrylamide gels of labelled proteins at various times after infection with wild type T4 and [sur] (data not shown). Although we have not studied the various means of suppression, we have noted that

suppression by [sur] does not appear to involve an increase of the excision repair pathway. Dominance tests involving [sur] and the wild type allele suggested that [sur] might either be involved in a stoichiometric function rather than a catalytic one (Snustad, 1980) or that there were dominance interactions at the polypeptide level. Host DNA degradation studies indicated that both [sur] and [das] mutations led to an increased degradation of host DNA (Hercules and Wiberg, 1971; and Figure 23) but that these mutations affected different steps in the degradation process. Possibly the increased degradation of host DNA was not the basis of suppression but rather the degradative activities reflected the increased activity of an alternative DNA repair-recombination pathway.

5. The Coupling of DNA Repair-Recombination Functions with DNA Replication: uvsU and the "Switch" Model

The interaction of the T4 phage DNA repair, recombination and replication functions has been generally accepted but the mechanistic basis for the relationships are unclear. We have been concerned with five genes (59, 46, 47, uvsX and uvsY) of the uvsX-uvsY DNA repair-recombination pathway, whose functions appear to be primarily involved in the DNA processing pathway (Results, section 2; Wakem and

Ebisuzaki, 1981). Mutants in these five genes initiate DNA replication, but later in infection DNA replication is shut off (Wiberg et al., 1962; Wu et al., 1972; Dewey and Frankel, 1975; Cunningham and Berger, 1977). We have also been concerned with two other genes, uvsU and uvsW, whose functions are closely related to the functions of the above five genes. Although the [uvsU] and [uvsW] mutants are also defective in DNA repair, nevertheless they maintain a wild type level of DNA replication (Hamlett and Berger, 1975; Results, section 5). In addition, the [uvsW] mutant suppressed the DNA replication defect of the [46], [47] and [59] mutations and similarly the [uvsU] mutant suppressed the replication defect of the [uvsX] mutation (Cunningham and Berger, 1977; Results, section 5).

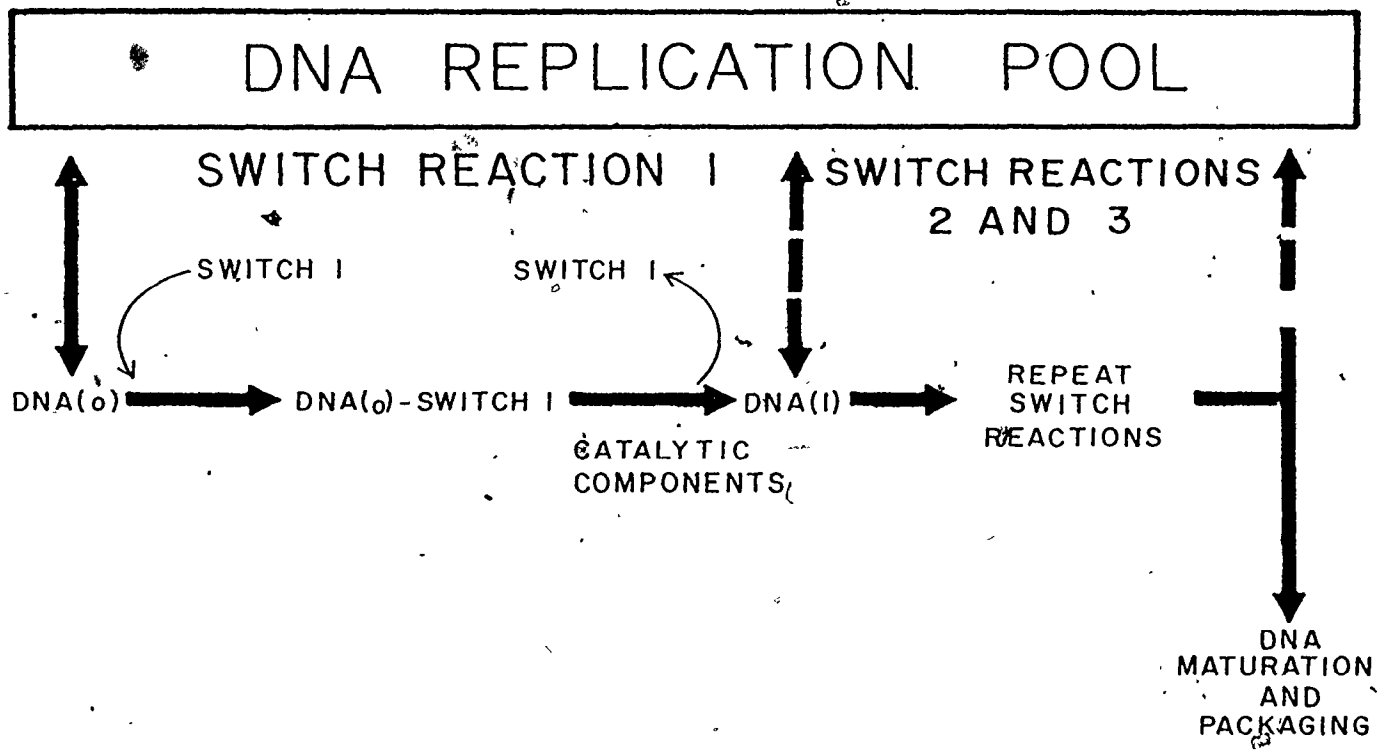
5a. "Switch" model

To account for some of the above observations, we propose that the arrest in DNA replication observed with some of the mutants of the uvsX-uvsY DNA repair pathway result because the DNA intermediates are removed from the replication pool and become unavailable for replication purposes. In this model, the gene products of uvsW and uvsU function as "switches" that divert the DNA intermediates from the DNA replication pool to the DNA repair-

recombination pathway (Figure 33). The suppression of the [59], [46] and [47] mutants by [uvsW] and the [uvsX] mutant by [uvsU] suggests that the corresponding gene products might function as a complex. If such complexes were composed of wild type proteins, the DNA intermediate binds to the "switch", undergoes a reaction(s) catalyzed by the other components of the complex and dissociates. The transit of the DNA intermediates via these complexes proceeds until the DNA repair-recombination reactions are completed and the DNA intermediate either returns to the replicating pool or is processed further for eventual packaging (Results, section 2; Wakem and Ebisuzaki, 1981). However, if a "switch" (either uvsU or uvsW) were defective, the DNA intermediates are retained in the replicating pool resulting in the wild type levels of DNA replication. On the other hand, if the catalytic components (59, 46, 47 or uvsX) were defective, the DNA intermediate remains bound to the "switch" component and unavailable for replication. A continuation of this process results in a depletion of the DNA templates and a cessation in replication. By having such a two step process, a defect in the first step ("switch") would have a dominating effect such that a further mutation in the second ("catalytic") component(s) would be innocuous. This would account for the suppression of the DNA replication defects by uvsW and uvsU.

FIGURE 33

The "switch" model for coupling DNA replication with the DNA repair-recombination pathway. DNA intermediates (DNA-O) from the replication pool either bind to or react with a "switch" component and are converted by the catalytic components to the product, DNA(1). Subsequently, both the DNA product and the "switch" component dissociate. The DNA product either returns to the replication pool or is further processed by the additional switch reactions, maturation and packaging into phage particles. Switch reaction 1, refers to the initial set of reactions involving the "switch" component exemplified by the uvsW gene product and the catalytic components by the gene 59, 46 and 47 products. Switch reactions 2 and 3 refer to a similar complex composed of the uvsU and uvsX gene products and another complex involving the uvsY gene product.


DNA REPLICATION POOL
SWITCH REACTION 1
SWITCH REACTIONS 2 AND 3
SWITCH 1
SWITCH 1
DNA(o)
DNA(o)-SWITCH 1
DNA(I)
REPEAT SWITCH REACTIONS
CATALYTIC COMPONENTS
DNA MATURATION AND PACKAGING

5b. Support for the model

The "switch" model pre-supposes that if recombination or DNA repair is continuously initiated and aborted, the DNA template pool becomes depleted. For this to happen, each DNA intermediate must undergo one or more recombinational events. This possibility is supported by estimates that each phage chromosome undergoes about three (Doermann and Parma, 1967) or as many as 20 to 40 (Stahl et al., 1964) recombinational events. Further support is provided through the analysis of the DNA intermediates by alkaline sucrose gradient centrifugation. Cunningham and Berger (1977) showed that at a time when DNA replication was arrested (25 minutes at 30°C postinfection), most of the DNA strands were shorter than mature size DNA in [uvsX] or [uvsY] infections, whereas comparable studies with wild type T4 infection indicated that the DNA was mature size.

The [uvsU] mutant appears to be co-dominant over the [uvsX] mutant in mixed infections involving [uvsU] and [uvsX]. Although the model depicting a "switch" and "catalytic components" has no biochemical basis, the co-dominance relationships of the [uvsU] mutant seems to be more compatible with a stoichiometric (such as binding) function than a catalytic one. A similar dominance relationship may also occur with [uvsW]. Melamede and Wallace (1980) have noted that in DNA synthesis experiments,

[uvsW] was dominant in mixed infections involving [uvsX] and [uvsY].

The functional features of the model involve a complex composed of a "switch" and catalytic components. An argument for symmetry would require a similar switch for uvsY. Previously, we noted [uvsY] mutants as determined by complementation tests were composed of two types, those with a DNA arrest phenotype and those with a "wild" type level of DNA replication (Results, section 2; Wakem and Ebisuzaki, 1981). Mutants of the latter type appeared and preliminary experiments have indicated that crosses between such mutants and uvsY yielded a high frequency of uvs+ progeny. These results suggest that those [uvsY] mutants with a "wild" type DNA replication are similar to the [uvsU] mutant and a "switch" component might also exist for the uvsY function.

5c. Problems associated with the model

A number of problems related to the "switch" model will be discussed.

(1) If the sequence of the DNA repair-recombination pathway begins with the uvsW complex (Discussion, section 3), then the model predicts that a block in uvsW should block not only the functions of genes 59, 46 and 47 but also the functions of the distal genes, uvsX and uvsY. However,

the [uvsW] mutation does not suppress the DNA replication defect of the [uvsX] and [uvsY] mutants (Cunningham and Berger,1977). This could mean that the model is incorrect or that less potent, alternative pathways exist. Alternative pathways are possible since a large number of nucleases are induced after infection (Mathews, 1977) and some of the host DNA repair- recombination functions may be active.

(2) One of the puzzling aspects of these studies is the failure of [uvsU] and [uvsX] mutants to complement, despite the fact that they are located in different regions of the T4 map (Results, section 5). Clearly, more studies are needed to study this problem, particularly with different [uvsU] mutants.

(3) The Luder-Mosig Model. While the Luder-Mosig model is primarily concerned with the mechanisms for the initiation of DNA replication, it also deals with the coupling of recombination and DNA replication (Luder and Mosig, 1982). In their model, mutations in the maturation genes 33 and 55 allows for the continued function of RNA polymerase in the initiation of DNA replication. However, if the 33 and 55 genes were wild type, the modified RNA polymerase no longer functions in initiation and the recombination mode of initiation becomes necessary. This could explain the basis for the replication defect of the [46,47] mutants and restoration of DNA replication in the

[33,55,46,47] mutants. Since the [uvsW,46,47] mutant also shows a restoration of DNA replication while still retaining the recombination defect (Cunningham and Berger, 1977), could the role of uvsW be analogous to the 33, 55 maturation functions and be a component of the RNA polymerase complex? Gene 33 and 55 mutants are defective in late transcription, however the [uvsW] mutant showed no general defect in late protein synthesis as judged by the production of serum blocking power but the mutant does show a defect in packaging (Hamlett and Berger, 1975). However, the packaging defect might be the consequence of structural defects in DNA. The [uvsU] mutant shows no delay in phage formation (see Results). These observations suggest that the [uvsW] and [uvsU] mutants are not defective in maturation.

While the experimental evidence supporting the Luder-Mosig model does not appear to be inconsistent with a "switch" model, the two models focus on entirely different perspectives, so not suprisingly, the interpretations differ. A better understanding of the DNA repair-recombination process, particularly the role of uvsW and uvsU should help in clarifying the interrelationship of the repair-recombination and replication functions.

SUMMARY

This thesis has involved biochemical and genetic analyses of the uvsX-uvsY DNA repair pathway of bacteriophage T4. The studies were conducted to aid in understanding the repair mechanisms involved in the pathway, the functions and interactions of the repair-recombination gene products and the relationship of the uvsX-uvsY pathway to DNA replication and DNA maturation.

Our analyses of the uvsX-uvsY DNA repair pathway showed the following main results:

1. The uvsX and uvsY gene products are important in DNA joining reactions both in the formation of concatemers and in the repair and rejoining of nicked DNA following uv-irradiation.

2. The products of genes 46, 47, 59, uvsX and uvsY function in a common DNA repair pathway (uvsX-uvsY pathway).

3. Mutations in the DNA repair-recombination genes 46, 47, 59, uvsX and uvsY suppress mutations in the DNA maturation gene 49 but not mutations in genes 16 and 17.

4. A study of the [das], [uvsW(dar)], [sur] and [uvsU] mutations indicated the diverse activities of these suppressors of mutations in genes 59, 46, 47, uvsX and uvsY and in turn permitted us to predict the sequence involved in

the uvsX-uvsY pathway.

5. A new suppressor [sur], suppresses mutations in the five DNA repair-recombination genes and appears to be specific for the uvsX-uvsY DNA repair pathway. The possible mode of suppression was considered.

6. A new uv-sensitive mutation [uvsU], involved in the uvsX-uvsY pathway is described. The [uvsU] mutant specifically suppresses but does not complement [uvsX] mutations and is similar in phenotype to [uvsW] mutants.

Using the results from our pathway analysis experiments, we have proposed the following models concerning the uvsX-uvsY DNA repair pathway:

1. The products of the DNA repair-recombination genes may function in the following reaction sequence: 59→(46-47)→(uvsX-uvsY) (Table 6).

2. The products of genes 59, 46, 47, uvsX and uvsY function in a DNA processing pathway (Figure 31). Replicative DNA intermediates are processed into concatemeric DNA by the repair-recombination gene products. The concatemers are further processed by the products of the maturation genes 49, 16 and 17 to a form which is packageable into phage heads. The requirement for the gene 49 function is bypassed when any of the five DNA repair-recombination genes are mutated. However, the requirement for the gene 16 and 17 functions is not

bypassed..

3. The products of genes uvsW and uvsU couple DNA replication with the DNA repair-recombination functions (Figure 32). In our model, the uvsW and uvsU gene products act as switches to divert DNA intermediates from the replication pool into the DNA processing pathway. The uvsW gene product binds DNA intermediates in a complex with the gene 59, 46 and 47 products until the repair-recombination functions have reacted with the DNA. After the reactions are complete, the DNA is released from the complex and is free to be bound by the next complex in the sequence eg. uvsU with uvsX or "high y" with uvsY. This process continues until the DNA is either packaged or returns to the replication pool.

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