1973

Ultrastructural Studies Of Mitosis In Fungi

Edith Kathleen Mccully

Follow this and additional works at: https://ir.lib.uwo.ca/digitizedtheses

Recommended Citation
https://ir.lib.uwo.ca/digitizedtheses/654

This Dissertation is brought to you for free and open access by the Digitized Special Collections at Scholarship@Western. It has been accepted for inclusion in Digitized Theses by an authorized administrator of Scholarship@Western. For more information, please contact tadam@uwo.ca, wlswadmin@uwo.ca.
The author of this thesis has granted The University of Western Ontario a non-exclusive license to reproduce and distribute copies of this thesis to users of Western Libraries. Copyright remains with the author.

Electronic theses and dissertations available in The University of Western Ontario’s institutional repository (Scholarship@Western) are solely for the purpose of private study and research. They may not be copied or reproduced, except as permitted by copyright laws, without written authority of the copyright owner. Any commercial use or publication is strictly prohibited.

The original copyright license attesting to these terms and signed by the author of this thesis may be found in the original print version of the thesis, held by Western Libraries.

The thesis approval page signed by the examining committee may also be found in the original print version of the thesis held in Western Libraries.

Please contact Western Libraries for further information:
E-mail: libadmin@uwo.ca
Telephone: (519) 661-2111 Ext. 84796
Web site: http://www.lib.uwo.ca/
ULTRASTRUCTURAL STUDIES OF MITOSIS IN FUNGI

by

Edith Kathleen McCully

Department of Bacteriology and Immunology

Submitted in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

Faculty of Graduate Studies

The University of Western Ontario

London, Canada

September 1972

© Edith Kathleen McCully 1972
The cost of this study was supported by a grant to Professors C. F. Robinow and R. G. E. Murray from the Medical Research Council of Canada. The author expresses her thanks to the Council for this support.
ACKNOWLEDGMENTS

I wish to thank Professor C. F. Robinow whose wide knowledge and enthusiasm first made me interested in the problems of mitosis and who provided constant guidance and encouragement throughout the course of this work.

I also wish to thank Dr. C. E. Bracker, Purdue University, Lafayette, Indiana, U.S.A. for his advice about fixation techniques for electron microscopy.

Thanks are also due to Miss Julia Wang for technical assistance and to Mr. John Marak for maintaining the electron microscope at peak efficiency.

The typing of this thesis was done by Mrs. Jill Murray for whose excellent effort I am grateful.
## CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ACKNOWLEDGMENTS</td>
<td>iv</td>
</tr>
<tr>
<td></td>
<td>LIST OF ILLUSTRATIONS</td>
<td>vii</td>
</tr>
<tr>
<td></td>
<td>LIST OF TABLES</td>
<td>viii</td>
</tr>
<tr>
<td></td>
<td>ABSTRACT</td>
<td>ix</td>
</tr>
<tr>
<td>CHAPTER I</td>
<td>INTRODUCTION AND HISTORICAL REVIEW</td>
<td>1</td>
</tr>
<tr>
<td>CHAPTER II</td>
<td>STUDIES ON <em>Schizosaccharomyces pombe</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>INTRODUCTION</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>MATERIALS AND METHODS</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>OBSERVATIONS</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>DISCUSSION</td>
<td>23</td>
</tr>
<tr>
<td>CHAPTER III</td>
<td>STUDIES ON <em>Leucosporidium scottii</em> <em>(Candida scottii)</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>INTRODUCTION</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>MATERIALS AND METHODS</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>OBSERVATIONS</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>DISCUSSION</td>
<td>60</td>
</tr>
<tr>
<td>CHAPTER IV</td>
<td>STUDIES ON <em>Rhodosporidium sp.</em> <em>(Rhodotorula glutinis)</em> AND <em>Aessorporon salmonicolor</em> <em>(Sporobolomyces salmonicolor)</em></td>
<td>99</td>
</tr>
<tr>
<td>Chapter</td>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>- vi -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chapter V</td>
<td>STUDIES ON MUCOR HIEMALIS</td>
<td></td>
</tr>
<tr>
<td>Introduction</td>
<td></td>
<td>149</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td></td>
<td>152</td>
</tr>
<tr>
<td>Observations</td>
<td></td>
<td>153</td>
</tr>
<tr>
<td>Discussion</td>
<td></td>
<td>157</td>
</tr>
<tr>
<td>Chapter VI</td>
<td>SUMMARY AND FINAL DISCUSSION</td>
<td>187</td>
</tr>
<tr>
<td>Bibliography</td>
<td></td>
<td>199</td>
</tr>
<tr>
<td>Vita</td>
<td></td>
<td>215</td>
</tr>
</tbody>
</table>
# LIST OF ILLUSTRATIONS

## DIAGRAMS

<table>
<thead>
<tr>
<th>Diagram</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
</table>
| Fig. 18 | Diagrammatic Interpretation of Mitotic Events in
          *Leucosporidium scottii.*                                             | 68   |
| Fig. 43 | Diagrammatic Interpretation of Spindle Formation and the Separation of Daughter Nuclei in
          *Rhodosporidium sp.*                                                   | 114  |
| Fig. 81 | Diagrammatic Interpretation of Mitotic Events in
          *Mucor hiemalis*                                                        | 164  |
| Fig. 82 | Three Interpretations of the Morphology of the
          Microtubule Organizing Centre in Dividing Nuclei of
          *Mucor hiemalis*                                                        | 166  |

## ELECTRON MICROGRAPHS

<table>
<thead>
<tr>
<th>Figs.</th>
<th>Description</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 17</td>
<td><em>Schizosaccharomyces pombe</em></td>
<td>38-51</td>
</tr>
<tr>
<td>19 - 42</td>
<td><em>Leucosporidium scottii</em></td>
<td>69-98</td>
</tr>
<tr>
<td>44 - 69</td>
<td><em>Rhodosporidium sp.</em></td>
<td>115-136</td>
</tr>
<tr>
<td>70 - 80</td>
<td><em>Aessosporon salmonicolor</em></td>
<td>137-148</td>
</tr>
<tr>
<td>83 - 101</td>
<td><em>Mucor hiemalis</em></td>
<td>167-186</td>
</tr>
</tbody>
</table>
# LIST OF TABLES

| TABLE I  | Previous Ultrastructural Studies of Ascomycetes and Basidiomycetes at Mitosis and Meiosis | 9 |
| TABLE II | Names Used for Spindle-Associated Organelles at Mitosis and Meiosis | 24 |
| TABLE III | Characteristics of Stages of Mitosis in *Schizosaccharomyces pombe* | 32 |
| TABLE IV | Proposed Steps in the Evolution of the Mitotic Apparatus | 192 |
ABSTRACT

Mitosis has been studied in five species of fungi with electron microscopy. In all these fungi, mitosis differs from the "classical" pattern.

In Schizosaccharomyces pombe, an ascomycetous yeast, the nucleus divides by elongation and constriction. The nucleolus stretches out within the unbroken envelope of the nucleus as it elongates and finally becomes more or less equally distributed to the daughter nuclei. A mitotic spindle consisting of a narrow bundle of parallel microtubules elongates in step with the nucleus. The ends of the spindle are associated with disc-shaped osmophilic organelles which are attached to the outside of the nuclear envelope. Such organelles have previously been seen by others in ascomycetes and basidiomycetes and have been given many different names including "microtubule organizing centres" and "kinetochore equivalents". I have proposed that the term "microtubule organizing centre" is generally applicable to spindle-associated organelles in fungi but that the more specific term "kinetochore equivalent" may apply to the organelles in S. pombe because there are indications that the chromosomes are attached to them. It is proposed that the major cause of genome separation in S. pombe is the growth of the nuclear envelope between the attachment points of the two daughter sets of chromosomes.

In Mucor hiemalis, a terrestrial phycomycete, mitosis also takes
place within an intact nuclear envelope and involves a narrow intra-
nuclear spindle. The nucleolus also persists and stretches out within
the elongating nucleus. The ends of the spindle are associated with
regions of amorphous electron-dense material which are chiefly
inside the nuclear envelope. Such regions, which are referred to
as microtubule organizing centres, are described here for the first
time in a terrestrial phycomycete. A considerable elongation of the
nucleus occurs while the spindle is still quite small. Later, the
ends of the spindle reach the ends of the nucleus and further
elongation of the spindle occurs in step with nuclear elongation.

Mitosis in the heterobasidiomycetous yeast Leucosporidium scottii
(formerly Candida scottii) resembles mitosis in mycelial basidiomycetes
and differs in many ways from mitosis in S. pombe and M. hiemalis.
At mitosis, the nucleus of L. scottii appears to grow into the bud.
The nuclear envelope then becomes discontinuous. Inside the bud, a
spindle forms and elongates within the partially torn nuclear envelope.
This spindle has the shape of a double cone and it consists of both
pole-to-pole and chromosomal microtubules. The nucleolus-containing
portion of the nucleus remains in the mother cell and disintegrates
when the envelope breaks down. After chromatin division in the bud,
one daughter nucleus returns to the mother cell. A microtubule
organizing centre consisting of two globular ends and a bridge-like
middle piece is located in the cytoplasm near the interphase nucleus.
This organelle accompanies the advancing tips of nuclei as they enter
the bud. After breakdown of the nuclear envelope, the two spherical components of the organelle are seen at opposite poles of the mitotic spindle.

Mitosis in another heterobasidiomycetous yeast, Aessosporon salmonicolor (formerly Sporobolomyces salmonicolor) seems to be identical to mitosis in L. scottii. The same type of mitosis with a few variations occurs in a third heterobasidiomycetous yeast Rhodosporidium sp. (formerly Rhodotorula glutinis).

It is proposed that studies of unusual forms of mitosis such as the ones reported here might make three contributions apart from their general interest. They might have taxonomic significance. They might provide clues about the fundamental mechanisms of "classical" mitosis. They might provide clues about the evolution of the mitotic apparatus.
CHAPTER I

INTRODUCTION AND HISTORICAL REVIEW

The precise division of a duplicated parent genome into two identical daughter genomes is a prerequisite for development and differentiation in multicellular organisms. Therefore an understanding of mitosis is central to an understanding of growth and differentiation both normal and pathological.

Mitosis is not a single phenomenon. It is a complex process of many integrated cellular events. As such, it is a difficult process to study but at the same time it is very rewarding since in the words of Mazia (1965) "in the observation of mitosis we are given one of our clear visions of what the cell is beyond being a device for replicating DNA and making proteins and ATP".

Mitosis in both plant and animal cells is accomplished by the same sequence of events which may be summarized as follows:
- the duplicated chromosomes coil and shorten
- the nuclear envelope breaks down
- the nucleolus disappears
- a spindle apparatus is formed which is composed of both pole-to-pole and chromosome-to-pole fibres
- the chromosomes move to the equator of the spindle
- sister chromatids separate to opposite poles
the nuclear envelope reforms around the daughter nuclei
in each daughter nucleus the chromosomes resume their
thread-like interphase form and the nucleoli reform.

This sequence of events is known as "classical" mitosis. Plant
cells have no structures at the poles of the spindle while animal
cells have polar regions of variable size known as asters or
centrosomes. Each of these consists of a central pair of centrioles
surrounded by a halo of membranes, vesicles and radiating micro-
tubules.

Despite much observational and experimental data accumulated
since 1880 when Walther Flemming described "classical" mitosis in
more or less modern terms, many questions about the mechanism of the
process remain unresolved. Modern techniques such as electron
microscopy, polarizing microscopy, chemical inhibition of spindle
formation, UV microbeam irradiation of spindle fibres, and micro-
manipulation of chromosomes have settled some of the old questions
asked by classical cytologists such as whether spindle fibres are
real structures or lines of force analogous to those of a magnetic
field. However, such techniques have not been able to resolve other
important questions such as whether the spindle microtubules provide
the force for chromosome movement or whether they are passive tracks
which direct movement that is produced by some other force.

Many cytological studies, especially those carried out since
the advent of electron microscopy, have shown that variations from
the "classical" pattern of mitosis are common in algae, protozoa and fungi. Some of these are large variations from the sequence outlined on Pages 1 and 2, while others differ from it in only a few details, e.g. the nuclear envelope may not break down.

Such variations are interesting in themselves but they may also give clues about the fundamental mechanisms of "classical" mitosis which other research approaches have failed to resolve. By studying only mitosis in plant and animal cells there is a danger of falsely attributing particular origins and, from constant association, particular functions to various components of the mitotic apparatus. Moreover, as Pickett-Heaps (1969) has persuasively argued, the study of mitosis in primitive eukaryotic organisms might provide clues about the evolutionary development of the complex "classical" mitotic sequence. Such a proposal is not new. E. B. Wilson (1896) considered that "the process of mitosis in the one-celled plants and animals has a peculiar interest, for it is here that we must look for indications of its historical origin".

It is interesting to note that E. B. Wilson gives examples of unusual forms of mitosis in several algae and protozoa but he is silent about fungi--the class of organisms dealt with in this thesis. The cytological literature reviewed by Wilson (1896) contained numerous descriptions of mitosis in plants, animals, algae, and protozoa but there were no comparable accounts of nuclear division in
fungal hyphae even although the presence of nuclei in these structures had been established several years earlier (e.g. Schmitz, 1879; de Bary, 1884). Reports of somatic fungal mitosis which appeared about the same time as Wilson’s famous book was published (e.g. Trow, 1895; Léger, 1896) were not intelligible in terms of mitosis as it was then understood and it is likely that Wilson would have chosen to disregard them had he been aware of them in time for inclusion in his work (they are not mentioned in later editions). Unequivocal accounts of mitosis in hyphal nuclei were not published until much later—in some species only within the last twenty years. This reflects the technical difficulties which are associated with observing fungal mitosis with light microscopy.

The small size of somatic fungal nuclei and the unusual staining characteristics of their chromosomes has led to much controversy about fungal mitosis in the literature (see Robinow, 1957; Robinow & Bakerspigel, 1965; Crackower, 1969; Matile, Moor & Robinow, 1969 for discussions of this subject). Some of the controversy arose because the large meiotic nuclei in asci and basidia stain readily with conventional staining techniques and have chromosome configurations typical of dividing plant and animal cells (e.g. Harper, 1905; Colson, 1935) and it was sometimes assumed that the same thing takes place in somatic nuclei but on a much smaller scale which is difficult to demonstrate (e.g. Olive, 1953). It now seems clear however, that in most fungal nuclei at mitosis the chromosome configurations vary considerably from the "classical" pattern. Exceptions to this
generalization are *Basidiobolus ranarum* (Robinow, 1963; Tanaka, 1970) and *Catenaria anguillulae* (Ichida & Fuller, 1968) where the chromosomes are typically arranged at the equator of the spindle prior to separation to opposite poles.

Light microscopy of the 5 fungal species dealt with in this thesis was carried out by Dr. Robinow either prior to or simultaneously with the electron microscope studies. Throughout the thesis, reference is made to published accounts of these light microscopical observations whenever they clarify what has been seen with the electron microscope.

Not only has light microscopy of fungal nuclei involved certain technical problems, but also there have been difficulties associated with the preparation of many fungi, especially yeasts, for electron microscopy. The work presented in this thesis was begun with the idea of overcoming some of these technical problems in order to clarify the process of mitosis at the ultrastructural level. The following paragraphs describe the rationale behind the selection of five particular fungi for study out of the many possible species which could have been chosen. Previous ultrastructural studies of fungal mitosis and meiosis are also briefly reviewed.

Robinow & Marak (1966) had studied mitosis in the ascomycetous yeast *Saccharomyces cerevisiae*. They had confirmed earlier reports
based on the study of permanganate fixed cells that mitosis takes place within an intact nuclear envelope (see review by Hawker, 1965). They had also described for the first time, the presence of an intranuclear mitotic spindle which is associated with bar-shaped electron-dense organelles located in enlarged pores of the nuclear envelope. Their observations raised questions about the relationship of the chromosomes to the spindle since the tetraploid species they worked with has at least 64 chromosomes (by genetical analysis, Mortimer & Hawthorne, 1969) and the intranuclear spindle has only about 15 microtubules.

The ascomycetous yeast *Schizosaccharomyces pombe* was the first species selected for my study because it was hoped that more information could be obtained about the relationship of the chromosomes to the spindle. Unfortunately, in *S. pombe* (as in *S. cerevisiae*) no chromosomes were visible with electron microscopy so any information about the relationship of the chromosomes to the spindle had to be derived indirectly by comparing light micrographs with electron micrographs.

It has been suggested that yeast chromosomes are not visible in thin sections viewed with electron microscopy because they are small and thread-like and do not become condensed during division (Moens & Rapport, 1971b; Ris, 1972 personal communication). With light microscopy of stained preparations, it can be seen that the chromosomes of *S. cerevisiae* are less densely stained at mitosis.
than they are at interphase and that \textit{S. pombe} chromosomes are
stained with approximately the same intensity at all stages in the
cell cycle. Assuming that intensity of staining is a measure of
how condensed the chromosomes are, the yeast \textit{Candida scottii} seemed
a worthwhile second species to study. In this yeast, the chromatin
is much more densely stained with aceto orcein at certain stages in
the division process than it is at interphase.

\textit{Candida scottii} had come to the attention of Dr. Robinow
because of a report by Eckstein (1958) which described individual
chromosomes at mitosis. Dr. Robinow had observed that in \textit{C. scottii}
all the chromatin moves into the bud before division. In the bud,
the chromatin divides and one daughter nucleus moves back to the
mother cell. This sequence of events had been overlooked by
Eckstein. As expected from the increased density of stained
chromatin at mitosis, the chromosomes of \textit{C. scottii} are visible in
the electron microscope at some stages of mitosis. However, several
unique features e.g. the partial breakdown of the nuclear envelope
suggested that mitosis in \textit{C. scottii} was quite different from
mitosis in \textit{S. pombe} and that the relationship of the chromosomes to
the spindle was probably different in the two organisms. These
differences were more readily understood when it was learned that
\textit{C. scottii} was taxonomically very different from \textit{S. pombe}. \textit{C. scottii}
had been recognized as the sporidial stage of a heterobasidiomycete by
Fell, Statzell, Hunter & Phaff (1969) and reclassified in the genus
**Leucosporidiun.** The differences between mitosis in *C. scottii* and
*S. pombe* are consistent with at least two differences that have
previously been observed to exist between dividing ascomycetes and
basidiomycetes as set out in Table I.

Since the type of mitosis found in *C. scottii* had not been
previously observed in a yeast, it seemed logical to investigate
whether any other yeasts might divide in a similar way. Therefore
the third and fourth species selected for study were the yeasts
*Rhodotorula glutinis* and *Sporobolomyces salmonicolor* which have
also recently been reclassified as heterobasidiomycetes and placed
in the genera *Rhodosporidiun* and *Aessosporon* respectively (Fell,
1970; vanderWalt, 1970). It had been previously reported that
mitosis in *R. glutinis* was a process of elongation and constriction
within an intact nuclear envelope (Thyagarajan, Conti & Naylor, 1962).
However, this account seems to have been incorrect and it was found
that mitosis in both *R. glutinis* and *S. salmonicolor* is very similar
to mitosis in *C. scottii* and to the mycelial basidiomycetes that
have previously been studied by other workers.

The fifth species chosen for study was a member of the
phycomycetes—*Mucor hiemalis*. This is a coenocytic mycelial fungus
which was chosen for the particular reason that it produces non-
motile sporangiospores. Most of the phycomycetes in which mitosis
had been previously examined with electron microscopy were those
which form motile zoospores namely: *Catenaria anguillae* (Ichida &
 Fuller, 1968), *Allomyces macrogynus* (Robinow & Bakerspigel, 1965),
<table>
<thead>
<tr>
<th>BASIDIOMYCETES</th>
<th>ASCOMYCETES</th>
<th>Characteristics of the nuclear envelope around dividing nuclei</th>
<th>Shape of Spindle-Associated Organelles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coprinus (meiosis)</td>
<td></td>
<td>partial breakdown</td>
<td>globular</td>
</tr>
<tr>
<td>Lu (1967)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lerbs &amp; Thielke (1969)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lerbs (1971)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Armillaria (mitosis)</td>
<td></td>
<td>partial breakdown</td>
<td>globular</td>
</tr>
<tr>
<td>Boletus (meiosis)</td>
<td></td>
<td>partial breakdown</td>
<td>globular</td>
</tr>
<tr>
<td>McLaughlin (1971)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polystictus (mitosis)</td>
<td></td>
<td>partial breakdown</td>
<td>globular</td>
</tr>
<tr>
<td>Girbardt (1968)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schizophyllum (mitosis)</td>
<td></td>
<td>partial breakdown</td>
<td>globular</td>
</tr>
<tr>
<td>Raudaskaski (1970)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saccharomyces (mitosis)</td>
<td></td>
<td>remains intact</td>
<td>bar-shaped</td>
</tr>
<tr>
<td>Robinow &amp; Marak (1966)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saccharomyces (meiosis)</td>
<td></td>
<td>remains intact</td>
<td>bar-shaped</td>
</tr>
<tr>
<td>Moens &amp; Rapport (1971a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus (mitosis)</td>
<td></td>
<td>remains intact</td>
<td>bar-shaped</td>
</tr>
<tr>
<td>Robinow &amp; Caten (1969)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascobolus (meiosis)</td>
<td></td>
<td>remains intact</td>
<td>bar-shaped</td>
</tr>
<tr>
<td>Wells (1970)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Podospora (meiosis)</td>
<td></td>
<td>remains intact</td>
<td>bar-shaped</td>
</tr>
<tr>
<td>Neotiella (meiosis)</td>
<td></td>
<td>remains intact</td>
<td>bar-shaped</td>
</tr>
<tr>
<td>Westergaard &amp; von Wettstein (1970)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pustularia (meiosis)</td>
<td></td>
<td>remains intact</td>
<td>bar-shaped</td>
</tr>
<tr>
<td>Schrantz (1967)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xylophaera (meiosis)</td>
<td></td>
<td>remains intact</td>
<td>globular at interphase bar-shaped at mitosis</td>
</tr>
<tr>
<td>Beckett &amp; Crawford (1970)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Blastocladiella emersonii (Lessie & Lovett, 1968) and Saprolegnia ferax (Heath & Greenwood, 1968, 1970), all of which form an intra-nuclear spindle and have centrioles located outside the intact nuclear envelope at the poles of the spindle. Centrioles have also been seen in association with the nuclei of two other zoospore-producing phycomycetes namely: Allomyces arbusculus (Renaud & Swift, 1964) and Albigo candida (Berlin & Bowen, 1964), but in these species the mitotic process was not examined.

The only phycomycete not producing motile zoospores that had been carefully examined with electron microscopy at the time when I began my investigations on Mucor was Basidiobolus ranarum (Tanaka, 1970). In this fungus at mitosis, a large barrel-shaped spindle is formed and the chromosomes line up on a metaphase plate. The nuclear envelope breaks down in the equator region of the spindle and the poles of the spindle are characterized by a labyrinth of membranes. There seem to be no associated centrioles.

Basidiobolus, because of its large nuclei which are located singly in walled-off regions of cytoplasm can not be considered typical of the phycomycetes which do not have a motile stage. Mucor hiemalis, with its coenocytic mycelium containing numerous small nuclei, is a much more typical species. Moreover, when examined with light microscopy, division in Mucor hiemalis (Robinow, 1957) seems to be almost identical with division in at least three species of the Saprolegniaceae (Bakerspigel, 1960) including
Saprolegnia ferax, a species known to have centrioles at the poles of the spindle (Heath & Greenwood, 1968, 1970). Study of whether or not there are also centrioles in Mucor would seem to be an interesting test of the hypothesis put forward by Pickett-Heaps (1969). He has suggested that centrioles, even in animal cells, may not be necessary for spindle formation and that they are associated with the spindle simply to ensure their distribution to daughter cells which require them as basal bodies for cilia or flagella.

According to Pickett-Heap's hypothesis, no centrioles should be found in fungi which do not have a motile stage in their life cycle. Enough ascomycetes and basidiomycetes have now been examined to practically rule out the possibility that members of these groups of fungi might have centrioles associated with the mitotic spindle. However, at the time when Mucor was selected for study, not enough phycomycetes that do not have a motile stage had been examined thoroughly enough to rule out this possibility.

Preliminary observations on the ultrastructure of Mucor hiemalis had previously been made by Robinow & Marak (unpublished). Because they found no centrioles but did find some evidence of a spindle-associated organelle analogous to the bar-shaped or globular-shaped structures found at the poles of mitotic and meiotic spindles in ascomycetes and basidiomycetes, it was decided that this organism should be more carefully examined. My work has confirmed these observations and is of particular interest since Girbardt (1971) has
now reported that he examined several non-motile phycomycetes including a species of *Mucor* and found neither centrioles nor any other spindle-associated structure.

The five organisms which have been studied are dealt with in different chapters of this thesis. These are arranged in the order in which they were investigated as described above.
CHAPTER II

STUDIES ON SCHIZOSACCHAROMYCES POMBE

INTRODUCTION

The fission yeast, Schizosaccharomyces pombe a fungal organism now increasingly studied by cell physiologists and geneticists (Mitchison, 1970; Leupold, 1970) is also well suited for observations on mitosis. Large numbers of dividing cells are readily obtained, and the mitosis of the single central nucleus is related in a regular manner to the cell's cycle of growth and division (Mitchison, 1970). Dividing nuclei can thus be expected in cells exceeding a certain length.

Previous studies of mitosis in S. pombe have been carried out exclusively with the light microscope. Early literature on the subject has been reviewed by Schopfer, Wustenfeld & Turian (1963). These authors, on the basis of observations made on cells stained with Giemsa solution after hydrolysis, concluded that "mitosis appears to be accomplished without the help of a spindle apparatus and without the formation of typical metaphase plates". It has since been shown that acid fuchsin in acid solution selectively stains a narrow intranuclear spindle in S. pombe (see Appendix to Chapter 7 by Robinow in Mitchison, 1970), as it does in S. cerevisiae (Robinow & Marak, 1966).
Until now, fixatives used in studies of the fine structure of *S. pombe* (Maclean, 1964; Schmitter & Barker, 1967; Osumi & Sando, 1969; Oulevey, Deshusses & Turian, 1970; Heslot, Goffau & Louis, 1970), have been unsuitable for the preservation of nuclear organization. Observations have now been made with the electron microscope which confirm and add to the previous knowledge of mitosis in this organism based on light microscopy. New observations on the structure and behaviour of the mitochondria have also been made.
MATERIALS AND METHODS

Materials

Observations were made on a diploid strain of *S. pombe* supplied to Dr. Robinow by Dr. H. Gutz of the University of Texas. Because diploids are considerably larger than haploids they are easier to examine and photograph with light microscopy although nuclear structure and behaviour appear to be the same as in normal wild type haploids. To obtain comparable observations with electron microscopy I used the same diploid species that had been studied with light microscopy by Dr. Robinow. The yeast was grown on a medium consisting of Difco yeast extract 0.5g, glucose 2.0g, and agar 1.5g per 100 ml. of water.

Preparation for Electron Microscopy

A modification of the method of Karnovsky (1965) was used. Monolayers of growing cells in Petri dishes were flooded with Karnovsky's formaldehyde-glutaraldehyde mixture. The cells were scraped off the plates, centrifuged, and resuspended in fresh fixative for 14 hours at room temperature. The fixative was removed with 8-10 washes of .1 M cacodylate buffer at pH 7.2. The cells were post fixed for 6 hours at room temperature in 1.33% osmium tetroxide in collidine buffer. They were then washed twice in collidine buffer and twice in distilled water before being stained in 0.5% aqueous uranyl acetate for 2 1/2 hours.
The cells were next suspended in molten 1.5% water agar at 47°C and quickly centrifuged into a pellet before the agar could solidify. After cooling, the pellet of cells thus embedded in solid agar, was cut into \( \frac{1}{2} \) mm cubes. These were dehydrated in a graded alcohol series followed by anhydrous acetone and embedded in Araldite 6005 (Richardson, Jarett & Finke, 1960). Silver or grey sections, cut with a diamond knife, were mounted on ionized carbon films and double stained for 20 minutes with 1% aqueous uranyl acetate followed by 8 minutes with lead citrate (Reynolds, 1963).

Specimens were viewed with a Phillips EM 200 at 60 KV.

OBSERVATIONS

Cytoplasmic Features

The cytoplasm of log phase cells is closely packed with ribosomes and has many vacuoles scattered throughout (Figs. 1, 2, 16). With the electron microscope it can be seen that these vacuoles are partially filled with an extremely electron-dense material and are bounded by a unit membrane (see especially Fig. 15).

After fixation for light microscopy, the vacuole contents stain red with toluidine blue at a low pH; a characteristic which may indicate the presence of polyphosphate (McCully & Robinow, 1971).
The cytoplasm of growing cells also contains a few spherical inclusions about 0.2-0.3 μm in diameter which tend to be in clusters at the ends of the cell. These have a low electron density and are not membrane bound (Figs. 2 & 16). Inclusions of the same size and relative quantity per cell are recognizable in the light microscope. They stain blue with Sudan Black B and are obviously lipid inclusions (McCully & Robinow, 1971).

The mitochondria are short in cells of the early growth phase (Fig. 1) and become longer as the cell grows. In long cells (which are therefore close to nuclear division), the mitochondria may stretch the entire length of the cell (Fig. 2), or they may be highly branched. They remain long during mitosis and appear to divide just before the inward growth of a transverse septum achieves the formation of two daughter cells. Continued fragmentation of the mitochondria after closure of the septum (Fig. 17) seems to produce the short mitochondria that are characteristic of cells in the early growth phase.

The mitochondria in cells from growing cultures have a few long convoluted cristae surrounded by a fine grey matrix and ribosome-like particles (see especially Figs. 6, 7, 8). They also have mesosome-like membrane invaginations (Figs. 3, 4, 5). These membranes often seem to be covered with amorphous electron-dense material and they usually encircle an electron-transparent region. There may be several of these membranous inclusions in one mitochondrion (Fig. 2).
The regular association of a mitochondrion with the nucleus is dealt with below in the section which describes nuclear features seen with electron microscopy.

**Nuclear Features**

The following account describes changes in nuclear morphology which occur during the growth cycle of *S. pombe* as seen with electron microscopy. Two criteria have been used to arrange these observations on fixed cells into a life-like sequence. First; characteristics such as cell length and the presence or absence of a septum which, in *S. pombe* are correlated with the growth cycle (Mitchison, 1970) and second; knowledge of mitosis in living cells derived from phase contrast microscopy (McCully & Robinow, 1971).

The early interphase nucleus is spherical, with an excentrically placed nucleolar region containing ribosome-like particles dispersed among amorphous electron-dense material, and a chromatin-containing region which is of uniformly low electron density showing nothing recognizable as chromosomes (Fig. 1). Located on the outside of the nuclear envelope, adjacent to the chromatin-containing region and roughly opposite the position of the nucleolus is a structure which, for several reasons to be outlined in the discussion, has been called the KCE (kinetochore equivalent). The position of this organelle at interphase is always closer to one of the side walls of the cell than it is to the cell's longitudinal axis, and it is
always lying in a narrow ribosome-free zone which is bounded by
the outer nuclear membrane on one side and a mitochondrion on
the other. Serial sections of the early interphase KCE show
that it is a curved disc about 220 nm in diameter with a bulge
in the centre of its concave side which is in contact with the
nuclear envelope (Figs. 6, 7). This curved disc has two regions:
the one is an electron-dense portion consisting of at least three
layers plus the bulge, and the other is a fuzzy grey portion
covering the convex surface of the electron-dense region. There
is also some amorphous electron-dense material inside the
nuclear envelope beneath the site where the KCE appears to be
attached, which has the effect of making this region stand out
from the rest of the envelope.

At late interphase, the nucleus is enlarged and usually
ovoid (Fig. 2). The KCE at this stage is in the same position
but has a different morphology than the previously described
interphase KCE. It has become longer and more bar-shaped and it
lacks the fuzzy grey surface portion (Fig. 8). Like the early
interphase KCE, this type of KCE is accompanied by amorphous
electron-dense material underlining the inner aspect of the nuclear
envelope.

On rare occasions, I have observed what appears to be a
divided KCE located beside an otherwise normal-looking late inter-
phase nucleus. This structure can best be described as two short
osmophilic bars which lie very close and almost parallel to each other in a ribosome-free indentation of the nuclear envelope with their long axes at right angles to the membrane surface (Fig. 9).

Nuclei in early stages of mitosis look similar to interphase nuclei but are characterized by the presence of a short peripheral spindle which runs through the chromatin region and joins two KCE's located a short distance apart on the outside of the envelope. The short segment of envelope between the two KCE's always appears to be convoluted or bulged (Figs. 10, 11, 12). However, over the greater part of their surface, these nuclei still retain the rounded or oval contours that are seen in interphase nuclei. The peripheral spindle seems to be a narrow bundle of parallel microtubules which is slightly curved when the KCE's are a short distance apart (Fig. 10), and straight when the KCE's are more widely separated (Figs. 11, 12). At this stage of the peripheral spindle and at all subsequent stages of spindle formation, the KCE's have a non-layered disc-like appearance and seem to be closely pressed to the nuclear envelope. As in interphase, KCE's at the ends of intranuclear spindles are located in ribosome-free zones and each one is closely associated with a mitochondrion. These KCE's have approximately the same diameter as the curved disc-shaped KCE's of early interphase but they are considerably longer than the two component bars of the proposed divided KCE which is described above. The fact that none of the intermediate steps which must occur between the divided KCE stage (Fig 9) and the short, curved, peripheral spindle
stage have been observed, (Fig. 10), may indicate that this change occurs very rapidly.

A later stage in mitosis is characterized by a nucleus which has a narrow, elongated, almost rectangular shape (Fig. 13). The nucleolus persists and is stretched out in the interior of the nucleus. The spindle microtubules occupy the longitudinal axis and seem to run parallel to each other, without interruption, between two KCE's located at opposite poles of the nucleus.

Subsequently the more or less rectangular nucleus becomes further elongated into a dumbbell shape with two rounded ends joined by a long narrow channel containing the spindle microtubules. (Figs. 14, 15, 16). These microtubules are stretched in a long, straight, parallel bundle between the widely separated KCE's located at opposite dumbbell ends. Before the long dumbbell stage (Fig. 16) is reached, the nucleolus apparently becomes divided between the rounded daughter ends so that no nucleolar material is found in the narrow connecting channel. The whole dumbbell shaped unit is surrounded by an intact nuclear envelope. This membrane is typically double in most places, but along the envelope of the spindle channel, there are several minute regions of high electron density which can be resolved into fold-like arrangements with four unit membranes instead of two. (Arrows Fig. 14 and inset). As reported by Oulevey et al. (1970), and shown in Fig. 16, the cell septum is sometimes beginning to form at this stage before nuclear separation is complete.
Two of the final steps in the division process are the breaking down of the spindle channel, and the return of each KCE from its central position at a pole of the division axis, to its interphase position on the surface of the nucleus facing a side wall of the cell (as in Figs. 1, 2). The observations seem to indicate that both these steps are achieved at the same time by a pivoting movement of each daughter nucleus. This movement may be inferred from the shape and position of daughter nuclei in cells with an almost completed or a fully completed septum. Whereas the daughter ends of the dumbbell shaped unit are elongated in the pulling-out direction with each portion of the divided nucleolus located nearly opposite its respective polarly-positioned KCE, a newly separated daughter nucleus is usually seen to be elongated almost perpendicularly to the division axis with the nucleolus now opposite the KCE in its new interphase position at the side of the nucleus (compare shape of nuclei and the positions of the nucleoli and KCE's in Fig. 16 and Fig. 17). The two KCE's (and nucleoli) of recently separated nuclei are always seen to lie facing opposite side walls of the cell, suggesting that final separation of daughter nuclei may involve pivoting movements in opposite directions by end portions of the dumbbell shaped nucleus.

After separation has occurred, mitochondria which accompany the polar KCE's in the dumbbell stage are now consistently located in close apposition to the KCE's in their new interphase positions (compare again Fig. 16 & Fig. 17). At this stage the morphology
of the KCE resumes that of a curved, clearly layered disc which
has been described as a characteristic of early interphase cells.

DISCUSSION

Why "Kinetochore Equivalent"?

Since the osmophilic structures that have now been seen
several times at the poles of mitotic and meiotic intranuclear
spindles in ascomycetes and basidiomycetes have been given
different names by different authors, as set out in Table II
the use of Girbardt's term "kinetochore equivalent" (KCE) for
the spindle-associated organelle in _S. pombe_ must be justified.

All these names, except KCE and archontosome, imply that
the spindle-associated structures at the poles of dividing fungal
nuclei are analogous to centrioles since even the term "centrosome"
as defined by Boveri, 1901, refers to the hyaline regions at the
poles of mitotic spindles which contain smaller, deeply staining
centrioles (Boveri's definition is quoted and discussed by Cleveland,
1963). Since the advent of electron microscopy, the term "centriole"
has acquired a purely descriptive meaning devoid of functional or
topographical connotations. The typical centriole is a short hollow
cylinder which, in cross section, has nine triplet sets of tubules
arranged around its circumference. A more detailed description of
centriole structure can be found in Stubblefield & Brinkley (1967) and
<table>
<thead>
<tr>
<th>Names Given</th>
<th>Studies of Somatic Nuclear Division</th>
<th>Studies of Meiosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;modified or poorly preserved centrioles&quot;</td>
<td>Motta (1967) Armillaria</td>
<td></td>
</tr>
<tr>
<td>&quot;Centriole ähnliche Körper&quot;</td>
<td>Lerbs &amp; Thielke (1969) Coprinus</td>
<td></td>
</tr>
<tr>
<td>&quot;undifferenzierten Centriolen&quot;</td>
<td>Lerbs (1971) Coprinus</td>
<td></td>
</tr>
<tr>
<td>&quot;Centrosomal plaques&quot;</td>
<td></td>
<td>Zickler (1970) Ascobolus, Podospora</td>
</tr>
<tr>
<td></td>
<td></td>
<td>McLaughlin (1971) Boletus</td>
</tr>
<tr>
<td>&quot;Disque central d'un aster&quot;</td>
<td></td>
<td>Schrantz (1967) Pustularia</td>
</tr>
<tr>
<td>&quot;KCE&quot;</td>
<td>Girbardt (1968) Polystictus</td>
<td></td>
</tr>
<tr>
<td>&quot;Archontosome&quot;</td>
<td></td>
<td>Beckett &amp; Crawford (1970) Xylospahaera</td>
</tr>
</tbody>
</table>
de Harven (1968). Centrioles associated with mitotic spindles are identical with those that form the bases of cilia and flagella. None of the spindle-associated organelles in ascomycetes and basidiomycetes have a structure which resembles that of a centriole. If they have often been given names which suggest an analogy with centrioles, it is because they are assumed to have the same function as centrioles. It is further assumed that this common function is the formation of the mitotic spindle.

Although it is commonly thought that centrioles are responsible for the formation and orientation of mitotic spindles, this view has recently been disputed (Dietz, 1966; Pickett-Heaps, 1969, 1971; Friedländer & Wahrman, 1970; Bajer & Molè-Bajer, 1971). Numerous observations strongly indicate that centrioles are not necessary for the formation of spindle microtubules even though their close association with mitotic spindles in animal cells might suggest that they are.

Plant cells, for example, are able to form spindles without the participation of centrioles. Early light microscopists explained this inconsistency by the idea that plant cells probably did have centrioles which were too small to resolve with their microscopes. Electron microscopy has now established that centrioles are definitely not present in plant cells (e.g. Pickett-Heaps & Northcote, 1966) except in a few primitive plants such as ferns and liverworts which form motile gametes (Mizukami & Gall, 1966; Moser & Kreitner, 1970).
It could be argued that plant cells have lost the ability to form centrioles and have evolved some other method of organizing microtubules. However, in many cells which have centrioles functioning as basal bodies of cilia or flagella, there are no centrioles functioning as division centres. This is the case in the alga *Chlamydomonas reinhardi* in which the centrioles are located near the cleavage furrow rather than at the poles of the spindle during division. (Johnson & Porter, 1968). Then too, in ciliated protozoa which have thousands of basal bodies available to them for use as division centres, centrioles are never associated with the dividing nuclei (e.g. Tucker, 1967; Jenkins, 1967).

On the basis of his study of meiosis in crane flies, Dietz (1966) has argued persuasively that even in cells which do have spindle-associated centrioles, these centrioles may be dispensible. By flattening the cells and thus preventing the movement of the asters (and their contained centrioles) to opposite poles of the future division axis, Dietz observed that a normal spindle could be formed and normal chromosome separation could occur without the participation of centrioles. Moreover, many ultrastructural studies of cells with spindle-associated centrioles have shown that the microtubules are very rarely in direct contact with the centrioles but appear to arise either from small bar shaped "centriolar satellites", strikingly illustrated by Szollosi (1964) and de Harven (1968), or from the amorphous osmophilic material which
has been frequently noted in the vicinity of centrioles (e.g. by Buck, 1967).

The evidence quoted above seems to indicate that the function of the centriole is not directly related to spindle formation. It would therefore be inappropriate to use terms relating to centrioles to designate the organelles in fungi which are more consistently and more intimately associated with microtubules than are centrioles and do therefore seem to play a more obvious role in spindle formation. By a similar reasoning, Beckett & Crawford (1970) choose to call the spindle associated organelle in Xylosphaera polymorpha an "archontosome" (Greek, meaning organizing or controlling body). This would seem to be an acceptable term but it would be better to have some other term whose meaning is more readily apparent.

Pickett-Heaps (1969) proposes that the spindle-associated organelles in fungi should be called "microtubule organizing centres", a general term which he also uses for similar osmophilic microtubule-associated regions in plant and animal cells including, among many examples, the kinetochore regions of chromosomes and the small electron dense "nucleating centres" in ectodermal cells of sea urchin blastulae (Tilney, 1968). Experimental proof that the "nucleating centres" are indeed sites of microtubule assembly has since been provided by Tilney and Goddard (1970).
Although "microtubule organizing centre" and "archontosome" appear to be acceptable general terms for spindle forming organelles in fungi, it is proposed that use of the specific term "kinetochore equivalent" is justifiable in *S. pombe* for the following three reasons:

1. The organelle in *S. pombe* appears to organize microtubules.

Many observations have been recorded which, viewed together, support the contention that the kinetochore region of chromosomes is also capable of organizing microtubules.

Single chromosomes that are separated from the spindle by spontaneous or induced division abnormality are able to form small independant "chromosomal" spindles and sister chromatids can separate to opposite poles of these spindles (Dietz, 1969).

Studies of mitotic nuclei recovering from the variously induced breakdown of the spindle apparatus have shown that new spindle fibres begin to elongate from the kinetochore region as soon as the inhibitory agent is removed. This has been observed by Pease (1946) in *Tradescantia paludosa* microsporocytes recovering from pressure treatment, by Roth (1967) in the giant amoeba *Chaos carolinensis* after cold treatment, and by Molié-Bajer (1969) in *Haemanthus katherinae* endosperm following chloral hydrate treatment.

The conclusion that kinetochore spindle fibres are assembled at the kinetochore also finds support in studies on living cells irradiated with a UV microbeam and observed with polarizing microscopy. If a small
region between the chromosomes and the polar region of crane fly spermatocyte nuclei at metaphase I is briefly irradiated, the birefringence in that region disappears. The birefringence then begins to move from the region of the chromosomes into the irradiated area while the part of the spindle fibre with reduced birefringence shifts poleward (Forer, 1965). This observation is in accord with the hypothesis that spindle fibre material is continuously being assembled at the kinetochore and disassembled at the pole (Inoué, 1964).

#II The structure of the spindle-associated organelle in *S. pombe* resembles that of a kinetochore. The layered appearance of the organelle in *S. pombe* and even more so of the comparable "centriolar plaque" in *S. cerevisiae* (see Fig. 28 in Matile et al. 1969) is reminiscent of the distinctly layered kinetochores of certain animal cells (e.g. Jokelainen, 1967; Brinkley & Stubblefield, 1966), as well as of the chromosomes in an unidentified species of the alga *Oedogonium* (Pickett-Heaps & Fowke, 1969) and of the chromosomes in the hypermastigid flagellate *Barbulanympha ufulula* (Hollande & Valentin, 1968). The latter are even more similar to the organelle in question in *S. pombe* because they are also attached to the nuclear envelope. However, their attachment is to the inside of the nuclear envelope while the organelle in *S. pombe* appears to be attached to the outside of the nuclear envelope.
III The organelle in S. pombe is attached to the chromosomes.
The evidence for this statement is, admittedly, not conclusive since
the chromosomes of S. pombe cannot be seen in the electron microscope.
However, comparison of the chromosome configurations in stained
preparations with electron micrographs of dividing nuclei (e.g.
compare Fig. 11 in McCully & Robinow, 1971 with Fig. 13), gives the
impression that the chromosomes move apart in two clusters because
they are attached to two opposing points on the nuclear envelope and
that these points are the spindle-associated organelles. Therefore
it is suggested that chromosomes are attached to the organelle just
as chromosome material is attached to a true kinetochore.

Support for this hypothesis is provided by the observations
that the spindle in the light microscope appears to have the shape of
a thin wire instead of a double cone (see Fig. 36 in McCully & Robinow,
1971), and that in the electron microscope it seems to consist of
microtubules which run parallel to each other without interruption
between the two organelles. Apparently, no discontinuous microtubules
are present like the ones shown by Heath & Greenwood (1970) in
Saproleagia ferax (in which, as in S. pombe, chromosomes are not visible
with electron microscopy). One would expect to see similar discontinuous
microtubules at anaphase in S. pombe if the chromosomes were not, as I
have suggested, directly attached to the terminals of the microtubule bundle.

Thus, in the light of these arguments, Girbardt's term
"kinetochore equivalent" has been used for the electron-dense organelles
found at the ends of the intranuclear spindle in S. pombe.
This term is definitely not applicable to the spindle-associated organelles of all fungi. There is no doubt from the observations of Lu (1967) in *Coprinus lagopus* and of Wells (1970) and Zickler (1970) in *Ascobolus stercorarius* and *Ascobolus immersus* that during meiosis in these fungi, separate chromosomes and chromosomal fibres exist. Their so-called "centrosomes" and "centriolar plaques" may be best termed "microtubule organizing centres" and may be analogous to centriolar "satellites" instead of to kinetochores. For reasons which will be made clear later, "microtubule organizing centre" is the term that has been used to refer to spindle-associated structures which have been seen in the other four fungi to be described in subsequent chapters of this thesis.

**Significance of Membrane Growth, Microtubule Assembly and "Cytoplasmic Forces" in the Process of Nuclear Elongation and Division**

To clarify the discussion of these matters, the stages of mitosis in *S. pombe* are summarized in Table III.

It is proposed that the two KCE's which are seen at opposite poles of short peripheral spindles in Stage II nuclei arise from the division (via the parallel bar stage) of the single KCE associated with the interphase nucleus and that they have been moved apart to their Stage II positions by a differential membrane growth of a region of nuclear envelope between them. This conclusion is based on the convoluted or bulging appearance of the short stretch of
<table>
<thead>
<tr>
<th>Stage of Nuclear Cycle</th>
<th>Shape of Nucleus</th>
<th>Position of Nucleolus</th>
<th>Position of Spindle and of the KCE</th>
<th>Illustration Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTERPHASE</td>
<td>spherical</td>
<td>excentric, nearly opposite position of the KCE.</td>
<td>No spindle: KCE located on the surface of the nucleus facing a side wall of the cell.</td>
<td>1, 2</td>
</tr>
<tr>
<td>MITOSIS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage I (duplication of the KCE)</td>
<td>spherical or oval</td>
<td>excentric</td>
<td>No spindle: Duplicated KCE's in interphase position as above. They are parallel bars which lie perpendicular to the nuclear envelope.</td>
<td>9</td>
</tr>
<tr>
<td>Stage II</td>
<td>spherical or oval with a bulging segment.</td>
<td>excentric</td>
<td>KCE's are at the poles of a peripheral intra-nuclear spindle.</td>
<td>10, 11, 12</td>
</tr>
<tr>
<td>Stage III</td>
<td>elongated (roughly rectangular)</td>
<td>central</td>
<td>Spindle now occupies the long axis of the nucleus. KCE's are at the spindle poles. i.e. the ends of the nucleus.</td>
<td>13</td>
</tr>
<tr>
<td>Stage IV</td>
<td>elongated (dumbbell shaped)</td>
<td>distributed to daughter ends of the dumbbell.</td>
<td>Spindle occupies the long axis of the nucleus. KCE's are at the spindle poles. i.e. the ends of the nucleus.</td>
<td>14, 15, 16</td>
</tr>
<tr>
<td>Stage V (after separation of daughter nuclei before cell separation)</td>
<td>two spherical</td>
<td>both excentric each one in a position nearly opposite their respective KCE.</td>
<td>No spindle: KCE's in interphase position facing opposite sides of the cell.</td>
<td>17</td>
</tr>
</tbody>
</table>
nuclear circumference between the KCE's at Stage II which seems to indicate that this region has expanded while the rest of the nuclear envelope has not expanded and has therefore retained its round or oval interphase contours.

Further, it is proposed that this differential membrane growth could also achieve the change from Stage II to Stage III since membrane growth exclusively in the region bounded off by the peripheral spindle would ultimately move the KCE attachment points into polar positions and shift the excentrically placed nucleolus into a central position without any actual nucleolar movement.

After Stage III has been achieved by differential membrane growth, Stage IV may be the expression of a general expansion of the nuclear envelope. This could cause a further separation of the KCE's since pressures in the cytoplasm resulting from the close proximity of the cell's side walls would ensure that the growing nucleus expanded towards the ends of the cell. If it is assumed that the chromosomes and perhaps the nucleoli, too, via nucleolar chromosomes, are in some way all linked to the two KCE's then, as the KCE's are moved farther and farther apart, there is eventually not enough nuclear material left in the central region of the nucleus to prevent the collapse of the nuclear envelope into a tight-fitting sleeve around the central part of the spindle. (Nucleolar chromosomes have been shown to occur in *S. pombe*. See Figs. 16 - 19 in McCully & Robinow, 1971).
The fold-like regions along the nuclear envelope of the spindle channel at Stage IV can be interpreted in two ways. They could be localized regions of membrane growth which, had the cell not been fixed, would soon have slid apart into an elongated stretch of normal double membrane. However, there is no published description of membrane growth in this way, and these fold-like areas are not seen at any other stage in the elongation process. Therefore it seems more likely that increased resistance to movement encountered by the daughter ends of the nucleus at Stage IV as they approach the ends of the cell may cause the membrane, which is continuing to expand in the spindle channel region, to buckle back on itself.

It does not seem likely that the spindle is generating the force to cause the change from Stage I to Stage IV. The observations show that the KCE especially during spindle formation, is closely associated with the nuclear envelope and appears to be attached. In Saccharomyces, attachment of the comparable "centriolar plaques" has been even more clearly established by Robinow & Marak (1966) who showed that they were "set into enlarged pores of the nuclear envelope". If the growing spindle were generating the force to separate the membrane-attached KCE's, the shape of the nucleus at each stage would be different from what has been observed. At Stage II the segment of envelope bounded by the peripheral spindle would be flatter, not more bulging than the rest of the nuclear envelope. At Stage III and IV the growing spindle would push out the poles of the nucleus into sharp points. On the contrary, it was observed that the
ends of nuclei at Stage III and IV are rounded with the KCE's sitting in slight membrane depressions.

It is proposed therefore, that spindle growth is keeping in step with rather than causing KCE separation, and that the spindle microtubules hold the separated KCE's rigidly apart. Microtubules are known to provide rigidity in many types of cells and indeed, during mitosis in the dinoflagellate *Gyrodinium cohni* and in several hypermastigid flagellates including *Spirotrichonympha hispiga* and *Barbulanympha uvalula*, there is good evidence amenable to the interpretation that this is the chief function of the microtubules while the chromosomes are moved apart by growth of the nuclear envelope to which they are attached (Kubai & Ris, 1969; Cleveland, 1938, see especially his Plate IV; Hollande & Valentin, 1968). Since the chromosomes seem to be attached to the KCE in *S. pombe*, the proposed membrane growth which moves KCE's apart may be causing genome separation just as it seems to do in dinoflagellates and hypermastigid flagellates. Chromosomes are also attached to the nuclear envelope during mitosis in the sleeping sickness trypanosome, *Trypanosoma rhodesiense* (Vickerman & Preston, 1970). However, in this organism it was proposed that an intranuclear bundle of microtubules plays an active role in chromosome separation by pushing the two halves of the nucleus apart. My view of the spindle in *S. pombe* as a relatively passive participant in chromosome separation is strengthened by the results of Crackower (1972). He observed that after disruption of the mitotic spindle in *Aspergillus nidulans* by the drug griseofulvin, anaphase chromosomes continue to move apart although they do so in a disorganized fashion.
The final separation of daughter nuclei occurs between Stage IV and Stage V. It seems very likely that the microtubule precursors may have become depleted by the time the long spindle of Stage IV has become assembled and that the spindle may therefore start to break down shortly after this stage has been reached. However, the consistent pivoting of daughter dumbbell ends in opposite directions as evidenced by the shape of daughter nuclei and the positions of their KCE's and nucleoli in electron micrographs of Stage V, together with phase contrast observations at this stage which show the nucleoli at opposite sides of the daughter nuclei, (see Fig. 6 in McCully & Robinow, 1971), suggests that other forces besides spindle breakdown must be at work between Stage IV and V. Girbardt (1968), has been led by detailed cinematographic evidence to postulate the existence of "cytoplasmic forces" which act on the KCE's of dividing hyphal nuclei of *Polystictus versicolor*. Perhaps such forces also act on the KCE's of *S. pombe* after spindle breakdown. It needs only the further assumption of an intrinsic polarity of the organization of the KCE's to account for their being moved in opposite directions by the putative cytoplasmic forces.

*Unanswered Questions About Mitochondria*

Mitochondria have not been seen in close association with the spindle forming organelles of other fungi. In contrast, every KCE that has been seen in *S. pombe* both in interphase and at mitosis has
been located in a narrow ribosome-free zone which is separated from the rest of the cytoplasm by a mitochondrion. Since there is no reason to suspect that the mitotic process in *S. pombe* requires more energy than it does in other fungi, the KCE-associated mitochondria may be playing another role in addition to energy production. It is impossible to suggest with any certainty what this role might be.

It is difficult to know whether the unique mesosome-like membranes inside the mitochondria of log phase *S. pombe* cells are real structures or artifacts of fixation. It is almost certain however, that they correspond to the electron transparent "nucleoid" regions described by Osumi & Sando (1969) in *S. pombe* mitochondria after permanganate fixation, because the "nucleoids" in their material are similar in size and position to the "mesosomes" in my material. Moreover these workers did not clearly demonstrate that DNA was present in their proposed "nucleoids". It is interesting to note that these structures are not present in the mitochondria of normal stationary phase cells (my own observation; and Heslot et al. (1970)). This fact seems to indicate that these mitochondrial regions have some functional significance.
Fig. 1  *S. pombe* - Early interphase (as determined by the length of the cell). The kinetochore equivalent (KCE), which has two regions of different electron density, is located on the outside of the nuclear envelope adjacent to the electron-transparent chromatin region (Chr) on the side opposite to the nucleolus (N). A few short mitochondrial profiles can be seen (M). One of these lies next to a ribosome-free zone around the KCE. The cytoplasm also contains vacuoles (V), partially filled with electron-dense material.  X 17,500.

Fig. 2  *S. pombe* - Late interphase. At this stage the nucleus is larger and the kinetochore equivalent (KCE) has a more uniform electron density than in the cell shown by Fig. 1. The position of the KCE relative to the chromatin region (Chr), the nucleolus (N), and the cell's side wall is similar to that seen in Fig. 1. A long mitochondrion (M) runs almost the complete length of the cell and seems to separate the ribosome-free zone around the KCE from the rest of the cytoplasm. Inside this mitochondrion, several inclusions are present which at this low magnification appear to consist of a central transparent region and an electron-dense periphery. Prominent vacuoles (V) and clusters of lipid droplets (L) are present in the cytoplasm.  X 17,500.

Figs. 3, 4 & 5  *S. pombe* - These micrographs show the mesosome-like appearance of the mitochondrial inclusions when they are seen at high magnification. Fig. 3 shows one of the inclusions in the long mitochondrion seen in Fig. 2 (the one indicated by the arrow). The magnification in each micrograph is  X 88,300.
Figs. 6 to 10. *S. pombe* - Sequence showing the change in kinetochore equivalent morphology between early interphase and the initial stages of spindle formation at mitosis. A portion of the nucleus can be seen towards the bottom of each micrograph. The osmophilic KCE's at each stage are located on the outside of the nuclear envelope in a ribosome-free zone and are accompanied by a mitochondrion (M) on their cytoplasmic side. All Figs. 6 to 10 X 80,70°.

Figs. 6 & 7. Adjacent sections of an early interphase KCE showing that it is a curved layered disc with a bulge in the centre of its convex surface which touches the nuclear envelope. Amorphous electron-dense material underlines the inner surface of the nuclear envelope in the region where the KCE is in contact with the membrane.

Fig. 8. KCE in late interphase. In contrast to the KCE in Figs. 6 & 7, this type of KCE is longer and flatter and does not have a pronounced layered appearance. Electron-dense material on the inside of the nuclear envelope also accompanies the KCE at this stage.

Fig. 9. This micrograph shows what is interpreted as a recently divided KCE. Note the two short nearly parallel bars which lie in an indentation of the nuclear envelope. They are flanked by accumulations of osmophilic material which may be spindle precursors.

Fig. 10. Shows two KCE's joined by a slightly curved bundle of microtubules (Sp), and separated by a convoluted stretch of nuclear envelope. At this stage the KCE's appear to be closely pressed to the nuclear envelope and they do not have a pronounced layered appearance. The length of the spindle shown in this micrograph is 1.15 μm.
Fig. 11  *S. pombe* - Slightly later stage of spindle formation than in Fig. 10. In this nucleus the nucleolus (N) and the chromatin (Chr) have the same relative positions to each other as they do in interphase. A straight bundle of parallel microtubules (Sp) crosses the chromatin region. Because it is sectioned slightly obliquely, only the kinetochore equivalent (KCE) at the spindle end near the top of the micrograph is visible but the ribosome free zone around the other KCE (lower arrow) can be seen at the spindle end near the bottom of the micrograph. The close association between mitochondria and the ribosome free zones around the KCE's can be clearly seen in this micrograph. The short segment of nuclear envelope between the KCE's and bounded by the peripheral spindle has a markedly convoluted appearance in contrast to the smooth contours of the rest of the nucleus. The length of the spindle is 1.65 µm. X 54,000.
Fig. 12  *S. pombe* - The nucleus in this micrograph is at a slightly later stage of mitosis than the one in Fig. 11, but the nucleolus (N) and the chromatin (Chr) still have the same relative positions to each other as they do in interphase. The length of the peripheral spindle is 1.75 μm. Note how the spindle seems to be a parallel bundle of microtubules running through the chromatin region between two terminal kinetochore equivalents (KCE's), and how the short segment of nuclear envelope that is bounded by this spindle bulges out from the more or less oval contours of the rest of the nucleus.

A mitochondrion (M) is located close to the spindle pole near the top of the micrograph. Although the KCE at the lower spindle pole appears to not have an associated mitochondrion, further sections of this nucleus showed that one was present. X 67, 250.
Fig. 13  *S. pombe* - A dividing nucleus which is elongated into a more or less rectangular shape. Note how the chromatin (Chr) is located at the ends of the nucleus while the nucleolus (N) is stretched out in the central region. The spindle which is 3.85 μm in length, occupies the longitudinal axis of the nucleus. Note how the kinetochore equivalents (KCE's) at the ends of the spindle are each associated with a mitochondrion (M). X 44,200.
Figs. 14 & 15  *S. pombe* - Two portions of a dumbbell shaped nucleus shown whole in Fig. 16.

Fig. 14 shows part of the spindle channel (Sp). Note that the microtubule bundle is bounded by an intact nuclear envelope composed of the usual two membranes, except for several small regions of high electron density (arrows). If these regions are sectioned at a favourable angle, it can be seen that they are fold-like arrangements of four unit membranes continuous with the two membranes on each side of them. (Inset and interpretive drawing). Fig. 14  X 107,600; inset Fig. 14 X 228,900.

Fig. 15 shows a section of one of the daughter dumbbell ends. The arrows near the bottom of the micrograph indicate where the envelope narrows down into the spindle channel. Note how the microtubules run from the mouth of the spindle channel, through the nucleolus (N) and the chromatin region (Chr) towards that region of the nuclear envelope where the kinetochore equivalent (KCE) appears to be externally attached. A mitochondrion (M) is associated with the ribosome-free zone around the KCE.

The ribosome-like particles in the nucleolus are clearly visible at this magnification. Note also the unit membrane (VM) around the vacuole (V) at the top right of the micrograph. Fig. 15  X 80,900.
Fig. 16  *S. pombe* - A late stage of division showing a nucleus which is elongated into a dumbbell shape. The nucleolar material (N) and the chromatin (Chr) has been distributed to the daughter dumbbell ends which remain joined by a long channel containing the spindle. The small arrows in the central portion of the cell indicate the narrow spindle channel. Note how the daughter dumbbell ends are slightly elongated in the pulling-out direction and how the spindle-associated kinetochore equivalents (KCE's) are at the opposite poles of the nucleus (i.e. near the ends of the cell). Each of the KCE's is associated with a mitochondrion (M). The length of the spindle (from KCE to KCE) in this nucleus is 9.25 μm.

Several vacuoles (V) and a few lipid droplets (L) can be seen in the cytoplasm of the cell. Asterisks indicate where the cell septum is starting to grow inwards from the side walls of the cell.  X 13,800.

Fig. 17  *S. pombe* - One daughter end of a cell with a completely formed septum (S). In contrast to the daughter dumbbell ends in Fig. 16, the newly separated daughter nucleus seen in this micrograph is elongated almost at right angles to the former pulling-out direction. The nucleolus (N) now faces the cell wall on the right side of the micrograph, while the kinetochore equivalent (KCE) is located beside the nucleus near the left cell wall. At this stage the KCE has two regions of different electron density as it does in early interphase cells.

Note the mitochondrion which appears to have recently pinched off into two portions. One of these is associated with the KCE at the side of the nucleus. The prominent electron-transparent region inside the mitochondrion close to the position of the KCE is one of the mesosome-like mitochondrial inclusions described in the text. X 31,300.
CHAPTER III

STUDIES ON LEUCOSPORIDIIUM SCOTTII (CANDIDA SCOTTII)

INTRODUCTION

As outlined in Chapter I, the budding yeast *Candida scottii* came to Dr. Robinow's attention because of a report by Eckstein (1958) which described densely staining, undivided chromosomes at mitosis. The work with this yeast has not fulfilled a hope of being able to study the behaviour of chromosomes in a *Saccharomyces*-like intranuclear division process, but it has, nevertheless, yielded results of considerable interest. It has been found that mitosis in *C. scottii* involves breakdown of the nuclear envelope and differs in several additional ways from mitosis in ascosporogenous yeasts such as *Saccharomyces cerevisiae*, *Wickerhamia fluorescens*, and *Schizosaccharomyces pombe*, which is achieved by elongation, constriction and equipartition of nuclear components between daughter nuclei without breakdown of the nuclear envelope (Robinow & Marak, 1966; Matile, Moor & Robinow, 1969; Chapter II, this thesis). By contrast, the behaviour of the dividing nucleus in *C. scottii*, is similar to mitotic events in mycelial basidiomycetes. The events described here are the first reports of such behaviour in a yeast.
Until recently, *Candida scottii* was considered to be asporogenous but the finding of a perfect stage (Fell, Statzell, Hunter & Phaff, 1969) has resulted in its reclassification as a heterobasidiomycete in the genus *Leucosporidium*. The observations on mitosis described here are in accord with this new taxonomic assignment. In conformity with accepted usage, *Candida scottii* shall henceforth be referred to by its new name of *Leucosporidium scottii*. 
MATERIALS AND METHODS

Material

A smooth strain of Candida scottii Didens et Lodder No. 4025 in the yeast collection of the yeast collection of the Centraalbureau voor Schimmelcultures at Delft, Netherlands was studied. This strain is a subculture of the strain which the collection received from B. Eckstein. Cultures were maintained and propagated for all purposes on a single medium consisting of "Difco" yeast extract 0.5 g, glucose 2.0 g, and agar 1.5 g per 100 ml of water.

Preparation for Electron Microscopy

Monolayers of cells on agar plates were flooded with cold (4°C) 3% glutaraldehyde in 0.1 M cacodylate buffer pH 7.2. The cells were scraped off the agar, centrifuged into a pellet and resuspended in fresh cold fixative. Fixation was carried out on ice for 3 hours. The glutaraldehyde was washed away with 10 - 15 changes of cacodylate buffer. Post fixation was in 1% osmium tetroxide dissolved in cacodylate buffer. This fixation was done either at 4°C (Figs. 19, 20, 25, 26, 40) or at room temperature (all the other electron micrographs of L. scottii) for 4 - 6 hours. Then the cells were washed 2 times in cacodylate buffer and 2 times in distilled water before being stained in a 0.5% aqueous solution of uranyl acetate for 2.5 hours. Details
of incorporation into agar blocks, dehydration, embedding and
staining of thin sections are the same as has been previously described
for *S. pombe* in Chapter II.

OBSERVATIONS

The cytoplasm contains a high concentration of ribosomes,
several mitochondria, 1-3 prominent vacuoles partially filled with
electron-dense material, and electron-transparent regions located
just underneath the cytoplasmic membrane (Fig. 19). The latter
regions seem to be the sites of some storage material since they are
much larger in stationary phase cells than they are in budding cells.

Near the tips of buds, there are prominent regions of vesicles,
(Figs. 19, 20, 23, 28). These serve as useful bud markers since
oblique sections of budding cells often do not reveal the distinctive
shape of buds which can be seen with light microscopy.

Buds and mother cells are also recognizable by their respective
cell wall characteristics. The outer layers of the mother cell wall
seem to rupture at the site of bud initiation and the bud wall is a
continuation of only the inner layers of mother cell wall. Consequently,
the wall of the bud usually appears to be slightly thinner than the
wall of the mother cell. A collar composed of the ruptured mother cell
wall layers can often be detected at the base of the bud (Figs. 27, 29,
36).
The nucleus in interphase cells is spherical or ovoid in shape and it consists of two regions (Fig. 19). The one is a region of low electron density which can be identified as the site of chromatin by comparison with aceto orcein stained preparations (see Fig. 4 in McCully & Robinow, 1972a). The other is a nucleolus composed of ribosome-like particles dispersed among amorphous electron-dense material. The nucleolus is spherical and seems to occupy 1/4 - 1/3 of the total nuclear volume. When buds are present, the nucleolus is regularly located at the side of the nucleus that is most distant from the bud.

Located in a ribosome-free zone near the outside of the nuclear envelope and sometimes associated with cytoplasmic microtubules, is a structure which, in view of its later activity during mitosis, has been called a "microtubule organizing centre" (MTOC) following Pickett-Heaps (1969). This organelle, shown at high magnification in Figs. 21 & 24, is composed of two electron-dense globular ends and a bridge-like middle part which is of medium electron density. Each of the globular ends is surrounded by a halo of medium electron-dense material which is continuous with the bridge-like middle part. The diameter of each globular end is about 80 nm and the total length of the bipolar organelle during interphase is about 260 nm. In budding cells the position of the MTOC is regularly outside that portion of nucleus which is closest to the base of the growing bud. Although single globular MTOC components were often seen in randomly selected
sections, serial sectioning has always revealed another globular component at a different level. No structure which could positively be identified as a single globular MTOC was observed in association with interphase nuclei.

The first indications of impending nuclear division are changes in the shape and size of interphase nuclei. They become pear-shaped with the nucleolus occupying part of the rounded basal portion and the MTOC located outside the pointed tip portion, which extends in the direction of the bud (Figs. 22, 23). The size of the nucleus seems to increase in such a way that the volume of its rounded basal portion remains approximately the same as its original total volume.

The pointed tip of the pear-shaped nucleus later becomes greatly elongated with the result that a long protrusion of the nucleus approaches (Figs. 25, 26), and enters into the bud (Figs. 27, 28, 29, 30, 33, 34). It is accompanied by the MTOC. This bipolar organelle is regularly located on one side of the nuclear projection near its tip (see especially Figs. 27 & 34). The bridge of amorphous material joining the two globular MTOC components is longer than it was in interphase and the ribosome-free area around the organelle now contains profiles of the endoplasmic reticulum and vesicles with a distinctive morphology (Figs. 31, 32, 34). These vesicles usually consist of several small vesicles inside a larger one and resemble the so-called multi-vesicular bodies described by Girbardt (1968) in the vicinity of
spindle forming organelles in Polystictus versicolor. Cytoplasmic microtubules extend from the region of the MTOC both backwards towards the mother cell and forwards ahead of the nuclear projection into the bud (Figs. 25, 26, 27, 29, 34). At this stage the chromatin appears to be almost uniformly of low electron density like the chromatin region of spherical interphase nuclei.

Subsequent mitotic events which seem to correspond to the period when the nucleus becomes invisible with phase contrast microscopy (see Figs. 2 & 3 in McCully & Robinow, 1972a), can be summarized as follows. The nuclear envelope on one side of the nuclear projection into the bud becomes discontinuous (Figs. 34 & 35). This breakdown may well be the cause of the sudden loss of phase contrast between nucleus and cytoplasm in living cells. The globular ends of the MTOC move into the chromatin region and a spindle-shaped bundle of microtubules forms between them (Figs. 36, 37, 38). The chromatin condenses into distinct electron-dense aggregations which are scattered along the spindle. The formation of these aggregations corresponds to the stage at which the chromatin becomes intensely stained with aceto orcein (see Fig. 4 in McCully & Robinow, 1972a). The spindle consists of both pole-to-pole and chromosomal fibres. No kinetochore regions of the chromosomes were observed. Chromosomal microtubules simply end in fork-like structures within the chromosomes (Fig. 39).

Before breakdown of the nuclear envelope, at the time when there is a long projection of the nucleus into the bud, a considerable
portion of the nucleus including the nucleolus is left in the mother cell. Concurrent with the breakdown of nuclear envelope, the nuclear material in the mother cell seems to disintegrate. Remnants of nuclear envelope (Fig. 38) and what may be a disintegrating nucleolus (Fig. 37) can be seen in the mother cell at the time when a spindle is present in the bud.

Separation of daughter sets of chromosomes seems to progress as the spindle elongates. This elongation has the effect of moving one end of the spindle farther into the bud and the other end of the spindle back towards and into the mother cell. At this time the nuclear envelope seems to lie around the daughter ends of the spindle and along one side of it but the other side of the spindle is open to the cytoplasm (Fig. 40).

It is not clear how daughter nuclei finally become separated and at what stage an intact nuclear envelope is formed around each of them. The observations suggest that a considerable portion of the original nuclear envelope is utilized in this process.

It is also not clear what becomes of the single globular MTOC's at the ends of the telophase spindle. At the time when daughter nuclei are moving apart to their respective places in the bud and mother cell and are still connected through a narrow spindle corridor, MTOC's at the ends of the spindle seem to lie inside the new daughter nuclei (Fig. 41). These single MTOC elements may become relocated outside the nucleus before final closure of the envelope or they may disintegrate inside the nucleus.
By the time two daughter nuclei are clearly recognizable as separate entities, they are already spherical with well formed nucleoli. The chromatin in these nuclei is once again in the diffuse electron-transparent interphase condition (Fig. 42). I have searched diligently for any structure recognizable as an MTOC outside daughter nuclei but without any success. However, the possibility that the organelle is present at this stage cannot be completely ruled out.

These events are summarized in the diagram shown in Fig. 18.

DISCUSSION

Mitosis in \textit{L. scottii} is different in many ways from mitosis in \textit{Saccharomyces cerevisiae} although superficially these two budding yeasts look quite similar. In both yeasts at mitosis, the nuclei elongate and start to advance into the bud. However, division in \textit{Saccharomyces} is then accomplished by constriction in the narrow neck between bud and mother cell with half of the nuclear contents reaching the bud and half remaining in the mother cell. The nucleolus does not break down during mitosis; it stretches out within the elongating nucleus and is evenly distributed between the daughter nuclei. Moreover, division takes place within an intact nuclear envelope (Robinow & Marak, 1966).
A previous report of mitosis in *L. scottii* (*Candida scottii*) described a similar process of nuclear elongation and constriction in the region between bud and mother cell (Eckstein, 1958). That author did not recognize that all the chromatin enters into the bud before division. Although Eckstein has tried to arrange her illustrations in a uniform manner with the bud uppermost, some, such as her Figs. 11, 16 and 17 are upside down (mother cell uppermost). She considered these configurations to be in the mother cell because she did not recognize that buds invariably have more parallel sides than mother cells and may sometimes even be larger than the mother cell. Therefore she did not observe that many of the important mitotic events in this yeast are taking place in the bud.

Ultrastructural studies of nuclear division in basidiomycetes have been made by Girbardt (1968) in the dikaryotic hyphae of *Polystictus versicolor*, by Motta (1969), in the rhizomorphs of *Armillaria mellea*, by Lu (1967) in the basidia of *Coprinus lagopus* and by Lerbs and Thielke (1969) and Lerbs (1971) in the basidia of *Coprinus radiatus*. A common feature of division in these fungi is the breakdown of the nuclear envelope. Therefore the breakdown of envelope that has been observed in *L. scottii* is not surprising in view of its reclassification as a heterobasidiomycete (Fell, Statzell, Hunter & Phaff, 1969).

Certain other features of the mitosis in *L. scottii* strikingly resemble what Girbardt has observed in *Polystictus versicolor* using
phase contrast microscopy (Girbardt, 1961) and electron microscopy (Girbardt, 1968). Since I wish to stress that mitosis in this newly classified heterobasidiomycetous yeast is similar to what has been observed in other basidiomycetes, Girbardt's observations will briefly be described and compared with what has been seen in *L. scottii*.

One of the two nuclei in a dikaryotic cell of *P. versicolor* divides in the hypha and Girbardt has not yet described this mitosis in detail. The other nucleus just prior to mitosis begins to move into the clamp connection preceded by an electron-dense cytoplasmic structure consisting of two globular ends joined by a flat middle piece. This structure is morphologically similar to what is referred to in *L. scottii* as the "microtubule organizing centre". After part of the nucleus has entered the clamp connection, the nuclear envelope breaks down and much of the rear portion of the nucleus including the nucleolus is left behind and becomes dissolved in the cytoplasm of the hypha. Inside the clamp connection, the two globular ends of the electron-dense cytoplasmic structure move apart to form the poles of a bundle of microtubules (the "Zentralstrang"). The chromatin becomes condensed along this microtubule bundle and as the microtubules increase in length, the two daughter sets of chromosomes are separated to opposite poles. At the end of mitosis one daughter nucleus remains in the clamp connection and the other daughter nucleus moves back to the hypha.
One difference between *P. versicolor* and *L. scottii* is the following: In *P. versicolor*, the nuclear envelope which breaks down after the chromatin portion has entered the clamp, is later repaired to form a closed vesicle ("Bläschen") in which mitosis is carried out, whereas in *L. scottii*, the spindle is formed and mitosis takes place within a partially torn nuclear envelope which regularly has a large gap on one side of the spindle.

The electron-dense cytoplasmic structures with two globular ends seem to have similar roles in the two organisms. First, in accompanying the tips of nuclei which extend into the bud or the clamp connection, and secondly in organizing the mitotic spindle. However, in two respects the structure that is called a "microtubule organizing centre" in *L. scottii* differs from the structure that Girbardt (1968) has called a "kinetochore equivalent" in *Polystictus versicolor*. Girbardt finds that this organelle is "firmly attached to the nuclear envelope" and "coherent with the chromosomes" through pores in the nuclear envelope. The corresponding structure in *L. scottii*, although morphologically similar to the KCE in *P. versicolor*, is located close to but apparently not attached to the nuclear envelope. In contrast to what Girbardt has described in *P. versicolor* and what seems to be the case in *S. pombe* (see Chapter II) there is no evidence that the organelle in *L. scottii* is directly attached to chromatin inside the nucleus. During spindle formation, the chromosomes in *L. scottii* are clearly not attached directly to the MTOC's but are connected to them
by chromosomal microtubules. Therefore the term "kinetochore equivalent" cannot be properly used for the spindle forming structure in L. scottii as it has been for the comparable organelles in P. versicolor and S. pombe. It is therefore referred to as a "microtubule organizing centre", a general term proposed by Pickett-Heaps (1969) for osmophilic structures which are associated with microtubules.

There is evidence that the organelle in question is capable of doing other things besides serving as a "nucleating centre" for the assembly of microtubules (Tilney, 1968). Girbardt (1968) in his account of mitosis in P. versicolor calls it the "activity centre" of the nucleus. Observations on living and stained nuclei of Ceratocystis fagacearum and Fomes annosus have conveyed the distinct impression that "the centriole (MTOC in my terminology) pulls the nucleus through the cytoplasm with the nucleolus lagging behind" (Wilson & Aist, 1967). At first sight such behavior would also seem to be reflected in the geometry of the nuclei L. scottii. However, close examination of electron micrographs and of Dr. Robinow's micrographs of living dividing nuclei (see Fig. 2 in McCully & Robinow, 1972a) has led me to the conclusion that the interphase nucleus is first carried a short distance from the centre of the mother cell to the neck region, probably by the current of materials that is flowing towards the expanding bud. Starting from this position, it would seem that the nucleus does not then migrate into the bud; it grows into the
bud. This is immediately suggested by the unchanging shape and dimensions of the lower half of "migrating nuclei". Awareness of this is enhanced by looking at the nuclei of Figs 22, 29, 30, 33 with their upper portions covered up. It is anyhow not easily seen how the MTOC of *L. scottii* can be pulling a nucleus which it accompanies but to which it is not attached.

It has been suggested (Motta, 1969) that the spindle forming organelle in *Armillaria mellea* might function as a nuclear membrane organizing body and in the previous chapter the idea of differential membrane growth between separating MTOC's as the initial event in elongation of the intact mitotic nucleus of *Schizosaccharomyces pombe* has been advanced. The present observations on *L. scottii* lend support to the view of a possible role for the MTOC in the regulation of localized membrane growth.

It is proposed that prior to mitosis, the MTOC could direct the formation of nuclear envelope in that region of the ovoid interphase nucleus that is close to where the MTOC is located (i.e. the region of the ovoid nucleus that is close to the base of the bud). This would result in the formation of first a pear-shaped nucleus and then, of a long sac-shaped nucleus with a rounded base and a neck which extends into the bud. It is further proposed that the two globular MTOC elements of nuclei which have reached the interior of the bud and which are consistently placed on one side of the nuclear extension could cause the nuclear envelope to break down on that side perhaps by
temporarily ceasing to perform a putative membrane maintaining function. After spindle formation the daughter sets of chromosomes move to opposite poles where one of the two original MTOC globular components is present. At this time the organelle may again direct the synthesis (and maintenance?) of new nuclear membrane so that each daughter set of chromosomes becomes enclosed by an intact envelope. Until more is known about the chemical composition of spindle forming organelles and how membranes are formed, a membrane-regulating role for the spindle forming organelle in _L. scottii_ must remain a hypothesis that can not be proven.

In _L. scottii_ the observations suggest that after chromosome separation the partially formed daughter nuclei may be pushed apart to their respective places in the bud and mother cell by the elongation of spindle microtubules. Additional evidence for the importance of microtubule growth in the separation of daughter nuclei is provided by the observations on _Rhodotorula glutinis_ described in the following chapter.
Fig. 18  Diagrammatic interpretation of mitotic events in *L. scottii*. (A) Interphase. (B), (C), (D) Stages in the movement of a large portion of the nucleus into the bud. It is accompanied by a bipolar microtubule organizing centre and cytoplasmic microtubules. (E) (F) Spindle formation and chromosome separation inside the bud. Note that the nuclear envelope is not present along one side of the spindle. A portion of the nucleus containing the nucleolus has remained in the mother cell and is disintegrating at this stage. (G) Further elongation of the spindle. One end of the spindle (i.e. a daughter nucleus) is now located in the mother cell. (H) Reconstruction of intact daughter nuclei.
Fig. 19  

*L. scottii* — A mother cell and small bud. The nucleus which is located in the middle region of the mother cell is spherical and has two regions: a chromatin-containing region (Chr) of low electron density and an electron-dense nucleolus (N). In the cytoplasm are mitochondria (M), vacuoles (V), electron-transparent regions under the plasma membrane which seem to be the sites of some storage material (SM), and a region of vesicles (VR) near the tip of the bud. Outside the nuclear envelope is a bipolar microtubule organizing centre (MTOC). X 25,000.
Fig. 20  A bud of *L. scottii* shown at high magnification to reveal structural details of the vesicle region (VR) at its tip. \( \text{(M)} = \text{mitochondrion.} \)  X 67,000.
Fig. 21  *L. scottii* - Interphase nucleus. Note the microtubule organizing centre (MTOC) outside the nuclear envelope. In this cell, cytoplasmic microtubules (MT) run along the surface of the nucleus from the region of the MTOC although this is not a regularly observed feature of interphase cells. The nucleolus which is always present in interphase nuclei is not visible in this section. X 72,000.
Fig. 22  **L. scottii** – First indications of impending nuclear division (compare shape of this nucleus with the one shown in Fig. 19). This nucleus is pear-shaped with its pointed tip portion extending towards the bud. Like the spherical inter-phase nucleus, this nucleus contains an electron-transparent chromatin region (Chr) and a nucleolus (N) in which both amorphous electron-dense material and ribosome-like particles can be seen. The microtubule organizing centre (MTOC) is located outside the tip portion of the nucleus. (V) = vacuole. X 42,900.
Fig. 23  **L. scottii** - At the bottom right of this micrograph, a portion of the nucleus can be seen. Like the nucleus in Fig. 22, the nucleus in this cell has started to extend in the direction of the bud (which can be identified by the region of vesicles (VR) near its tip). The microtubule organizing centre which is associated with the nucleus is shown at higher magnification in Fig. 24. (N) = nucleolus  (Chr) = chromatin-containing region  X 30,000

Fig. 24  **L. scottii** - Details of the microtubule organizing centre (MTOC) which is shown at low magnification in Fig. 23. The organelle consists of two globular electron-dense ends and a bridge-like middle part. Note that it is located close to but apparently not attached to the nuclear envelope.  X 94,000.
Fig. 25 & 26  *L. scottii* - Adjacent sections of a nucleus which has extended closer to the neck of the bud than the two nuclei shown in Figs. 22 & 23.

A microtubule organizing centre (MTOC) is located on one side of the nucleus near its tip. The two globular ends of the MTOC are now farther apart than they were at interphase, and in this cell the bridge-like middle part of the MTOC is not present. This, however, may be due to the plane of sectioning.

Microtubules (MT) run from the region of the MTOC along one side of the nucleus. A multi-vesicular body (MVB) is also visible in the region of the MTOC. Both Figs. 25 & 26  X 67,000.
Fig. 27  \textit{L. scottii} - The tip of this nucleus has advanced into the bud. One globular component of the microtubule organizing centre (MTOC) is visible on one side of the nucleus. Microtubules (MT) run in the cytoplasm from the region of the MTOC along the side of the nucleus.

The arrows at the base of the bud point to the outer layers of mother cell wall which have broken open during bud formation.  X 67,000
Figs. 28 & 29  *L. scottii* - Two nuclei which extend into the bud. The relative positions of the nucleolus (N) and the chromatin-containing region (Chr) are characteristic of nuclei at this stage. In each micrograph, one component of the bipolar microtubule organizing centre (MTOC) is visible near the tip of the nucleus.

In Fig. 28 a region of vesicles (VR) is visible near the tip of the bud and in Fig. 29 the outer layers of mother cell wall form a collar around the base of the bud (arrows). These two features positively identify which cell is the bud.  Fig. 27  X 27,300

Fig. 29  X 36,000.
Fig. 30  **L. scottii** - A similar stage to those shown in Figs. 28 & 29. Again, the chromatin-containing region (Chr) extends into the bud while the nucleolus (N) remains in the mother cell within the rounded base of the nucleus.

The region outlined by the rectangle marks the position of the microtubule organizing centre. X 29,000.

Figs. 31 & 32  Adjacent sections of the region marked by the rectangle in Fig. 30. One component of the microtubule organizing centre (MTOC) is visible in Fig. 32. Associated with the MTOC are many multi-vesicular bodies (MVB) and cytoplasmic microtubules (MT). These are seen most clearly in Fig. 31. Both Figs. 31 & 32 X69,000.
Fig. 33  **L. scottii** - Another nucleus which extends from the mother cell into the bud. A large vacuole (V) seems to be hindering the movement of the nucleus. Notice that the contours of the rounded base of this nucleus are similar to those of an interphase nucleus while the portion which extends around the vacuole and into the bud seems to be an extension which has grown on to the rounded rear portion.  X 26,000.

Fig. 34  **L. scottii** - Portion of a protruding nucleus in the bud. A bipolar microtubule organizing centre (MTOC) is located on one side of the tip. The bridge-like middle part of this organelle is now longer than it was in interphase cells. Vesicles (Ve) and endoplasmic reticulum (ER) are visible in the ribosome-free area around the MTOC. Cytoplasmic microtubules (MT) extend from the region of the MTOC along one side of the nucleus. Notice the discontinuities in the nuclear envelope near the MTOC (large arrows). These may be initial stages of envelope breakdown prior to spindle formation.  X 68,000.
Figs. 35 & 36  \textit{L. scottii} - Spindle formation in the bud.
The relationship of the spindle to the rest of the cell is shown at low magnification in Fig. 35.

Fig. 36 shows details of the spindle (Sp). Notice that the nuclear envelope is partially broken down. The two globular components of the microtubule organizing centre (MTOC) are seen at opposite poles of the spindle. Scattered along the spindle are masses of electron dense material which are interpreted as chromosomes (Chr).

Arrows point to regions where the outer wall layers of the mother cell have broken open during bud formation. Fig. 35 $\times$ 18,700  Fig. 36 $\times$ 67,000.
Figs. 37  **L. scottii** - Spindle formation in the bud.

Notice that the nuclear envelope is present along the left side of the spindle (Sp) but the right side is open to the cytoplasm.

A region of amorphous electron-dense material is located in the mother cell near the base of the bud (arrows). This is interpreted as the nucleolus (N) which is disintegrating at this stage.  X 33,000.
Fig. 38  **L. scottii** - Spindle formation in the bud. Note that the nuclear envelope is present along the left side of the spindle (Sp), but the right side of the spindle is open to the cytoplasm. A single globular microtubule organizing centre is visible at one end of the spindle. This region is shown at higher magnification in Fig. 39. The microtubule organizing centre at the opposite end of the spindle is not visible because the spindle is sectioned obliquely.

Arrows near the bottom of the micrograph point to remnants of nuclear envelope in the mother cell.

X 35,000.

Fig. 39  **L. scottii** - Spindle microtubules in close association with a single globular microtubule organizing centre (MTOC) that is shown at lower magnification in Fig. 38. A microtubule running from the region of the MTOC ends in a fork-like structure (near the arrow) within a mass of amorphous electron-dense material which is interpreted as a chromosome (Chr).

X 96,000.
Fig. 40  **L. scottii**  - A later stage in spindle elongation than what has been shown in Figs. 36, 37 & 38. As the spindle tubules elongate, one daughter end is pushed back into the mother cell and the other end is pushed farther into the bud. The nuclear envelope is present along the right side of the spindle (Sp) but not along the left side. The new daughter nuclei appear as rounded areas at the ends of the spindle. These are partially surrounded by nuclear envelope.  X 42,000.

Fig. 41  **L. scottii**  - A slightly more advanced stage of daughter nuclei separation than in Fig. 40. One daughter nucleus is visible in the bud at the top of the micrograph and a glancing section of the other daughter is seen in the mother cell near the bottom of the micrograph. Although it is not apparent in this section, adjacent sections showed that the two daughter nuclei are still connected by spindle microtubules and a partially intact channel of nuclear envelope. A small portion of this channel (between arrows) is visible near the bottom of the micrograph. A single spherical MTOC can be seen in association with spindle (Sp) microtubules in the daughter nucleus which is located in the bud. Notice how this MTOC seems to be inside the nuclear envelope.  X 44,000.
Fig. 42  **L. scottii** - At the end of nuclear division a spherical daughter nucleus with a well defined chromatin region (Chr) and nucleolus (N) is located in both the mother cell and the bud. Although several sections of this mother cell and bud were examined, no MTOC was observed in association with either daughter nucleus. X 23,000.
CHAPTER IV

STUDIES ON RHODOSPORIDIUM SP. (RHODOTORULA GLUTINIS) AND AESSOPORON SALMONICOLOR (SPOROBOLOMYCES SALMONICOLOR)

INTRODUCTION

The preceding chapter described a sequence of mitotic events, previously unknown in a yeast, in which spindle formation and chromosome separation take place within the bud. This chapter describes a similar type of mitosis in Rhodotorula glutinis and Sporobolomyces salmonicolor; two yeasts which, like L. scottii, have recently been reclassified as heterobasidiomycetes (see review by Fell, 1970). These findings indicate that "mitosis in the bud" has taxonomic significance as a distinctive characteristic of yeasts which are related to mycelial basidiomycetes.

Rhodotorula glutinis shall henceforth be referred to as Rhodosporidium sp. since R. glutinis is known to be the vegetative phase of at least three distinct species of Rhodosporidium (Fell, 1970). Sporobolomyces salmonicolor shall be referred to as Aessosporon salmonicolor in conformity with its new taxonomic assignation (van der Walt, 1970).

A few interesting differences have been found between mitosis in Rhodosporidium and L. scottii. In Rhodosporidium I have also
obtained additional information as to how a daughter nucleus returns to the mother cell after chromosome separation.

The nuclear behavior of *A. salmonicolor* resembles *L. scottii* more closely than it does *Rhodosporidium*. This widespread contaminant offers a third example of "mitosis in the bud" and its large size made it particularly suitable for detailed comparative studies with light microscopy. (See Figs. 2-10 in McCully & Robinow, 1972b).
MATERIALS AND METHODS

Materials

*Rhodotorula glutinis* (Fres.) Harrison, strain NRRL Y-2502 was received from the yeast collection of the U.S. Dept. of Agriculture at Peoria, Illinois. *Sporobolomyces* was encountered as a contaminant on an agar plate. Its identification as *S. salmonicolor*, Kluyver and van Niel, was performed at the Yeast Division of the C.B.S. at Delft, The Netherlands. Cultures were maintained and propagated on a medium composed of yeast extract 0.5g, glucose 2.0g, and agar 1.5g per 100 ml of water.

Preparation for Electron Microscopy

All procedures were the same as those described for *L. scottii* in Chapter III with one exception. Both glutaraldehyde and osmium fixatives were dissolved in .05 M potassium phosphate buffer pH 6.5 instead of cacodylate buffer. It was found that there was a dense sprinkling of fine black particles within the cells of both *Rhodosporidium sp.* and *A. salmonicolor* when fixatives dissolved in cacodylate buffer were used.

Most of the cells illustrated here were fixed in cold (4°C) glutaraldehyde and room temperature osmium. Fig. 73 shows a cell that was fixed with both glutaraldehyde and osmium at 4°C.
OBSERVATIONS

Cytoplasmic Features of Rhodosporidium sp. and A. salmonicolor

Both species are similar to L. scottii in that they have a high concentration of ribosomes and accumulations of electron transparent material underneath the plasma membrane (Figs. 44, 46, 70, 71). In Rhodosporidium this material was also regularly seen in the interior regions of both buds and mother cells (Fig. 64).

Outside the cell wall of Rhodosporidium is a granular electron-dense capsule layer (Fig. 46). The interior of cells with thick capsules tended to be poorly fixed.

Buds in both Rhodosporidium and A. salmonicolor are, as in L. scottii recognizable by the presence of a cluster of vesicles near their tip (Figs. 46, 70). Buds in the two species are also recognizable by their relatively thin cell wall in comparison to the wall of mother cells (see especially Figs. 59 and 77).

Nuclear Features of Rhodosporidium sp.

The Interphase Nucleus (Fig. 44). During interphase the nucleus is ovoid and consists of two regions. One is of low electron density and by comparison with aceto orcein stained cells may be identified as the site of chromatin although it contains no structures recognizable as chromosomes. The other region is a centrally placed, spherical
nucleolus. It consists of ribosome-like particles dispersed among amorphous electron-dense material and is never set off from the chromatin region by a membrane as has been previously wrongly reported for this organism (Conti, Thyagarajan and Naylor, 1963).

**Microtubule Organizing Centres (MTOC's)**

In a ribosome-free zone beside the interphase nucleus but clearly not attached to it is a structure which is referred to as the microtubule organizing centre (MTOC) (Figs. 44, 45). It consists of two bar-shaped electron-dense ends each with dimensions of about 130 nmX 40 nmX 40 nm. These two bars are surrounded by amorphous material of medium to high electron density which also forms a bridge between them. A few microtubules usually extend from the region of the MTOC along the surface of the nucleus (Fig. 44). In cells with no buds or small buds the MTOC always consists of two bar shaped elements. When a single bar was seen as in Fig. 44, another bar was invariably present in an adjacent section. MTOC's were found in a variety of positions relative to the nucleus but in cells with large buds in which mitosis was presumably imminent, the MTOC was regularly found outside that portion of nucleus that was closest to the base of the growing bud.

When the nucleus extends into the bud, the MTOC is regularly located outside its advancing tip (Figs. 46 to 52). Microtubules extend from the region of the MTOC backwards towards the mother cell
along the surface of the nucleus (Figs. 48, 49) and forwards ahead of the nucleus into the bud (Fig. 46). At this time, the ribosome-free area around the MTOC is considerably larger than it is around MTOC's associated with ovoid interphase nuclei. This area now contains proliferations of the endoplasmic reticulum and distinctive vesicles which have been called "multi-vesicular bodies" following Girbardt (1968) who observed similar structures in the vicinity of the spindle-forming organelles in Polystictus versicolor. These multivesicular bodies consist of several small vesicles inside a larger vesicle which is about 120 nm in diameter (Figs. 48, 51).

**Spindle Formation in the Bud** (see diagram Fig. 43 B-F). Before spindle formation takes place, the nuclear envelope appears to break down in the tip region of the nucleus adjacent to the MTOC. The amorphous electron-dense bridge between the two bar-shaped MTOC components disappears and the two bars sink into the chromatin region (Fig. 53). Subsequently within the discontinuous nuclear envelope, a bundle of microtubules forms between the two bars (Fig. 54). When the spindle increases in length, discontinuities in the nuclear envelope are no longer seen and for a time, both the nucleolus-containing portion of the nucleus in the mother cell and the spindle-containing portion in the bud seem to be enclosed in one intact nuclear envelope (Figs. 55, 56).

Later, the spindle-containing portion seems to pinch off from the rest of the nucleus which then disintegrates in the cytoplasm of
the mother cell. At this time an intact nuclear envelope is seen around the spindle (Fig. 57, 58). Remnants of nuclear envelope are seen around the portion of the nucleus which remains in the mother cell.

Further increase in the length of the spindle results in the formation of a membrane-enclosed, dumbbell-shaped region within the bud (Fig. 59). Some spindle microtubules seem to end in accumulations of electron-dense material (chromosomes?) located in the rounded dumbbell ends (Fig. 61).

Separation of Daughter Nuclei (see diagram Fig. 43-G). This event seems to be accomplished by the breakage of the nuclear envelope in the central channel region of the dumbbell and a large increase in the length of the spindle fibres between the two MTOC elements. These microtubules run through the cytoplasm between the new daughter nuclei and are not enclosed in a membranous sheath as during the telophase of Schizosaccharomyces pombe (compare Figs. 62, 64, 65 with Figs. 14, 16). During the separation process, the envelope around the new daughter nuclei is not completely intact. It is discontinuous on one side of the nucleus near the position of the MTOC and at the opposite side in a region through which the shaft of microtubules passes. (Figs. 63, 65).

Final Reconstruction of Daughter Nuclei (see diagram Fig. 43-H). After one daughter nucleus has reached a position in the central portion of the mother cell, the linking shaft of microtubules between the two
nuclei is broken down. Closure of the nuclear envelope seems to take place in such a way that the single spindle-associated MTOC in each daughter nucleus becomes located on the outside of the envelope in a prominent invagination (Figs. 66 to 69). The daughter nuclei become considerably larger than they were during the moving apart process and a new nucleolus is formed in them. Recently separated daughter nuclei are recognizable by their irregular contours. Later, the nuclei become spherical in shape.

Single MTOC elements were seen many times outside the daughter nuclei of *Rhodosporidium*. However, it is not known how and when the formation of a bipolar MTOC is accomplished.

**Nuclear Features of *Aessosporon salmonicolor***

Mitotic events in *A. salmonicolor* are similar to what has been described for *Rhodosporidium* with the following exceptions. As the nucleus moves into the bud (Figs. 71, 72), the position of the MTOC is on one side near the tip of the nucleus rather than directly at the tip as in *Rhodosporidium*. Breakdown of nuclear envelope occurs on one side of the nucleus and this membrane remains open during spindle formation (Figs. 73, 74, 75). Elongation of the spindle results in the formation of a dumbbell-shaped configuration inside the bud (Fig. 76). However, the nuclear envelope remains open over a long stretch of this dumbbell while in *Rhodosporidium*, the similar dumbbell shaped formation is surrounded by an intact nuclear envelope.
(compare Fig. 76 with 59). Daughter nuclei in _A. salmonicolor_ are spherical in shape and do not seem to have an associated MTOC (Fig. 77). The irregularly shaped daughter nuclei accompanied by single MTOC's that were a common feature of _Rhodosporidium_ were not seen in _A. salmonicolor_.

**Ballistospore Formation in Aessosporon salmonicolor**

As described by Buller (1933), cells of _A. salmonicolor_ (Sporobolomyces) sometimes stop budding and produce a ballistospore on a sterigma.

In the course of my observations on budding cells of _A. salmonicolor_, ballistospore-forming cells were sometimes encountered. In this process the nucleus seems to grow into the ballistospore preceded by the MTOC, just as the nucleus seems to grow into the bud (Figs. 79, 80).

The outgrowing tip of the sterigma and of the developing ballistospore contain vesicle regions like the ones seen near the tips of buds (Figs. 78, 80).

**DISCUSSION**

The finding of a mode of mitosis involving elongation (growth?) of the nucleus into the bud, partial breakdown of the nuclear envelope, loss of the rear portion of the nucleus including the nucleolus, division of the chromatin portion along a spindle apparatus inside the
bud, and return of one daughter nucleus to the mother cell in Rhodosporidium sp. and A. salmonicolor is consistent with recent findings which have revealed many other similarities between these two including their taxonomic affinity with basidiomycetes (see review by Fell, 1970).

The reader is referred to the Discussion Section of the preceding chapter for a detailed discussion of the striking similarities which exist between mitosis in these three heterobasidiomycetous yeasts and mitosis in the dikaryotic hyphae of Polystictus versicolor (Girbardi, 1961, 1968). The observations indicate that nuclear division in L. scottii and A. salmonicolor follows identical courses. However, some differences exist between the course of mitosis in these two and the third member of the group, Rhodosporidium. In one way mitosis in Rhodosporidium is even more similar to mitosis in Polystictus than the other two yeasts. In Rhodosporidium and Polystictus the nuclear envelope partially disintegrates after the chromatinic portion of the nucleus has entered the bud and is then repaired on a smaller scale so that the mitotic spindle comes to lie within a region surrounded by an intact nuclear envelope. By contrast, spindles in L. scottii and A. salmonicolor are regularly formed in a region bounded by the partially torn remnants of the nuclear envelope.

Another difference between Rhodosporidium and the other two yeasts concerns the MTOC. In all three species, a bipolar MTOC is found in association with resting nuclei but only in Rhodosporidium
can its origin be traced to the MTOC inherited during the previous division. In the other two yeasts single MTOC elements are seen inside the nuclear envelope early during the separation of daughter nuclei but neither inside nor outside are they encountered thereafter until the new cells have separated. Since in Rhodosporidium many daughter nuclei were seen with a single associated MTOC component and no MTOC's associated with daughter nuclei of the other two yeasts in spite of much careful searching, it is proposed that there may be real differences between these yeasts with respect to the continuity of MTOC's. In view of differences between the origin and continuity of spindle-forming organelles in two mycelial basidiomycetes, this possibility does not seem unlikely. In Polystictus versicolor a single spindle-forming element is present outside the envelope of newly separated daughter nuclei and soon after division a new bipolar entity forms at the base of the single structure which then disintegrates (Girbardt, 1968). In Armillaria mellea, however, a single spherical spindle-forming element arises "apparently de novo" in the cytoplasm just prior to mitosis and soon afterwards it divides into two (Motta, 1969).

Another interesting morphological similarity exists between these three yeasts and two mycelial basidiomycetes described in the literature. This is the presence in their cytoplasm of multi-vesicular bodies consisting of several small vesicles enclosed in a larger one. These multi-vesicular bodies are regularly seen in the ribosome-free vicinity of the MTOC when the nucleus extends into the bud. Their
significance is not known, but it seems worthwhile to point out that they have been seen in the vicinity of spindle-forming organelles by Girbardt (1968) in *P. versicolor* (see his Fig. 5G) and by Raudaskoski (1970) in *S. commune* (see her Figs. 5 & 6).

In *L. scottii* it is suggested that the movement of the nucleus into the bud may be an expression of localized membrane growth in the region of the MTOC. The observed shape of the nuclei of *Rhodorosporidium* and *A. salmonicolor* as they enter into buds, is consistent with the hypothesis that the same type of localized membrane growth occurs in *Rhodorosporidium* and *A. salmonicolor*.

Observations on *L. scottii* showed that the moving back of one daughter nucleus to the mother cell was accompanied by the growth of microtubules between daughter ends of the spindle. However, in this organism and in *A. salmonicolor*, the nuclear envelope persists along one side of the spindle as the rounded ends move to their respective positions in the bud and mother cell. Therefore it can not be completely ruled out that this nuclear membrane might be assisting in the telophase movements. By contrast, in *Rhodorosporidium* there is no nuclear membrane associated with the shaft of microtubules which connects daughter nuclei as they separate. Microtubules are known to be rigid structures and it seems possible that their growth between the daughter nuclei might cause the one daughter to be squeezed through the narrow neck between bud and mother cell and to be further pushed to a position in the central region of the mother cell.
A previous account of mitosis in *Rhodotorula glutinis* (Thyagarajan, Conti, & Naylor, 1962) described nuclei which extend into the bud in a comparable way to the nuclei shown in Figs. 46 & 47. This study used permanganate fixation for electron microscopy, a fixation method that is now known to destroy microtubules and spindle forming organelles. Therefore it is not surprising that these workers dismissed occasional openings seen in the nuclear envelope as fixation artifacts not realizing that these openings are the result of real mitotic events leading to the formation of a spindle apparatus within the bud. Instead, they concluded, erroneously, that mitosis in *R. glutinis* is a "simple process of elongation and constriction within an intact nuclear envelope in the region between bud and mother cell".

A recent electron microscope study using permanganate fixation on yeast-like haplonts of the heterobasidiomycete *Tremella mesenterica* (Bandoni & Bisalputra, 1971) has described a process of nuclear division involving elongation of the nucleus into the bud and division in the narrow isthmus between bud and mother cell. Large gaps in the nuclear envelope are illustrated but their significance is not discussed. It is likely that a similar type of mitosis to what I have described in three heterobasidiomycete yeasts also takes place in *Tremella*.

The widespread use of potassium permanganate as a fixative in ultrastructural studies of fungi and failure to correlate electron
micrographs with adequate light microscopy may be reasons why the distinctive differences between ascomycete related yeasts and basidiomycete related yeasts have remained undetected until now. These differences are so striking that they should be readily seen in either aceto orcein preparations examined with the light microscope or in glutaraldehyde-fixed cells examined with the electron microscope. Therefore it is suggested that the study of mitosis may be a useful method of finding taxonomic relationships in asporogenous yeasts. Those related to ascomycetes probably divide like Saccharomyces cerevisiae and Schizosaccharomyces pombe (Robinow & Marak, 1966; Chapter II, this thesis) while those related to basidiomycetes probably divide like L. scottii, Rhodosporidium sp. and A. salmonicolor.

Consistent with this generalization is mitosis in the budding yeast-like sporidia of Ustilago violacea which has recently been described by Day & Jones (1972) on the basis of light microscopical observations on hydrolysed, giemsa stained cells. Since Ustilago violacea is related to the basidiomycetes it is not surprising that its mitosis involves the movement of all the chromatin into the bud; division in the bud; and return of one daughter nucleus to the mother cell.

The same type of division was also noted by Holliday (1965) in the course of a study about the effect of ultra violet light on mitotic crossing-over in Ustilago maydis (see his Fig. I). This report came to my attention some time after the observations reported here had been made.
Fig. 43  Diagrammatic interpretation of spindle formation and
the separation of daughter nuclei in *Rhodosporidium* sp.

A. The nucleus extends into the bud. A microtubule
organizing centre consisting of two bars and a bridge-
like middle piece is located outside the tip of the
nucleus. The nucleolus remains in the mother cell.
The reader is referred to the diagram in the preceding
Chapter on *L. scottii*, Fig. 18 A-D for stages in the
process of nuclear movement into the bud. They are
similar in *Rhodosporidium*.

B. The nuclear envelope breaks down in the tip region of
the nucleus. The bridge-like middle piece of the MTOC
disappears and the two bars sink into the nucleus.
The density of the nucleolus decreases.

C. A spindle is formed between the two MTOC components.
The envelope is still discontinuous in the tip region.

D. The spindle elongates. The envelope becomes continuous
around the whole nucleus in both mother cell and bud.

E. The spindle-containing portion pinches off from the rest
of the nucleus. The envelope is intact around the
spindle and discontinuous around the portion of the
nucleus which remains in the bud.

F. The spindle further elongates and a dumbbell-shaped
membrane enclosed region is formed within the bud.
The portion of nucleus remaining in the mother cell
disintegrates.

G. The nuclear envelope in the central channel region of
the dumbbell seems to break and the two daughter nuclei
move apart to their respective places in bud and mother
cell. They are linked by a long straight bundle of micro-
tubules. The nuclear envelope in separating daughter
nuclei is discontinuous near the single MTOC component at
the end of the spindle and opposite the MTOC in the region
through which the shaft of microtubules passes into the
cytoplasm.

H. The microtubules break down. The nuclear envelope
becomes intact around the daughter nuclei in such a way
that the single MTOC that was at the end of the spindle
is positioned outside the envelope. A new nucleolus is
formed in each daughter nucleus.
Fig. 44 Interphase nucleus of *Rhodospiridium* showing an electron-dense nucleolus (N) surrounded by a chromatin-containing region of low electron density (Chr). A single bar-shaped component of the microtubule organizing centre (MTOC) is visible near the top of the micrograph. A similar bar was present in an adjacent section. Microtubules (MT) are present in the cytoplasm along the left side of the nucleus. Accumulations of electron-transparent storage material (SM) lie just underneath the plasma membrane. Part of a large vacuole (V) containing electron-dense material is visible at the top of the micrograph. X 56,800.

Fig. 45 A microtubule organizing centre beside an interphase nucleus of *Rhodospiridium* similar to the one in Fig. 44. It consists of two electron-dense bars joined by a flat middle piece. It is close to but apparently not attached to the nuclear envelope (NE). An electron-transparent region separates the organelle from the rest of the cytoplasm. X 100,500.
Figs. 46 & 47  Two budding cells of *Rhodosporidium* in which the chromatin-containing region of the nucleus (Chr) extends into the bud. The spherical electron-dense nucleolus (N) is located in the portion of the nucleus which remains in the mother cell.

A single bar shaped component of the microtubule organizing centre (MTOC) is visible at the tip of the nucleus in Fig. 46. Microtubules (MT) extend from the region of this MTOC into the cytoplasm.

Both components of the MTOC are visible in Fig. 47. This organelle is shown at higher magnification in Fig. 50.

In Fig. 46 note the region of vesicles (VR) at the tip of the bud, the electron-dense capsular material (C) around the cell outside of the cell, and the accumulations of electron-transparent storage material (SM) under the plasma membrane.

Fig. 46  X 22,800;  Fig. 47  X 32,500.
Figs 48 to 52  *Rhodosporidium* - Details of microtubule organizing centres and the surrounding cytoplasm at the tips of nuclei which extend into buds. Fig. 50 is the same MTOC that is shown at low magnification in Fig. 47. Figs. 51 & 52 are adjacent sections of the same cell.

In Figs. 48 and 52 only one component of the MTOC is visible. In both cells the other MTOC component was present in an adjacent section.

In Figs. 49 and 50 both components of the MTOC are visible at the same level of sectioning. Notice that the bridge of electron-dense material joining the two bar shaped MTOC components is longer at this stage than at interphase (compare Figs. 49 and 50 with Fig. 45).

Notice that all the MTOC's shown here are located close to but apparently not attached to the nuclear envelope.

Associated with the MTOC's are cytoplasmic microtubules (MT) and multi-vesicular bodies (MVB). The latter, shown most clearly in Fig. 51, consist of several small vesicles inside a larger one.

All Figs. 48 to 52  X 67,300.
Fig. 53  *Rhodosporidium* - Nuclear envelope breakdown prior to spindle formation. Notice that the envelope is discontinuous at the end of the nucleus which projects into the bud. The two bar-shaped components of the microtubule organizing centre (*MTOC*) are no longer connected by a bridge of amorphous electron-dense material and they have moved into the chromatin-containing region (*Chr*). The nucleolus (*N*) is faintly visible in the portion of the nucleus which remains in the mother cell (outlined by the arrows). Although the contrast between the nucleolus and the chromatin-containing portion of the nucleus is less than at the stage shown in Figs. 46 & 47, the nucleolus is still recognizable by the presence of ribosome-like particles.  X 51,200.

Fig. 54  *Rhodosporidium* - Within the portion of nucleus which extends into the bud, a bundle of microtubules (*Sp*) is present between the two bar-shaped components of the microtubule organizing centre (*MTOC*). Notice that the nuclear envelope is discontinuous around the spindle.  X 67,300.
Figs. 55 & 56  **Rhodosporidium** - Two adjacent sections of a spindle-containing nucleus. The spindle (Sp) lies between two bar-shaped microtubule organizing centres (MTOC) which are visible in Fig. 56. In Fig. 56, due to a fold in the section, it is not possible to determine with certainty whether the nuclear envelope is intact along the left side of the spindle. An adjacent section shown here in Fig. 55, serves to illustrate that the nuclear envelope is intact around the whole nucleus which stretches between the mother cell and bud.

This stage should be contrasted with the slightly earlier stage of mitosis shown in Fig. 54 in which the envelope is discontinuous around the spindle.

Fig. 55  X 49,600;  Fig. 56  X 60,500.
Figs. 57 & 58  

**Rhodosporidium** - Two adjacent sections of a cell in which the spindle-containing portion of the nucleus has become detached from the rest of the nucleus.

Because the spindle (Sp) is sectioned slightly obliquely, only the microtubule organizing centre (MTOC) at the spindle-end nearest the tip of the bud is visible in Fig. 57 while the MTOC at the opposite end of the spindle is visible in Fig. 58.

Notice that the nuclear envelope around the spindle-containing portion is intact and that there are a few gaps in the envelope around the portion of the nucleus in the mother cell.

Both Figs. 57 & 58  X 54,000.
Fig. 59  **Rhodosporidium** - Further elongation of the spindle-containing (Sp) portion of the nucleus into a dumbbell shape. Notice that the nuclear envelope is intact along both sides of the spindle.

Arrows near the bottom of the micrograph point to regions where the outer wall layers of the mother cell have ruptured during bud formation. This feature establishes that the spindle is located inside the bud.  X 47,000.

Fig. 60  **Rhodosporidium** - At a similar stage of mitosis to the cell in Fig. 59 as seen with the light microscope after hydrolysis and staining with aceto orcein. Within the bud (recognizable by its smaller size) the chromatin (Chr) is in a dumbbell-shaped configuration while the mother cell is enucleate.  X 4,500.

This micrograph has been provided by Dr. Robinow.

Fig. 61  **Rhodosporidium** - Adjacent section of one end of the dumbbell shown in Fig. 59. Spindle microtubules (Sp) extend from the region of a single microtubule organizing centre (MTOC). Some of these microtubules seem to end in small electron-dense regions which are interpreted as chromosomes (Chr).  X 65,300.
Figs 62 & 63  *Rhodospiridium* - Return of a daughter nucleus to the mother cell. These two micrographs are nearly adjacent sections of a daughter nucleus that is squeezing through the narrow neck between bud and mother cell. The position of ruptured outer wall layers (at the large arrows) establishes that the mother cell is at the bottom of the micrograph.

In Fig. 63 a bundle of microtubules (MT) passes from the cytoplasm through a gap in the nuclear envelope (small arrows) and ends near the opposite side of the nucleus. Fig. 62 shows that these microtubules extend from the nucleus into the cytoplasm where they lie in a narrow bundle that is not surrounded by nuclear envelope. It was observed that they are connected to the other daughter nucleus which is located in the bud beyond the top of the micrograph. Both Figs. 62 & 62  X 66,000.
Figs 64 & 65  *Rhodosporidium* - A new daughter nucleus in the bud shown at two different magnifications. Notice the narrow bundle of microtubules (MT) which runs from the daughter nucleus through the cytoplasm into the mother cell. Examination of adjacent sections showed that the bundle of microtubules connects to the other daughter nucleus in the mother cell. The position of this nucleus is marked by an asterisk near the bottom of Fig. 64.

Notice that the microtubules pass through the daughter nucleus of the bud and seem to end in an electron-dense region which is interpreted as a glancing section of a microtubule organizing centre. The nuclear envelope is discontinuous near this region (upper pair of small arrows in Fig. 65). The nuclear envelope is also discontinuous where the shaft of microtubules passes out into the cytoplasm (lower pair of small arrows in Fig. 65).

Within the cytoplasm are prominent accumulations of electron-transparent storage material (SM). Fig. 64  X 23,000;  Fig. 65  X 64,000.
Figs 66 & 67  Rhodosporidium - Two buds with newly reconstructed nuclei. The nuclear envelope is now completely intact and a single microtubule organizing centre (MTOC) is located outside each nucleus in an invagination of the envelope. Each nucleus now again consists of two regions; a nucleolus (N) and a chromatin-containing portion (Chr).

A large vacuole (V) filled with electron-dense material is visible in Fig. 66.

Electron-dense capsular material (C) surrounds both cells especially the one in Fig. 67.

Both Figs. 66 & 67  X 27,200.
Figs. 68 & 69  **Rhodospiridium** - Two nuclei which, by their irregular shape and their location in cells with a nucleus in both bud and mother cell are identified as nuclei which are newly reconstructed after mitosis.

Associated with each nucleus is a microtubule organizing centre (MTOC). Serial sectioning showed that at this stage the MTOC consists of only a single bar-shaped structure.

The nucleolus (N) in the nucleus of Fig. 68 is of low electron density and may be at an earlier stage of reformation than the nucleolus in the nucleus of Fig. 69, which consists of both amorphous electron-dense material and ribosome-like particles. Both Figs. 68 & 69 X 64,000.
Fig. 70  *A. salmonicolor* - A mother cell and small bud. The nucleus is spherical and has two regions; a chromatin-containing region (Chr) of low electron density and an electron-dense nucleolus (N). Outside the nuclear envelope is a bipolar microtubule organizing centre (MTOC).

At the tip of the bud is a region of vesicles (VR) and under the plasma membrane are electron-transparent regions which seem to be the sites of some storage material (SM).  X 17,500.

Fig. 71  *A. salmonicolor* - First indications of impending nuclear division (compare the shape of this nucleus with the interphase nucleus in Fig. 70). This pear-shaped nucleus consists of a chromatin-containing region (Chr) and a nucleolus (N). A bipolar microtubule organizing centre (MTOC) lies outside the nuclear envelope.

Note the electron-transparent regions under the plasma membrane. These seem to be the sites of some storage material (SM).  X 43,000.
Fig. 72  \textit{A. salmonicolor} – Nuclear extension into the bud. Part of the chromatin-containing portion (Chr) extends into the bud while the nucleolus (N) remains in the mother cell. A single component of the microtubule organizing centre (MTOC) is located near the tip of the nucleus. Another MTOC component was present in an adjacent section. X 52,000.

Fig. 73  \textit{A. salmonicolor} – Spindle formation inside the bud. Because the spindle (Sp) is sectioned slightly obliquely, only the microtubule organizing centre (MTOC) at the spindle end closest to the mother cell is visible. Notice that there is a large gap in the nuclear envelope on the left side of the spindle. A considerable portion of the nucleus remains in the mother cell. The envelope around this portion of nucleus is also discontinuous. X 52,000.
Figs. 74 & 75  *A. salmonicolor*  - Two sections of a spindle (Sp) formed inside the bud. In Fig. 75, a microtubule organizing centre (MTOC) is visible at the end of the spindle that is nearest the top of the micrograph.

Notice that the nuclear envelope is discontinuous along the right side of the spindle. Notice too, that a large portion of the nucleus remains in the mother cell.

The nucleolus (N) is starting to disintegrate at this stage of mitosis but it can still be discerned at the base of the portion of nucleus which remains in the mother cell (between arrows in Fig. 75).

Both Figs. 74 & 75  X 36,800.
Fig. 76  **A. salmonicolor** - Spindle elongation in the bud. The spindle (Sp) lies in a dumbbell-shaped region within the bud. Notice that the nuclear envelope is present along the left side of the spindle but not along the right side. This micrograph should be contrasted with the similar stage of mitosis in *Rhodosporidium* (Fig. 59), in which there is an intact nuclear envelope on both sides of the spindle.

In the mother cell, notice the area that is of lower electron density than the rest of the cytoplasm (arrows). This is interpreted as the disintegrating nucleolus (N). X 32,500.

Fig. 77  **A. salmonicolor** - At the end of nuclear division a spherical daughter nucleus with a well defined chromatin region (Chr) and nucleolus (N) is located in both the mother cell and the bud. Although several sections of this mother cell and bud were examined, no microtubule organizing centre was observed in association with either daughter nucleus.

Notice how the cell wall of the bud is thinner than the cell wall of the mother cell and how the outer wall layers of the mother cell wall seem to form a collar around the base of the bud. X 18,000.
Fig. 78  *A. salmonicolor* - Early stage of ballistospore formation. A nucleus of interphase dimensions is located in the mother cell. A microtubule organizing centre (MTOC) is associated with this nucleus. Notice the prominent region of vesicles (VR) in the sterigma near the base of the developing ballistospore. Notice also the bud scar (BS) on the mother cell near the bottom right of the micrograph. X 25,300.
Figs. 79 & 80  
*A. salmonicolor* - Two adjacent sections of a somewhat later stage of ballistospore formation than the one shown in Fig. 78. A long projection of the chromatin-containing region of the nucleus extends towards the base of the ballistospore. The nucleolus (N) remains in the rounded base of the nucleus. A single bar-shaped component of the microtubule organizing centre (MTOC) is visible at the tip of the nucleus in Fig. 80. Microtubules (MT) run in the cytoplasm from the region of the MTOC along the side of the nucleus.

Notice the vesicle region (VR) near the tip of the ballistospore. Both Figs. 79 & 80 X 18,000.
CHAPTER V

STUDIES ON MUCOR HIEMALIS

INTRODUCTION

Members of the Mucorales were among the first fungi in which somatic nuclear division was studied and in which it was shown that fungal mitosis may be different from mitosis in plant and animal cells (Léger, 1896). Using hematoxylin, a dye which in 1896 was already known to stain both the chromosomes and the nucleoli of a wide range of plants, animals, algae, and protozoa, Léger found that in the nuclei of the Mucorales he could only stain a central spherical body which he correctly identified as the nucleolus while the peripheral regions of the nucleus remained colourless.

Several people who studied mitosis in the Mucorales after Léger, reported similar peculiarities while others tried to fit the same observations into a "classical" pattern by erroneously assuming that what was stained by hematoxylin was a densely packed cluster of chromosomes (e.g. Callen, 1940).

These early studies are discussed fully by Robinow (1957) who also described his own light microscopical observations on two species of Mucor including Mucor hiemalis, the species that I have examined with electron microscopy.
Although Robinow, using the Fuelgen technique and Giemsa staining after hydrolysis, was able to identify the peripheral nuclear region around the nucleolus as the site of DNA, he was not able to see individual chromosomes or to learn much more about the division process than Léger had done. Both of them concluded that division was "direct" by elongation and constriction without the help of a spindle apparatus.

Robinow's observation in 1957 that the chromatin of Mucor hiemalis appears homogeneous at all times during division still stands uncorrected although at the time he did not realize that this is not generally the case in other fungi - for example, in Lipomyces lipofer (Robinow, 1961), Allomyces arbuscula (Robinow, 1962), Schizosaccharomyces versatilis (Robinow & Bakerspigel, 1965), Aspergillus nidulans (Robinow & Caten, 1969), and Schizosaccharomyces pombe (McCully & Robinow, 1971). Using similar techniques of fixation and staining as were used in Mucor hiemalis, the chromatin-containing region of the nucleus in these fungi has now been shown to consist of several chromatinic rodlets which are arranged in unusual "non-classical" configurations at division. By contrast, the whole chromatin-containing region of the nucleus in Mucor seems to simply pull apart and become equally divided between the daughter nuclei with only a slight increase in the staining density quite late in the pulling-out stage and with no trace of individual chromosomes. These do, undoubtedly, exist but in a closely packed thread-like form too small to be resolved in stained preparations.
examined with the light microscope or, as I have recently found, in sectioned nuclei examined with electron microscopy.

The conclusion that no spindle apparatus is involved in the mitosis of *Mucor hiemalis* must be revised in the light of observations with electron microscopy that are reported here. A narrow bundle of microtubules is present in dividing nuclei although how this functions to separate daughter sets of chromosomes is not clear.

It has also been established that no centrioles are present in *Mucor hiemalis*. This is in contrast to the zoospore-producing phycomycete *Saprolegnia ferax* which has a *Mucor*-like "direct" form of division by elongation and constriction (Bakersspigel, 1960), but has centrioles associated with the ends of the mitotic spindle (Heath & Greenwood, 1968, 1970).

Girbardt, (1971) has recently reported that he found no centrioles in several phycomycetes which do not produce zoospores, including a species of *Mucor*. In these fungi he also did not find any other spindle-associated structures analogous to the microtubule organizing centres (he referred to them as "kinetochore equivalents") which he and others have found in a large number of ascomycetes and basidiomycetes. I have found that microtubule organizing centres are present in *Mucor hiemalis*. These structures are of particular interest because their major component is located inside the nucleus rather than outside as it is in ascomycetes and basidiomycetes.
MATERIALS AND METHODS

Materials

*Mucor hiemalis* strain NRRL B-1555(minus) was obtained from the collection of the U.S. Agricultural Research Service at Peoria Illinois. The mould was maintained and cultured on a medium consisting of Difco yeast extract 0.5 g, glucose 2.0 g, and agar 1.5 g per 100 ml of water.

Spores were harvested from a well grown mycelium (7 days at room temperature) by flooding it with 10 ml of yeast extract – glucose broth containing 1 drop of Tween 80 (to act as a wetting agent) and scraping the surface with a glass rod.

The spore suspension was then evenly spread over the surface of yeast extract-glucose agar plates. Excess inoculum was removed with a pipette and with pieces of blotting paper. The plates were incubated until the spores had developed germ tubes that were at least 2-3 times as long as the original diameter of the spore (incubation for approximately 12 hours at room temperature). Shorter germ tubes could not be satisfactorily fixed for electron microscopy. Good preservation could be achieved in longer germ tubes but they are less well suited for the study of mitosis because they contain more vacuoles and a lower concentration of nuclei.
Preparation for Electron Microscopy

Germinated sporangiospores growing on the surface of agar plates were flooded with 3% glutaraldehyde in 0.1 M cacodylate buffer pH 7.2 at room temperature. The germinated spores were immediately scraped off the agar, centrifuged into a pellet, and resuspended in fresh fixative for 13 hours. The glutaraldehyde was washed away with 10-15 changes of cacodylate buffer. Postfixation was in 1.33% osmium tetroxide dissolved in collidine buffer (.06M at pH 7.4) for 6 hours at room temperature. The material was then washed twice in collidine buffer and twice in distilled water before being stained in a 0.5% aqueous solution of uranyl acetate for 2.5 hours.

Details of incorporation into agar blocks, dehydration, embedding, and staining of thin sections are the same as described previously for S. pombe in Chapter II.

OBSERVATIONS

Germinating sporangiospores of Mucor hiemalis contain many nuclei in a common cytoplasm (Fig. 83). Mitosis in these nuclei is asynchronous.

The shapes of nuclei and the relative positions of the nucleolus and the chromatin-containing region as seen by electron microscopy are the same at both interphase and during mitosis as previously described by Robinow (1957, 1962) on the basis of observations made with light microscopy.
When viewed with electron microscopy, the nucleolus consists of ribosome-like particles dispersed among amorphous electron-dense material and the chromatin-containing region is of uniformly low electron density showing nothing recognizable as chromosomes even during division.

The contours of interphase nuclei are very irregular because in living nuclei the chromatin-containing region is constantly flowing into different shapes around the central spherical nucleolus. Rounded, oval or teardrop shapes are most common but the nuclei may also be angular or drawn out into narrow tails (Figs. 83, 85). This is in marked contrast to the smooth contours of interphase nuclei which were seen in the four other fungal species previously described in this thesis.

Nuclei divide by elongation and constriction and the nucleolus is divided at the same time and in the same way, half of it passing to each daughter nucleus. During the initial stages of elongation, the nuclei often assume very irregular shapes (Fig. 89). Ultimately, the elongating nuclei achieve a symmetrical, more or less rectangular shape (Figs. 91, 92, 95, 96). After further elongation (Figs. 98, 99), the nuclei finally constrict in their central regions and become dumbbell shaped (Fig. 100). Separation of daughter nuclei probably involves breakdown of the channel between daughter ends of the dumbbell.

In dividing nuclei, electron microscopy has revealed the presence
of a mitotic spindle composed of a narrow bundle of parallel micro-
tubules. Short spindles were observed in one corner of nuclei which
were not yet markedly elongated (Fig. 86). Short spindles were also
seen in one corner of nuclei which were already considerably longer than
interphase nuclei (Figs. 87, 88). By the time the nucleus has elongated
into a symmetrical rectangular shape, the spindle occupies the central
longitudinal axis of the nucleus and it runs through the elongated
nucleolus (Figs. 91, 92, 95, 96). Stages in the elongation and
constriction of the nucleus and the simultaneous elongation of the mitotic
spindle are summarized diagrammatically in Fig. 81.

Associated with the ends of the spindle are regions which I have
called "microtubule organizing centres" (MTOC's), following the
terminology of Pickett-Heaps (1969) who defined MTOC's as accumulations
of amorphous electron-dense material associated with the ends of
microtubules. Microtubule organizing centres are also present in inter-
phase nuclei. Neither interphase nor dividing nuclei are ever
associated with centrioles.

The morphology of the microtubule organizing centre in *Mucor
hiemalis* is difficult to describe with precision because it appears
different in different nuclei and these differences do not seem to be
related to particular stages in the division cycle. The appearance
of the interphase MTOC seems to be fairly constant (Figs. 84, 85). At
interphase the MTOC appears to be simply a small mass of amorphous
 electron-dense material which is approximately 150-200 nm in diameter at its widest point. The major portion of this material lies inside the nucleus in contact with the inner membrane of the nuclear envelope but there is also a small portion of it which is located outside the nucleus in contact with the outer membrane of the nuclear envelope. During division, the MTOC usually has much the same size and appearance that it does in interphase except that spindle microtubules emerge from the amorphous electron-dense material and extend into the nucleus (Figs. 93, 94, 99). Occasionally however, the microtubules appear to be in contact with a clearly defined bar-shaped structure which lies just underneath the inner membrane of the nuclear envelope (Figs. 97, 101). This bar is surrounded by a variable amount of amorphous electron-dense material. In one instance, as shown in Fig. 90, I have observed a distinct connection between the inner membrane of the nuclear envelope and the bar-shaped component of the MTOC. The attachment point on the nuclear envelope seems to be closely associated with a nuclear pore. Apparent differences in the morphology of the MTOC may be due to differences in the angle of sectioning or to differences in the degree to which amorphous electron-dense material obscures other more structured components of the organelle. Three diagrammatic interpretations of the morphology of the MTOC based on electron micrographs are shown in Fig. 82. It is my view that the one in Fig. 82C is likely to be the most accurate one.
DISCUSSION

Consideration of Earlier Observations on Mucor Hiemalis Made With Light Microscopy

Robinow (1957) reasoned that the "direct" form of division that he found in Mucor could only yield equal daughter genomes if "the chromosomes have divided and sister chromatids have segregated to opposite poles before the constriction of the nucleus". He proposed that this might be achieved by "endomitosis", a process by which chromosomes divide and separate inside a nucleus without the aid of a spindle.

Now, with the finding of a spindle, there is no need to invoke "endomitosis" to explain the unusual division process in Mucor. However, since tiny spindles are found in nuclei which are not yet markedly elongated, it would seem that Robinow was at least partially correct — sister chromatids may well be segregated to opposite poles of the tiny spindle early in the division process before constriction of the nucleus.

It might be expected that some evidence of this early segregation of chromosomes should be visible with light microscopy in suitably stained preparations — i.e. two masses of chromatin should be seen on one side of the nucleolus before the nucleus starts to elongate. This is not the case however, because in Mucor at division the
chromosomes never become markedly coiled and shortened as they do in plant and animal cells and in many species of fungi. The long thread-like chromosomes of Mucor are undoubtedly able to extend in all directions around the nucleolus to form a symmetrical chromatin shell even although they are attached to the spindle.

How are the Chromosomes of Mucor Hiemalis Attached to the Spindle?

In S. pombe where the chromosomes are visible as short rodlets in aceto orcein stained preparations, the appearance of two clusters of chromosomes very early in the division process before the nucleus becomes markedly elongated suggested to McCully & Robinow (1971) that the chromosomes are directly attached to the ends of the spindle. It was proposed therefore, that the microtubule organizing centres in S. pombe were "kinetochore equivalents" (see Chapter II for a discussion of this hypothesis). It is my view that the chromosomes in Mucor are also directly attached to the ends of the spindle and that the microtubule organizing centres also serve as "kinetochore equivalents". This conclusion is based on the fact that the number of microtubules in the spindle of Mucor hiemalis appears to be even smaller than in the spindle of S. pombe. One would expect a larger number of microtubules if the spindle consisted of both chromosomal microtubules and pole-to-pole microtubules. Nevertheless, instead of "kinetochore equivalent", I have chosen the more general term "microtubule organizing centre" for the electron-dense organelles at the poles of the spindle.
in *Mucor* because the evidence that the chromosomes are directly attached to them is not as convincing as it is in *S. pombe*.

Heath & Greenwood (1968, 1970) have reported that the spindle of *Saprolegnia ferax* consists of both pole-to-pole and chromosomal microtubules which end in small electron-dense bars which they interpret as kinetochores (the chromosomes themselves are, as in *M. hiemalis*, not visible). Since *S. ferax* has a *Mucor*-like "direct" form of division, Heath & Greenwood's observations might suggest that the spindle of *M. hiemalis* also consists of both pole-to-pole and chromosomal microtubules. However, I have rejected this possibility on the grounds that the mitotic spindles in these two organisms do not seem to be comparable. The spindle of *S. ferax* is more fusiform-shaped and contains considerably more microtubules than the spindle of *M. hiemalis*. Moreover, microtubules ending in small electron-dense bars were not seen in *M. hiemalis*.

**Proposed Mechanism of Chromosome Separation and Nuclear Elongation**

In *S. pombe* it was proposed that the spindle is not generating the force which causes elongation of the nucleus during division (see Discussion of Chapter II). However, in *S. pombe*, the nucleus does not become longer than it is in interphase until the ends of the elongating spindle have reached the ends of the nucleus. In *M. hiemalis*, evidence that the spindle does not generate the force which causes
elongation of the nucleus is even more conclusive than it is in
*S. pombe*. The nucleus of *M. hiemale* is often markedly elongated
when the spindle is still quite small.

In *Saprolegnia ferax*, the elongation of the spindle also does
not keep in step with the elongation of the nucleus. Therefore Heath
& Greenwood (1970) proposed that two different force-generating
mechanisms must be involved during division - one which separates the
chromatin and another which causes elongation of the nucleus. They
suggested that the spindle generates the force to separate the
chromatin and that the nucleus elongates by actively "crawling" along
cytoplasmic microtubules which they observed in close association with
the nuclear envelope of elongating nuclei. Such cytoplasmic microtubules
were not observed in *M. hiemale*.

Alternatively, I would propose that one mechanism - a rapid
increase in the rate of nuclear envelope growth at division could
account for both chromosome separation and nuclear elongation. The
spindle in *M. hiemale* as I see it, serves the same purpose as I have
previously suggested that it does in *S. pombe* -- it simply serves to
keep the daughter sets of chromosomes rigidly apart after they have
become separated by growth of the nuclear envelope.

**Centrioles and Microtubule Organizing Centres in Phycomycetes**

The absence of centrioles in *Mucor hiemale*, and the presence
of centrioles in *Saprolegnia ferax* and several other zoospore-producing
phycomycetes (see Pages 8 & 10 for references), would seem to support Pickett-Heaps (1969) in his view that centrioles are present at the poles of mitotic spindles merely to ensure their distribution to daughter cells which require them as basal bodies of flagella.

Like Pickett-Heaps, Heath & Greenwood (1970) believe that the centrioles in *S. ferax* may not play a major role in the formation of microtubules. They propose that subunits of spindle microtubules, appearing as diffuse concentrations of electron-dense material are formed by specialized regions ("polar pockets") of the nuclear envelope and accumulate inside the nucleus at the base of these pockets. Similar concentrations of electron-dense material inside the nucleus at the poles of the mitotic spindle of *Catenaria anguillae* were noted by Ichida & Fuller (1968) who, however, did not comment on their possible role in spindle formation. Pickett-Heaps (1972) believes this electron-dense material to be a form of microtubule organizing centre typical of aquatic phycomycetes.

In view of these findings, it is not too surprising that a major component of the spindle-associated structure in *Mucor hiemalis* which I interpret as a microtubule organizing centre is also located inside the nucleus. Further studies on other terrestrial phycomycetes are needed to determine if the type of microtubule organizing centre found in *M. hiemalis* is of general occurrence in these organisms. Girbardt's failure to discover "kinetochore equivalents" in five species of terrestrial phycomycetes (Girbardt, 1971) may, perhaps, be
due to his having looked for these structures outside the nuclear envelope.
Fig. 81  Diagrammatic interpretation of mitotic events in *Mucor hiemalis*.

A. - Interphase.

B. - Formation of a tiny spindle in a nucleus with interphase dimensions.

C. - G.Stages in the elongation and constriction of the nucleus and the elongation of the spindle. Notice that initially, the nucleus becomes elongated although the spindle is still quite short. Later however, when the spindle occupies the longitudinal axis of the nucleus, the spindle elongates in step with the nucleus.

H. - Separation of daughter nuclei.
Fig. 82 Three interpretations of the morphology of the microtubule organizing centre (MTOC) in dividing nuclei of *Mucor hiemalis* based on electron micrographs.

A. - (based on 15 observations e.g. Fig. 93)
suggests that the MTOC consists of an amorphous mass of electron-dense material.

B. - (based on 4 observations e.g. Fig. 97) suggests that the MTOC consists of both amorphous electron-dense material and a bar-shaped component.

C. - (based on 1 observation illustrated in Fig. 90) suggests that the MTOC consists of amorphous electron-dense material and a bar-shaped component which is suspended from the inner membrane of the nuclear envelope near a nuclear pore.
Fig. 83  Low power view of a recently germinated sporangiospore of *Mucor hiemalis*. All of my observations on mitosis in *M. hiemalis* were made in germinated spores at a similar stage of development. Arrows point to several nuclei, all of which appear to be in interphase. Note their irregular shapes. X 4,900.
Figs. 84 & 85  Two interphase nuclei of *Mucor hiemalis*. Each nucleus consists of a central nucleolus (N) and a peripheral chromatin-containing region (Chr).

In Fig. 85, the relative sizes of the nucleolus and the chromatin-containing region are consistent with their appearance in the light microscope. The irregular shape of the nucleus in Fig. 85 is also typical of interphase nuclei.

The nucleus in Fig. 84 has undoubtedly been sectioned through a peripheral region so that the nucleolus appears much smaller than it really is.

Both nuclei have a prominent microtubule organizing centre (MTOC). Notice that the major part of this region is located on the inside of the nuclear envelope.

Both Figs. 84 & 85  X 62,700.
Fig. 86  *Mucor hiemalis* - an early stage of spindle formation. Notice the small spindle (Sp between the arrows) which runs through one corner of the chromatin-containing region (Chr). This nucleus appears to be larger than the interphase nuclei in Figs. 83 & 84 but the rounded shape of its nucleolus (N) would seem to indicate that no elongation has yet taken place.  X 62,700.
Figs. 87 & 88  *Mucor hiemalis*. Two sections of the same elongated nucleus which contains a relatively short peripherally-located spindle.

Both the nucleolus (N) and the chromatin-containing region (Chr) are elongated.

The spindle (between the large arrows) is sectioned obliquely and is admittedly, not clearly visible. However, the microtubule organizing centre (MTOC) and a few microtubules can be seen on the left side of the spindle in Fig. 87 and the other MTOC can be seen on the right side of the spindle in Fig. 88.

Both Figs. 87 & 88  X 35,900.
Fig. 89  *Mucor hiemalis.* An elongated nucleus which is typically irregular in shape. It contains a peripherally located spindle (*Sp* - between arrows) which is longer than the one in Figs. 87 & 88.

The morphology of the microtubule organizing centre which is visible near the top end of the spindle is of special interest and is shown at higher magnification in Fig. 90.  X 43,000.

Fig. 90  The microtubule organizing centre (*MTOC*) in this micrograph appears to consist of a bar-shaped structure which is suspended from the nuclear envelope (*NE*) by a membrane which has the same size as the two membranes which make up the nuclear envelope. Notice that one of the spindle microtubules (*Sp*) seems to be attached to the bar-shaped MTOC.

The asterisk is placed in a region where the nuclear envelope is discontinuous. This is interpreted as a nuclear pore.

X 112,800.
Figs 91 & 92  **Mucor hiemalis** - Two sections of a nucleus which has elongated into a symmetrical, nearly rectangular shape and in which the spindle (Sp) occupies the longitudinal axis. The relative positions of the nucleolus (N) and the chromatin-containing region (Chr) in Fig. 91 are closely similar to how they appear in the light microscope at this stage.

The spindle is most clearly visible in Fig. 92. Notice that it is a narrow bundle of nearly parallel microtubules which runs through the nucleolus.

The microtubule organizing centre (MTOC) at one end of the spindle (top end) is visible in Fig. 92 and at the other end (near the bottom of the micrograph) in Fig. 91. These MTOC's are shown at higher magnification in Figs. 93 & 94.

Both Figs. 91 & 92  X 48,700.
Figs. 93 & 94  Mucor hiemalis - Details of the microtubule organizing centres that are shown at low magnification at opposite ends of the spindle in Figs. 91 & 92. These MTOC's seem to consist of only amorphous electron-dense material which is chiefly located on the inside of the nuclear envelope. The dense rod-shaped structure that is seen in association with the MTOC in Fig. 93 (small arrow) is a particle of uranyl precipitate.

Notice that the spindle microtubules (Sp) seem to emerge directly from the amorphous material of the MTOC's. This is most clearly visible in Fig. 93.

Both Figs. 93 & 94  X 83,800.
Figs 95 & 96  *Mucor hiemalis* - Two sections of a nucleus which is elongated into a more or less rectangular shape like the nucleus in Figs. 91 & 92. The nucleolus (N) is stretched out within the elongated chromatin-containing region (Chr) and the spindle occupies the longitudinal axis. The microtubule organizing centre (MTOC) is visible at the top end of the spindle in Fig. 96. This MTOC is shown at high magnification in Fig. 97.

Both Figs. 95 & 96  X 53,000.

Fig. 97  Details of the MTOC which is visible at the top end of the spindle in Fig. 96. Notice that in addition to amorphous electron-dense material, the organelle consists of a bar-shaped structure lying just inside the nuclear envelope (NE).  X 105,800.
Figs. 98 & 99  *Mucor hiemalis* - Two sections of a nucleus in which the nucleolus (N) is stretched out within the elongated chromatin-containing region (Chr) and the spindle (Sp) occupies the longitudinal axis. This nucleus is at a fairly late stage of elongation and it is starting to constrict in its central region.

Notice the microtubule organizing centre (MTOC) at the top end of the spindle in Fig. 99.

Both Figs. 98 & 99  X 45,000.
Fig. 100  **Mucor hiemalis** - A late stage in mitosis in which the nucleus has elongated into a dumbbell shape. The arrows mark the position of the narrow channel between the rounded daughter ends of the dumbbell. Because the nucleus is sectioned obliquely, only the chromatin-containing region (Chr) is visible in the daughter end on the right side of the micrograph. An adjacent section of this daughter end is shown in Fig. 101.  X 28,200.

Fig. 101  **Mucor hiemalis** - An adjacent section of one end of the dumbbell-shaped nucleus shown in Fig. 100. Notice the bar-shaped microtubule organizing centre (MTOC) and the spindle microtubules (Sp) which run through the nucleolus.  X 77,000.
CHAPTER VI

SUMMARY AND FINAL DISCUSSION

This thesis has dealt with mitosis in five species of fungi.

In *Mucor hiemalis*, a terrestrial phycomycete, mitosis takes place within an intact nuclear envelope and the nucleolus persists during division. The chief component of the microtubule organizing centre is located inside the nuclear envelope. The narrowness of the mitotic spindle in *M. hiemalis* has led me to propose that the chromosomes may be directly attached to the ends of the spindle rather than to chromosomal microtubules as they are in plant and animal cells and many fungi e.g. *Catenaria anguillalae* (Ichida & Fuller, 1968).

In *Schizosaccharomyces pombe*, an ascomycetous yeast, mitosis also takes place within an intact nuclear envelope and the nucleolus persists during division. The microtubule organizing centre is located outside the nucleus in contact with the outer membrane of the nuclear envelope. In *S. pombe* the evidence that the chromosomes are directly attached to the ends of the spindle (and by implication, to the microtubule organizing centres) is somewhat more conclusive than it is in *M. hiemalis*, and for this reason the microtubule organizing centres in *S. pombe* are referred to as "kinetochore equivalents".
Apart from differences in the location of their microtubule organizing centres, the most obvious difference between mitosis in *M. hiemalis* and in *S. pombe* is that in *M. hiemalis* a considerable elongation of the nucleus takes place while the spindle is still quite short, whereas in *S. pombe* the nucleus retains its interphase dimensions until the poles of the elongating spindle have reached the ends of the nucleus and thereafter, spindle elongation keeps in step with the elongation of the nucleus.

In *Leucosporidium scottii*, *Aessosporon salmonicolor* and *Rhodosporidium* sp., three heterobasidiomycetous yeasts, mitotic events are quite different from what has been observed in *M. hiemalis* and *S. pombe* but they closely resemble what has been seen by others in dividing nuclei of mycelial basidiomycetes e.g. *Polystictus versicolor* (Girbardt, 1961, 1968). Mitosis takes place within a partially broken nuclear envelope. The nucleolus disintegrates in the cytoplasm of the mother cell at the same time as all of the chromatin moves into the bud and divides there. After division, one daughter nucleus moves back to the mother cell and a new nucleolus appears in each of the daughter nuclei. The microtubule organizing centre is located outside the nucleus and in my material it does not seem to be attached to the nuclear envelope. In contrast to the narrow spindles that were seen in *M. hiemalis* and *S. pombe*, the shape of the spindle in these three heterobasidiomycetous yeasts is typically that of a double cone and it consists of both pole-to-pole and chromosomal microtubules. In these yeasts, the chromosomes at mitosis are visible in the electron microscope.
as diffuse accumulations of electron-dense material. Such accumulations were never detectable in *M. hiemalis* and *S. pombe*. This difference has been attributed to the fact that the chromosomes of *M. hiemalis* and *S. pombe* (especially of the former) do not become markedly coiled and shortened before mitosis as they do in *L. scottii*, *A. salmonicolor*, and *Rhodosporidium sp.* (as evidenced by their appearance in the light microscope after staining with aceto orcein) and are therefore not apparent in sections which are thin enough to be viewed with electron microscopy.

On the basis of striking differences between mitosis in the ascomycetous yeast *S. pombe* and mitosis in the three heterobasidiomycetous yeasts that I have studied, I have concluded that mitotic events could be useful taxonomic indicators especially in the large group of asporogenous yeasts whose taxonomic affinities are unknown at present. The phylogenetic significance of mitosis has been discussed by Leedale (1970) for members of the algae and by Pickett-Heaps (1972) for both algae and fungi. There is definitely a correlation between mitotic events and the taxonomic groups to which these organisms belong. However, as Leedale (1970) has cautioned, some mitotic events such as the breakdown of the nuclear envelope are not necessarily restricted to a particular group since the myxamoebal and the plasmodial stages of the slime mould *Physarum flavicomum* have an "open" and a "closed" type of mitosis respectively (Aldrich, 1969).

Apart from their contribution to our understanding of phylogenetic
relationships, studies of unusual "non-classical" forms of mitosis such as the ones reported here may, as was suggested in Chapter I, provide clues about the evolutionary development of "classical" mitosis and about the fundamental mechanisms of "classical" mitosis. By way of conclusion, the validity of these two suggestions will be briefly discussed in the light of my observations.

Possible Clues About the Evolution of "Classical" Mitosis That can be Derived From Studies of Unusual Forms of Mitosis

Although his evidence is fragmentary, Pickett-Heaps (1969, 1972) has argued that the complex sequence of mitotic events in plant and animal cells has evolved in a stepwise fashion from a bacteria-like type of mitosis and that "relics" of primitive ultrastructure may be found preserved and functioning in some of the algae, protozoa, and fungi. In bacteria, genome separation appears to be accomplished by growth of the cell membrane between the two membrane-attached regions of the duplicated bacterial chromosome (Ryter, 1968). Pickett-Heaps believes that membrane growth may have remained important in the mitosis of primitive eukaryotic cells after the evolution of genomes consisting of more than one chromosome and other typically eukaryotic features such as microtubules and a membrane-enclosed nucleus. Such a mitosis may have resembled the mitosis which is still found in the dinoflagellate Gyrodinium cohnii in which sister chromatids appear to be separated by the growth of the nuclear envelope to which they are
attached while microtubules are found in cytoplasmic channels which traverse the nucleus (Kubai & Ris, 1969). Pickett-Heaps proposes that the disassociation of the chromosomes from the nuclear envelope and their attachment to the spindle by chromosomal microtubules is a relatively recent evolutionary development. He also points out that there seems to be an evolutionary tendency from the "closed" mitosis as we see it in many primitive eukaryotic organisms towards the "open" mitosis that occurs in plant and animal cells. The sequence of evolutionary events as postulated by Pickett-Heaps is summarized in Table IV.

My observations on mitosis in five fungi seem to fit into this scheme. The type of mitosis found in *Mucor hiemalis* and in *Schizosaccharomyces pombe* seems to fit into category two. Evidence for the direct attachment of the chromosomes to the ends of the spindle in *S. pombe* based on my electron micrographs and on Dr. Robinow's light micrographs is, in my view, even more convincing than it was in *Saccharomyces cerevisiae*.

In agreement with the generally accepted idea that basidiomycetes are at a higher evolutionary level than other fungi (Savile, 1968), the type of mitosis found in the three heterobasidiomycetous yeasts described in this thesis seems to fit into category four.

The idea that the nucleolus tends to persist in primitive organisms and to disperse in more highly evolved organisms (Pickett-Heaps,


<table>
<thead>
<tr>
<th>Sequence of Evolutionary Steps (from Primitive to highly evolved)</th>
<th>Characteristic Features of the Mitotic Variations</th>
<th>Examples of Organisms in which the Different Types of Mitosis are Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. The spindle is outside the nuclear envelope which remains intact. Chromosomes are attached to the nuclear envelope and are moved apart by membrane growth.</td>
<td>Gyrodinium cohnii (Kubai &amp; Ris, 1969)</td>
<td></td>
</tr>
<tr>
<td>II. The spindle is inside the nuclear envelope which remains intact. Chromosomes are attached to the nuclear envelope and are moved apart by membrane growth.</td>
<td>Saccharomyces cerevisiae Pickett-Heaps' (1969) interpretation of the observations of Robinow &amp; Marak (1966)</td>
<td></td>
</tr>
<tr>
<td>III. The spindle is inside the nuclear envelope which remains intact. The spindle consists of both pole-to-pole and chromosomal microtubules.</td>
<td>micronuclei of ciliated protozoa e.g. Blepharisma (Jenkins, 1967) Catenaria anguillulae (Ichida &amp; Fuller, 1968)</td>
<td></td>
</tr>
<tr>
<td>IV. The spindle consists of both pole-to-pole and chromosomal microtubules. The nuclear envelope is partially broken down. (In different organisms this ranges from the formation of polar fenestrae to a more complete breakdown with the persistence of large membrane fragments).</td>
<td>Chlamydomonas reinhardi (Johnson &amp; Porter, 1968) Spirogyra (Fowke &amp; Pickett-Heaps 1969) Coprinus lagopus (Lu, 1967)</td>
<td></td>
</tr>
<tr>
<td>V. The spindle consists of both pole-to-pole and chromosomal microtubules. The nuclear envelope is completely broken down.</td>
<td>most higher plant cells and animal cells.</td>
<td></td>
</tr>
</tbody>
</table>
1970), is borne out by my observations that during division the nucleolus persists in the phycomycete *M. hiemalis* and the ascomycete *S. pombe* but disintegrates in the three heterobasidiomycetous yeasts.

Pickett-Heaps (1972) has also suggested that there is an evolutionary tendency for microtubule organizing centres to move from the inside of the nuclear envelope to the outside of the nuclear envelope and later to become detached from the envelope. Following the generally accepted order of evolutionary development in fungi i.e. phycomycete, ascomycete, basidiomycete (Savile, 1968), the position of the microtubule organizing centre in *M. hiemalis*, *S. pombe*, and the three heterobasidiomycetous yeasts is respectively: just inside the nuclear envelope; outside the nuclear envelope and attached to it; and outside the nuclear envelope but lying free in the cytoplasm. Other variations on the location of the microtubule organizing centre known from the observations of others are as in *Saccharomyces cerevisiae* (Robinow & Marak, 1966) inside a pore of the nuclear envelope and, as in *Physarum polycephalum* (Sakai & Shigenaga, 1972) inside the nucleus a considerable distance away from the nuclear envelope.

These ideas about the evolution of the mitotic apparatus are, at present, only interesting speculations but my results do seem to fit the predictions of Pickett-Heaps. In future, as more and more unusual forms of mitosis are studied, it may be possible to make more definite conclusions about the evolution of "classical" mitosis.
Possible Clues About the Fundamental Mechanisms of "Classical" Mitosis That can be Derived from Studies of Unusual Forms of Mitosis

As was pointed out in Chapter I, by studying only "classical" mitosis there is a danger of falsely attributing particular origins, and from constant association, particular functions to various components of the mitotic apparatus.

The fact that some eukaryotic protists have centrioles associated with the spindle and others do not have centrioles seems to cast doubt on the role of the centriole in spindle formation (see Pickett-Heaps, 1969, 1971; and the Discussion Section of Chapter II). My observation that the terrestrial phycomycete M. hiemalis has no centrioles associated with the mitotic spindle even although aquatic phycomycetes do have centrioles, supports the hypothesis that centrioles are only present in organisms that have the potential for producing flagellated cells.

Speculations on the role of centrioles derived from the study of mitosis in eukaryotic protists lend support to the conclusions of Dietz (1966) which were based on his experiments in crane fly spermatocytes. He found that a normal spindle could still be formed after he had prevented the separation of the centrioles to opposite poles by flattening the cells and he concluded that in these cells at least, centrioles are not necessary for spindle formation.

It is my view that something can also be learned about the
function of microtubules in the mitotic spindles of plant and animal
cells from the study of their role in the mitotic spindles of
eukaryotic protists such as *Schizosaccharomyces pombe*.

Since microtubules are present in every dividing eukaryotic
cell so far examined and since mitosis is disrupted by agents which disrupt
spindle microtubules, there is no doubt that microtubules are of major
importance in normal chromosome movement.

Many theories (e.g. Dietz, 1969) have proposed that the motive
force for chromosome movement is provided by the microtubules as they
change in length at different stages of the mitotic sequence. The
chromosomes do seem to be pulled along by the spindle tubules when a
living, dividing plant or animal cell is observed with polarizing
microscopy, since the kinetochore region of the chromosome which is
attached to the microtubules leads the way to the poles while the
chromosome arms trail behind. However, this observation is also
consistent with the possibility that spindle microtubules are passive
track-like structures which direct movement which is caused by some
other force. This second idea has not received the same general
acceptance as those theories which assume that the microtubules play
an active role in chromosome movement. However, the work of Forer (1966)
suggests that the microtubules are indeed not the force generating
elements of the mitotic apparatus and it is my view that this
conclusion can also be derived from the study of mitosis in some
eukaryotic protists.
On the basis of his studies in living cells irradiated with a UV microbeam and observed with polarizing microscopy, Forer (1966) has concluded that the mitotic apparatus consists of force generating elements which are distinct from the microtubules. He observed that UV microbeam irradiation of kinetochore fibres at anaphase sometimes affected chromosome movement without altering the birefringence (a feature known to be closely correlated with the presence of microtubules—see Luykx, 1970, pages 96 & 97 for references). He found that the effectiveness of irradiation in causing changes in birefringence appeared to vary with the wavelengths he used while the effectiveness of irradiation in causing changes in chromosome movement did not vary with the wavelength.

More recently, Forer & Goldman (1972) have rejected theories such as that of Dietz (1969) (which is based on the assumption that functional spindles are composed mainly of one protein) since they have shown that the isolated mitotic apparatus which does consist mainly of one protein contains only a small percentage of the mitotic apparatus material found in vivo. Forer & Goldman (1972) propose that chromosome movement along the spindle is due to forces generated by interactions between actin-like and myosin-like proteins. Actin has already been detected in meiotic spindles (Behnke, Forer & Emmersen, 1971).

I have proposed that the spindle microtubules in *S. pombe* and *M. hiemalis* perform a relatively passive supporting function while the motive force for chromosome movement is provided by the growth of the
nuclear envelope. This view is, admittedly, open to question but in the dinoflagellate *Gyrodinium cohnii* where the microtubules can not possibly engage the chromosomes because they are located outside the nucleus, the chromosomes are clearly moved by growth of the nuclear envelope while the microtubules only direct and support this movement (Kubai & Ris, 1969).

Force exerted by growth of the nuclear envelope is undoubtedly a primitive feature which has been abandoned by more highly evolved organisms in favour of other more efficient force generating mechanisms—possibly interactions between actin-like and myosin-like proteins as proposed by Forer & Goldman (1972). It seems to me very unlikely that if microtubules perform a relatively passive track-like function in primitive eukaryotic organisms, the same structures could have become active force generating elements in more highly evolved cells. Moreover, it is known that in many animal and plant cells the microtubules in the cytoplasm appear to act as passive tracks along which a directed cytoplasmic streaming takes place. An interesting example of this is the streaming of pigment granules along rows of cytoplasmic microtubules in melanophores of the fish *Fundulus heteroclitus* (Bikle, Tilney & Porter, 1966). It is therefore not unreasonable to suspect that microtubules in the nucleus also act as passive tracks.

Many questions about the mechanisms of "classical" mitosis remain unanswered. Some clues about the function of centrioles and microtubules are already obtainable from studies of unusual forms of
mitosis in eukaryotic protists. Future studies of mitosis in these organisms may well shed more light on these and other components of the "classical" mitotic apparatus.
BIBLIOGRAPHY


Colson, B. (1935). The cytology of the mushroom *Psalliota campestris*  

and intracytoplasmic structures of *Rhodotorula glutinis* as  
revealed by electron micrographs of serial sections. Expl.  


Crackower, S. H. B. (1972). The effects of griseofulvin on mitosis  

Day, A. W. & Jones, J. K. (1972). Somatic nuclear division in the  
sporidia of *Ustilago violacea* I. Acetic orcein staining. Can.  
J. Microbiol. **18**: 663-670.

Dietz, R. (1966). The dispensability of the centrioles in the  
spermatocyte divisions of *Pales ferruginea* (Nematocera). In  
*Chromosomes Today* vol. I (eds. C. D. Darlington & K. R. Lewis)  

**56**: 237-248.

Arch. Mikrobiol. **32**: 65-80.


I. Micronuclear mitosis in Blepharisma. J. Cell Biol. 34:
463-481.

in Chlamydomonas reinhardi: Basal bodies and microtubules.

Jokelainen, P. T. (1967). The ultrastructure and spatial organization
of the metaphase kinetochore in mitotic rat cells. J.
Ultrastruct. Res. 19: 19-44.

of high osmolality for use in electron microscopy. J. Cell


Poitiers: E. Druinaud, Libraire-Éditeur.

Lerbs, V. (1971). Licht- und elektronenmikroskopische Untersuchungen
an meiotischen Basidien von Coprinus radiatus (Bolt) Fr. Arch.
Mikrobiol. 77: 308-330.


   Chromosoma 26: 427-448.


   44: 454-458.

   Mycologia 59: 370-375.

   Mycologia 61: 873-886.


   J. Electron microsc. 18: 47-56.

   Protoplasma 70: 217-224.


