NUCLEAR DIVISION IN THE FUNGI

edited by

I. Brent Heath

NUCLEAR DIVISION IN THE FUNGI

Edited by
I. Brent Heath
Biology Department
York University
Toronto, Ontario
Canada

1978

ACADEMIC PRESS New York San Francisco London
A Subsidiary of Harcourt Brace Jovanovich, Publishers

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
24/28 Oval Road, London NW1 7DX

Library of Congress Cataloging in Publication Data

Mitosis Symposium, Tampa, Fla., 1977.
Nuclear division in the fungi.

Consists of expanded versions of papers presented in the Mitosis Symposium at the 2d International Mycological Congress, Tampa, Fla., Aug. 27-Oct. 3, 1977.
Includes bibliographies and indexes.
1. Fungi—Cytology—Congresses. 2. Mitosis—Congresses. I. Heath, Ian Brent. II. International Mycological Congress, 2d, Tampa, Fla., 1977.
III. Title.
QK601.M57 1977 589'.2'0487623 78-7437
ISBN 0-12-335950-3

PRINTED IN THE UNITED STATES OF AMERICA

LIST OF CONTRIBUTORS

Arthur Forer
Biology Department
York University
Toronto, Ontario, Canada

Manfred Girbardt
Akademie der Wissenschaften der DDR
Zentralinstitut für Mikrobiologie und Experimentelle Therapie
Jena, German Democratic Republic

I. Brent Heath
Biology Department
York University
Toronto, Ontario, Canada

Donna F. Kubai
Department of Zoology
Duke University
Durham, North Carolina

PREFACE

The contributions to this book are expanded versions of papers presented in the Mitosis Symposium at the Second International Mycological Congress held in Tampa, Florida, August 27–October 3, 1977. The objectives that stimulated the organization of that symposium and led to the selection of the speakers, two of whom have not worked on fungi, were as follows: (a) to bring to mycologists a critical "state of the art" outline of the most recent information and hypotheses available from current diverse approaches to mitosis in all organisms, including the fungi, (b) to explore possible ways in which mitosis can be used as an aid to understanding fungal phylogeny, a topic much beloved by mycologists and protistologists alike. These objectives seemed to have potential appeal to a broader audience than just those able to attend the symposium. Thus, with the additional objective of bringing to nonmycologists the current information concerning fungal mitoses, the preparation of this book was undertaken.

The papers presented here do not represent an encyclopedic account of all information available on the respective topics. Rather, they are intended to highlight what the authors perceive to be the most important current information and recent major milestones. The validity of this approach, the wisdom of the authors' selection of material, and the degree of success of the authors in fulfilling the above objectives can only be judged in the light of extensive critical reading and with the benefit of hindsight some years from now.

I should like to express my gratitude to two groups of people. Dr. H. C. Aldrich and his program committee were indulgent enough to permit me to

organize a mycological symposium in which only half of the speakers were mycologists. The speakers themselves deserve thanks, not only for participating in the symposium, but also for subsequently preparing their work for publication.

NUCLEAR DIVISION IN THE FUNGI

CONTENTS

NUCLEAR DIVISION IN THE FUNGI

HISTORICAL REVIEW AND INTRODUCTION

Manfred Girbardt

Akademie der Wissenschaften der DDR
Zentralinstitut für Mikrobiologie und experimentelle Therapie
Jena, Beuthenbergstr., DDR

I. INTRODUCTION

During the last 80 years fungal karyology has suffered the same fate, though at different times, as the karyology of protists in general. Meticulous investigators and audacious speculants were simultaneously impressed and embarrassed by the manyfold phenomena they were confronted with in the light microscope. Thus it was not surprising that under the influence of evolutionary thinking at about the turn of this century attempts were made to classify the shapes of nuclei (mainly of protozoa) into a phylogenetic succession (Hertwig, 1902, Schaudinn, 1903, Hartmann, 1911). It is to the credit of Belar (1926) that he ended a sometimes erroneous (but nevertheless prosperous) era by crystallizing out the facts and separating them from pseudo-facts. It was unavoidable that by discrediting many of the earlier results, discussion was slowed down and a "big silence" followed in the literature. Belar (1926) reviewed his extended investigations on the nuclei of protists and concluded that their seemingly atypical character is dependent to a high degree on the manyfold variations of the "achromatic" structures. "... the only characteristic of some nuclei of protists ... is the intranuclear position of the centrosome ...". Electron microscopy has confirmed this statement and forced the thinking

ISBN 0-12-335950-3

again into evolutionary lines. Indeed many people are presently searching for special features of "primitiveness" in the behaviour of the "activity centres" of the nuclei, that is the "lokomotorische Komponente" of Hartmann (1911). (Compare his preface of Belar's book.)

Two recent reviews (Kubai, 1975, Fuller, 1976) are available on problems dealt with in this book and the reader is referred to them. Similar attempts have been undertaken by Robinow and Bakerspigel (1965) and Olive (1953) so all eras are covered with competent discussions. It is left for me to add some historical dates from a standpoint of a cytologist who has taken part in the discussion period which started (again) about 20 years ago. This discussion has centered around the question, whether fungal mitosis is only a minor variation of a fundamental scheme or an evolutionary qualified principle which makes it different from eumitosis. At present the question is not settled and ingenious experiments will be needed for a final resolution. Therefore, whenever possible, future trends which should be followed (in the opinion of this author) will be mentioned.

II. CHROMATIN

The Feulgen test for interphase nuclei was rendered more difficult by a low DNA content and/or strong chromatin uncoiling and hydratation. In addition many nuclei proved to be almost completely euchromatic. Staining methods, perhaps with lower specificity, but a more intense staining reaction had to be found. C.F. Robinow and his coworkers developed methods which he had applied in bacteria so masterly before and which he now used with virtuosity for fungal nuclei. They were based mainly on hydrolysing the specimens and either staining them with basic dyes or mounting them in acid dyes. (Feulgen mounted in acetocarmine; HCl-Giemsa; HCl-Orcein). Fig. 1 represents nuclei of Mucor hiemalis, it is taken from one of the first fungal works of Robinow. The most important progress was that the huge nucleolus remained completely unstained whereas the peripheral chromatin was well stained (Robinow, 1957).

Iron-hematoxylin-methods, well suited to and widely used for histological work, in many cases stained only the

Fig. 1. Mucor hiemalis. Germ tubes with interphase and dividing nuclei. Chromatin is well stained in contrast to unstained nucleoli. Fixed with acidic acid alcohol, hydrolysed with N/HCl at 60°C and stained with Giemsa (Courtesy of Robinow, 1957 with permission of Nat. Res. Council of Canada).

nucleolus of the fungal nucleus. The chromatin remained unstained. Early investigators, being glad to see the small nuclei at all (Rosen, 1893, Harper, 1895, Juel, 1898) fell

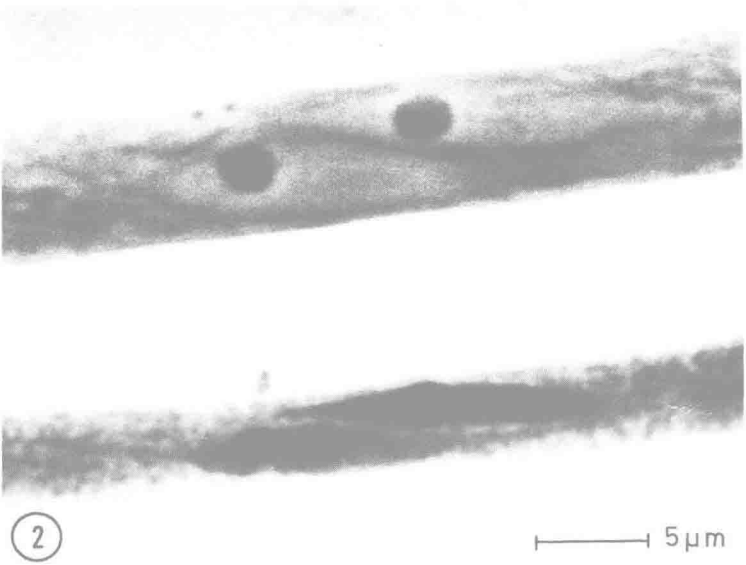

Fig. 2. Trametes (= Polystictus) versicolor. Interphase nuclei in the living cell (above) as seen by phase contrast and the same pair after fixation (CHAMPY) and staining (iron-hematoxylin) in bright-field (below). The bright halos of the phase-picture correspond with the chromatin (from Girbardt, 1955, with permission of VEB Fischer-Verlag, Jena).

into pardonable misinterpretation in as far as they took the nucleolus for the whole nucleus. As vacuolized nuclei are common in fungi, this error could be understood.

We then showed that the iron-hematoxylin-staining depends on the fixatives used. If the cells are fixed with fixatives containing mercury chloride or formaldehyde the chromatin remains completely unstained, surrounding the nucleolus with a bright halo. But in some cases (the most dangerous ones) the chromatin coagulates into the same granular-filamentous structures as the cytoplasm. The nucleolus then seems to be located immediately in the cytoplasm and chromatin cannot be seen at all (Girbardt, 1961a). Only after application of chrome-osmium-compounds are both chromatin and nucleolus stained (fig. 2). A comparison of fixed nuclei with the

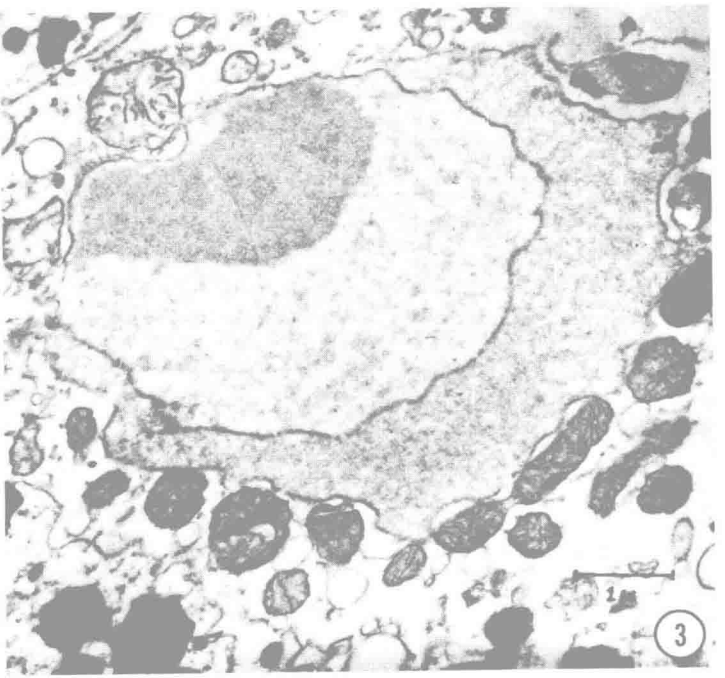

Fig. 3. Allomyces macrogynus. Section of a gamete. Fixation: OsO_4; embedding: methacrylate. Nuclear cap, envelope, chromatin and nucleolus are distinct (Courtesy of Turian and Kellenberger, 1956 with permission of Academic Press).

phase-contrast picture of the same nuclei in the living state clarified what structure corresponds to chromatin and nucleolus. This was needed as in early studies of living fungal cells similar confusions had arisen comparable to those with the hematoxylin-staining (MacDonald, 1949, Dowding & Baker-spigel, 1954).

So far electron-microscopy has delivered only small contributions concerning specialities of chromatin within the interphase nucleus. The reason is again the euchromatic nature of chromatin and the difficulties in fixing fungal cells. Areas which might correspond to heterochromatin are very rare, though in some species (e.g. Neurospora, Podospora, Fusarium, Coprinus) they are demonstrable (Girbardt, 1970).

The homogenous appearance of the interphase nucleus had

been realized in the first electron micrographs (fig. 3) of Turian & Kellenberger (1956). In this paper the standard for good preservation with osmium derivatives was set up. Earlier trials suffered from difficulties with permanganate (e.g. Agar & Douglas, 1955). The wall-less gametes of Allomyces used by Turian of course favoured good preservation, but from this time onward electron micrographs of wall-possessing hyphal forms also had to be measured by this quality. For many species it was unattainable for many years.

It is striking, that near the region of the nucleus-associated organelle (NAO = SPB) several authors have found a heavily contrasted material possibly representing less uncoiled chromatin (Girbardt, 1968, Heath & Greenwood, 1970, Aist & Williams, 1972). It would be very important to apply methods (e.g. annealing of repetitive DNA, Jones, 1970) for identifying these parts. If "centromeric heterochromatin" in fungi marks the position of kinetochores, this would help to uncoil the mystery not only of this chromatin but also that of structureless "kinetochores" found during some divisions.

The application of sensitive electronmicroscopical methods for detection of nucleic acids would also be important. They have been developed for other organisms and allow demonstration of DNA even in viral nucleoids. They are based on the Feulgen reaction using thallium ethylate (Moyne, 1973) or osmium amine (Gautier & Fakan, 1974). The expanded use of nucleases for indirect proof should also be considered. We expect nucleic acids in the NAO (Zickler, 1973) and the nucleolus. So far our results however are not satisfying.

Better results at the light microscopical level have been obtained by analyzing meiosis. Chromosome morphology during developmental steps of the generative phase was clearer much earlier than it was in somatic divisions. This was mainly due to the larger size of the meiotic nucleus and better stainability by basic dyes. Investigations showed most improvement after McClintock (1945) introduced the Orcein-squash-technique in fungal karyology (Olive, 1965). Particularly, more chromosomal details have been recognized during pachytene (Singleton, 1953) than have been resolved in mitotic chromosomes.

One point of agreement exists between fungal meiosis and mitosis: only very rarely are real metaphase plates found. This appearance has been discussed in length for many years. In the opinion of this author a plausible and generally accepted explanation is wanting. All trials to interpret the

mechanism of genome separation in fungi should consider these uncertainties. These problems are discussed from another point of view by Heath in this volume.

The synaptonemal complex which connects bivalents is very important for ultrastructural research (Westergaard & v. Wettstein, 1970). Its specific structure permits unquestionable identification and helps to explain mechanisms of crossing over. It is possible also, after reconstructing all synaptonemal complexes from serial sections, to count the chromosomes (Gillies, 1972). This is important for those cases where the exact chromosome number cannot be estimated by light microscopy.

Differentiation between chromatin and nucleolar substance in the electron microscope is also important, especially in cases where the nucleolus persists during division. We have obtained good results by treating glutaraldehyde-fixed specimens with RNAase before postfixation with osmium. Chromatin is then much more contrasted by lead post-staining than are the nucleolar substances. It is the same effect as has been described for staining with basic dyes by Pollister & Leuchtenberger (1949).

III. NUCLEOLUS

It has been proven that DNA is present within the nucleolus of higher organisms, for the vertebrate nucleolus discussions lasted until 1960 (Lettré, 1956). It had been very difficult to convince opponents of the presence of such small amounts of DNA within so much RNA and protein. Again I should like to praise the accuracy of the old protistologists who had for a long time defended the existence of chromatin within the nucleolus. Though their assumption was incorrect that this was a peculiarity of only some protistean nuclei ("Karyosom", "Amphinucleolus", "Binnenkörper" etc.) it is another example of the meticulous microscopical investigations of our forefathers (Belar, 1916, Doflein, 1916).

The sharply delimited nucleolus vanishes completely in most cases after permanganate fixation, widely used even today for fixation of fungi. Its substance must be spread over the whole nucleus as the space previously occupied in the living cell cannot be detected by the electron microscope. In some cases diffuse material can be present but we have never found

it in the original shape of the nucleolus.

After glutaraldehyde fixation, fibrillar and granular components embedded in a matrix can be demonstrated (Girbardt, 1970). In some species the granular component may surround the fibrillar one like shells. However it had not been possible to demonstrate nucleolus-associated chromatin. Compared with the success reached by cytochemical and biochemical analyses in describing nucleoli of higher organisms (Bernhard & Granboulan, 1968, Ghosh, 1976) the fungal nucleoli are still poorly understood. Fungi with large nucleoli, like Basidiobolus, should be suitable specimens for carrying out interesting trials. For example the mechanism of formation and extrusion of nucleolar vacuoles is still unexplained (Soudek, 1960, Erdelska, 1973).

During division nucleoli exhibit different behaviour as Pickett-Heaps (1970) has pointed out. Some "classes" have been established by him and evolutionary trends have been suggested. It would be therefore of interest to study more fungal species in this context. In Trametes (= Polystictus) the nucleolus can be largely dispersive. Its dispersion frequently occurs outside the fenestrated division in an excised part of the nucleus which is completely cut off from the chromatin-containing part. This seems to indicate that the nucleolar substance does not play a role during division. The same species however indicates that dispersion can also occur in the chromatin-containing part of the nucleus. Thus one might expect, in this case, coating of chromosomes with nucleolar material comparable to the behaviour in Spirogyra and Chara (Pickett-Heaps, 1970). So far however no morphological evidence for this assumption has been found. What should be shown by this example is that the "classes" of nucleolar behaviour must not have functional significance. Nevertheless fungal nuclear divisions would be well suited to further investigations of these questions provided better specific staining of the nucleolar substance becomes available.

Changes in nucleolar shape became evident during cinematographic recording of their behaviour in the living cell. In tissue cultures of animal cells, rotation of nuclei has been observed (Pomerat, 1953), the temporarily enclosed nucleus in the clamp of homobasidiomycetes also rotates (Girbardt, 1962a). In many cases the nucleolus was stretched in a drop-like fashion (compare fig. 4 Vph and fig. 6). This behaviour was extremely striking when the nucleus was preparing