MOLECULAR BIOLOGY OF THE FISSION YEAST

Edited by

Anwar Nasim
Paul Young
Byron F. Johnson

CELL BIOLOGY

A Series of Monographs

Molecular Biology of the Fission Yeast

Edited by

Anwar Nasim
Division of Biological Sciences
National Research Council of Canada
Ottawa, Ontario, Canada

Paul Young
Department of Biology
Queen's University
Kingston, Ontario, Canada

Byron F. Johnson
Molecular Genetics Group
Division of Biological Sciences
National Research Council of Canada
Ottawa, Ontario, Canada

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To Urs Leupold, J. M. Mitchison, and C. F. Robinow

Preface

The fission yeast, *Schizosaccharomyces pombe*, although known to science for less than a century (Lindner, 1893) and actively studied for only about forty years, has become one of the best characterized organisms. Since the early studies of Leupold, Mitchison, and Robinow, *S. pombe* has been considered the best defined yeast after *Saccharomyces cerevisiae*. In some areas, it has been the paradigm. A not-so-subtle implication is that the fission yeast has been "... some special object particularly suited for the study of each of the more important problems" (A. Krogh, Nobel Laureate, 1920). This volume attempts to show how the fission yeast has been that "special object" in a variety of important areas of modern research.

The diversity of experimental approaches and the ease with which the novel techniques of gene manipulation and cloning have been applied to *S. pombe* have obviously generated an ever-increasing interest in using the fission yeast as a biological system. Some recent experiments, whereby a human homolog of a cell cycle mutant of *S. pombe* has been found, have led to new ways of examining the functional similarities between simple unicellular eukaryotes and the highly differentiated complex systems.

This volume is the first attempt to assemble the lore of the fission yeast. It recognizes that a large body of literature has been accumulated and attempts to provide an overview of most of it, although inevitably some areas will be judged to have been treated too lightly.

The dominant themes for many years emphasized cell biology and genetics. Currently, a much broader interest in the molecular biology of *S. pombe* is developing. Findings regarding the conservation of some cell cycle genes, attributes of the RNA processing system, and the structure of the centromeres and chromosomes stimulate broad interest.

Among others, this book is addressed to the many new investigators and laboratories adopting this system. We hope it leads to the development of new molecular tools for investigating problems in *S. pombe* as well as to the definition of areas of metabolism and biology beyond the major themes of the past.

We are extremely thankful to all those who have contributed to this volume. It has been a great joy and feeling of personal satisfaction to have worked with all these colleagues. Along with all the other efforts being undertaken to focus on *S. pombe* as one of the organisms particularly suitable for genetics and cell biology, we hope this volume will help to focus on *S. pombe* as one of the organisms particularly suitable for modern research.

Several aspects of the molecular or cellular biology of the fission yeast cell have been reviewed recently, thus these aspects are not included, or, at least, are not emphasized in this volume. The references to those reviews are as follows:

Calleja, G. B. (1987). Cell aggregation. *In* "The Yeasts" (A. H. Rose and J. S. Harrison, editors), Volume 2 (2nd edition). Academic Press, London.

Phipps, J., Nasim, A., and Miller, D. R. (1985). Recovery, repair, and mutagenesis in *Schizosaccharomyces pombe*. *Advances in Genetics* **23,** 1–73.

Robinow, C. F., and Johnson, B. F. (1989). Yeast cytology. *In* "The Yeasts" (A. H. Rose and J. S. Harrison, editors), Volume 3 (2nd edition). Academic Press, London.

Anwar Nasim
Paul Young
Byron F. Johnson

Contents

4 Oncogene Homologs

S. A. Nadin-Davis, A. Nasim, Y. Fukui, and M. Yamamoto

5 Cell Cycle Controls

Peter A. Fantes

6 Cell Cycle Growth and Periodicities

J. M. Mitchison

7 Gene Cloning and Expression in Fission Yeast

Paul Russell

8 General Cytology of Fission Yeasts

C. F. Robinow and J. S. Hyams

9 Morphogenesis of Fission Yeasts

Byron F. Johnson, Machiko Miyata, and Hisao Miyata

10 Continuous Culture and Intermediary Carbon Metabolism

I. J. McDonald and C. S. Tsai

11 Plasma Membrane H^+-ATPase: Ion and Metabolite Transport in the Yeast *Schizosaccharomyces pombe*

André Goffeau, Alan Coddington, and Alain Schlesser

12 Taxonomy and Phylogenesis

M. Sipiczki

1

Genetics Overview

P. MUNZ,* K. WOLF,† J. KOHLI,* AND U. LEUPOLD*

*Institut für Allgemeine Mikrobiologie
Universität Bern
CH-3012 Bern, Switzerland

†Institut für Mikrobiologie und Weinforschung
Johannes Gutenberg-Universität
D-6500 Mainz, Federal Republic of Germany

I. INTRODUCTION

A. History

Schizosaccharomyces pombe was first isolated from an East African millet beer, called *Pombe,* by Lindner (1893) who described it as a yeast dividing by cell fission and forming four-spored linear asci. Studies on

MOLECULAR BIOLOGY OF THE
FISSION YEAST

ascus formation in the related eight-spored species *Schizosaccharomyces octosporus* led Beijerinck (1894) to believe that it was "nowhere clearer than here that the ascus and the ascospores are formed without a sexual act." This was disputed by Schiønning (1895) who thought if the possibility of a sexual act in the lower Ascomycetes was conceivable it would be precisely in this species in which ascus formation was found to be preceded by the pairwise fusion of cells. The sexual nature of these events became clearly established when Hoffmeister (1900) showed that the fusion of cells in *S. octosporus* is accompanied by nuclear fusion. Guillermond (1901) confirmed the results of Schiønning and Hoffmeister in a more detailed analysis of spore formation in both *S. octosporus* and *S. pombe*.

In spite of the demonstration of these and other clear cases of sexuality in yeasts that pointed to a life cycle involving a regular alternation of nuclear phases (Guillermond, 1905; Kniep, 1928), references to the possibility of a parthenogenetic formation of asci not preceded by sexual fusion of cells are repeatedly found in the literature on yeast of the following decades. The concept of regular alternation of haploid and diploid nuclear phases in the life cycle of yeasts was firmly established only when Winge and co-workers showed that sexual fusion of ascospores or cells derived from them was a regular feature of the life cycle of several yeast species of the genus *Saccharomyces* (Winge, 1935) and that sporulation of a cell clone derived from a strain of bakers' yeast was accompanied by the genetic segregation of morphological characters in each of the four-spored asci formed (Winge and Laustsen, 1937). This demonstrated that sexual fusion took place between spores or cells of haploid constitution and that it gave rise to diploid cell clones which under suitable conditions underwent meiosis to produce haploid ascospores again.

It was also Winge who suggested *Schizosaccharomyces pombe* as a potentially useful organism for genetic studies when the senior author of this chapter (U.L.) visited the Physiological Department of the Carlsberg Laboratory in Copenhagen as a young student in 1946 and again in 1948/1949. Considering the results obtained in *Saccharomyces*, it was clear that the early observations of Schiønning, Hoffmeister, and Guillermond, which showed that ascospore formation in *Schizosaccharomyces* immediately follows conjugation, pointed to a haplontic life cycle in which the diploid phase is restricted to the zygote formed by the sexual fusion of haploid cells. As in *Saccharomyces* and in higher Ascomycetes, ascospore formation was likely to be preceded by meiosis and to give rise to haploid ascospores which on germination would yield haploid cells again (Fig. 1).

The early observations on the conjugation of sister cells in *S. octosporus* and *S. pombe* (Schiønning, 1895; Guillermond, 1901, 1903, 1931)

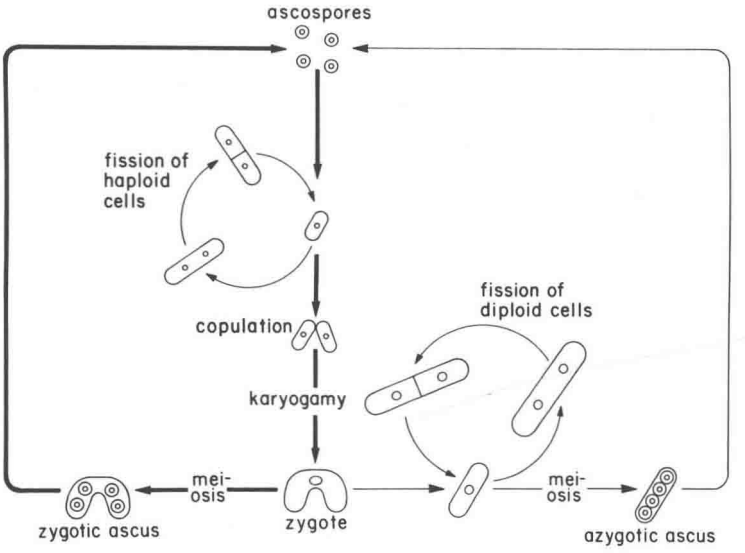

Fig. 1. Life cycle of *S. pombe* (homothallic strain). The left part of the figure (bold lines) shows the normal haplontic life cycle. The right part (thin lines) demonstrates the events that take place when zygotes develop into diploid cells. [Reproduced from "Handbook of Genetics" (R. C. King, ed.), Vol. 1, p. 396, Plenum Press, New York and London, 1974, by copyright permission of Plenum Publishing Corporation, New York.]

and on the ability of cell clones derived from single cells or spores of these yeasts to sporulate abundantly in pure culture (Beijerinck, 1897, 1898) had already made it clear that a homothallic mating behavior is common in the genus *Schizosaccharomyces*. In *Saccharomyces*, the finding that many cell clones derived from single ascospores were capable of early pairwise fusion of cells (Winge, 1935; Winge and Laustsen, 1937) pointed in the same direction. That heterothallic strains belonging to two self-sterile but cross-fertile mating types (called *a* and α) could also be found in this genus was only discovered several years later by Lindegren and Lindegren (1943), and it again took a few years before Winge and Roberts (1949) were able to show that a single pair of alleles *D*/*d* (now called *HO*/*ho*) is responsible for the early diploidization observed in homothallic clones and its lack in heterothallic clones derived from single spores.

B. Mating Types and Life Cycle

The first genetic analysis confirming the haplontic nature of the normal life cycle of fission yeast was carried out by Leupold (1950). The strain of *S. pombe* studied was obtained from the yeast collection of the Cen-

traalbureau voor Schimmelcultures in Delft. It had originally been isolated from grape juice by Osterwalder (1924) who first described it as a new species, *Schizosaccharomyces liquefaciens* Osterwalder. Since it differed from *Schizosaccharomyces pombe* Lindner only in its marked ability to liquefy gelatin, it was later renamed *Schizosaccharomyces pombe* Lindner, strain *liquefaciens* (Osterwalder), by Stelling-Dekker (1931).

From the Delft culture of this strain of *S. pombe*, Leupold (1950) isolated two types of homothallic clones differing in their fertility, of which only one (designated h^{90} because it formed about 90% spores in pure culture) has survived and needs to concern us here. In addition, heterothallic clones (designated h^+ and h^-) belonging to two opposite mating types, called (+) and (−), and some sterile clones were isolated from the same culture. In crosses between heterothallic strains of opposite mating type, the two parental types segregated 2 : 2 in each of the spore tetrads analyzed, and so did the two parental types in spore tetrads from the crosses of homothallic with heterothallic strains of either mating type. It was concluded that homothallism as well as heterothallism of the two mating types (+) and (−) were determined by a series of three alleles, h^{90}, h^+, and h^-. Rare spontaneous genetic events, interpreted to result from mutations among the three allelic states, were found to interconvert the three types of mating behavior, each of the three types giving rise to the other two types. However, h^- strains appeared to be able to do so only when derived from h^+ strains by secondary mutations. No mating-type mutants were observed in the original h^- isolates derived from the Delft strain or in the h^- progeny of their crosses with h^+ or h^{90} strains.

The two types of h^- strains, one stable and one unstable, were later recognized as two separate types, and additional secondary variants of homothallic and heterothallic strains (described in this volume, Chapter 2 by Egel, Mating-Type Genes, Meiosis, and Sporulation) were discovered. Their isolation was greatly facilitated when it was found that iodine, which stains sporulating colonies black and nonsporulating colonies or colony sectors yellow (Beijerinck, 1898), can be applied in the form of iodine vapor. A brief exposure will not kill the cells in the interior of the treated colonies and will therefore permit their isolation (Leupold, 1955).

The further elucidation of the genetic and physical basis of the inheritance of mating type in *S. pombe* has been one of the most fascinating topics of research in this yeast since the first analysis carried out by Leupold (1950). The development of the field may be traced by consulting the relevant sections in the reviews of Gutz *et al.* (1974) and Egel *et al.* (1980) on the genetics of *S. pombe* and in the review of Crandall *et al.* (1977) on the physiology of mating in yeasts. The present state of knowledge is summarized elsewhere in this volume (Chapter 2 by Egel, Mating-

Type Genes, Meiosis, and Sporulation). Suffice it to point out here that mobile genes assigned to three closely linked loci *mat1*, *mat2*, and *mat3* have turned out to exert the primary control of mating type in *S. pombe*. In homothallic h^{90} strains, they cooperate in a cassette mechanism very similar to that which has been found to underlie mating-type switching in homothallic strains of *Saccharomyces cerevisiae*. At the expression locus *mat1*, *P* (plus) information copied from the silent cassette *mat2-P* and *M* (minus) information copied from the silent cassette *mat3-M* are exchanged every few cell generations. In heterothallic h^+ and h^- strains, however, *P* or *M* information is stabilized at *mat1* as a result of aberrant recombination events in the mating-type region.

Judging from its mating-type constitution, h^{90} clearly represents the true wild type of *S. pombe*. Normal h^+ strains and stable h^- strains of the type originally isolated from the Delft culture of *S. pombe* strain *liquefaciens* (later called h^{+N} and h^{-S} to distinguish them from secondary heterothalic strains of the same mating type but differing in their mating-type interconversions) arise directly by rare but recurrent events from the homothallic h^{90} type. Although the precise genetic constitution of the homothallic wild-type and of several heterothallic variants including h^{+N} and h^{-S} with respect to the *mat* genes is known today from physical analysis, the symbols h^{90}, h^+, and h^- (with or without additional superscripts to indicate the various heterothallic subtypes) are still widely used as a short notation of the mating-type constitution of strains.

Although the haplontic nature of the normal life cycle of *S. pombe* was confirmed by the results obtained by Leupold (1950) in his first genetic analysis, subsequent studies have shown that it is nevertheless possible to propagate the organism vegetatively in the diplophase. This opened new possibilities for genetic analysis. It turned out that haploid strains regularly contain rare diploid cells arising presumably by endomitosis (Leupold, 1955). On solid media containing Magdala red (phloxin B), diploid cells develop into colonies that stain darker than haploid colonies, owing to a higher percentage of dead cells which are stained by the dye (Gutz *et al.*, 1974; Kohli *et al.*, 1977).

Diploid cell clones of constitution h^{90}/h^{90} isolated from haploid strains of the homothallic constitution h^{90} are capable of undergoing meiosis and spore formation directly, without preceding conjugation. The so-called azygotic asci thus formed retain the shape of the diploid cells from which they have arisen (Leupold, 1955). They are thus clearly distinguishable from the dumbell-shaped zygotic asci resulting from the conjugation of haploid (or diploid) homothallic or heterothallic cells. Diploid cell clones of constitution h^+/h^+ or h^-/h^- isolated from haploid strains of the heterothallic constitution h^+ or h^- are incapable of sporulation but retain the