THE MOLECULAR BIOLOGY OF THE YEAST SACCHAROMYCES

LIFE CYCLE AND INHERITANCE

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1981

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Printed in the United States of America
Book design by Emily Harste

Library of Congress Cataloging in Publication Data

Main entry under title:

The Molecular biology of the yeast
  saccharomyces, life cycle and inheritance.

  (Cold Spring Harbor monograph series,
ISSN 0270-1847 ; 11A)
  Includes index.
  1. Saccharomyces. 2. Molecular biology.
I. Strathern, Jeffrey N. II. Jones, Elizabeth W.
III. Broach, James R.
IV. Series.
QK623.S23M64 589.2'33 81-68895
ISBN 0-87969-139-5 AACR2

All Cold Spring Harbor Laboratory publications are available through booksellers or may be ordered directly from Cold Spring Harbor Laboratory, Box 100, Cold Spring Harbor, New York 11724.

SAN 203-6185

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Contents of companion volume

THE MOLECULAR BIOLOGY OF THE YEAST SACCHAROMYCES
METABOLISM AND GENE EXPRESSION

Preface

At the Molecular Biology of Yeast meeting at Cold Spring Harbor Laboratory in 1979, it was apparent that investigation of the biology of yeast was in a period of rapid and exciting growth. The meeting was alive with excitement over the fruits to accrue from the union of the developing technologies in molecular biology with the elegant genetic and phenomenological studies possible in yeast. Jim Watson perceived that it was an appropriate time to review the biology of yeast and attempt to define the direction in which the field would progress in the 1980s. He encouraged us to assemble this monograph.

Most of the volumes in the Cold Spring Harbor Monograph Series are organized as a combination of reviews and research papers. We felt, however, that the core of accumulated knowledge in yeast molecular biology was too large and the rate at which new information was being added was too rapid for the field to be covered adequately in a research paper format. Therefore, we decided to limit the monograph to reviews that would provide both a background in yeast molecular biology and an indication of the direction in which individual areas were proceeding. Our basic goal was about 24 reviews in about 750 pages, to be published in late 1980. The vigor of the field has been reflected in the rapid expansion of this monograph. The reviews have been divided into two volumes, which together will number nearly 1400 pages. In this volume, the cell cycle, the life cycle, non-Mendelian elements, and the mechanics of nuclear inheritance are discussed. The second volume will deal with metabolism and gene expression. These books are meant to serve both as general reviews and as references. Accordingly, the authors attempted to provide in their texts frameworks for the data from which guiding principles emerge. At the same time, the data had to be reviewed in sufficient detail so

that exceptions to such generalities would be apparent, both to guard against pitfalls and to serve as impetus for further research. With that in mind, rather extensive tables are included in several of the chapters. The authors have our deep appreciation for their efforts in attaining these twin goals.

There is a recurring theme in these chapters that the tools now exist to understand these topics in molecular detail. One gets the impression that the authors of these reviews returned to their laboratory benches with enthusiasm and great expectations. It is our hope that these volumes leave the reader with the same feeling of inspiration.

We wish to thank Nancy Ford and Nadine Dumser for their roles in editing the manuscripts and making this publication a reality. Furthermore, we would like to thank our colleagues Jim Hicks, Amar Klar, and George Zubenko for their support during these long months of editing.

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Contents

Development of Yeast as an Experimental Organism

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The editors of this volume have asked me to describe the landmark discoveries that have led to the prominence of *Saccharomyces cerevisiae*[1] as a eukaryotic organism of choice for a number of problems in molecular genetics. To place the beginnings of yeast genetics in proper perspective, it should be recalled that at the time that Ö. Winge was beginning his researches with yeast in the mid-thirties, the principal organisms then in use were *Drosophila*, corn, and *Neurospora*. The dazzling successes of the prokaryotic era were more than a decade away.

Yeast was already well known as an important tool in biochemical research. The interest in yeast stemmed at first from its role in alcohol production in the making of wine and beer. Early in this century, when it was found that alcoholic fermentation could be carried out by an extract from yeast, the fractionation of the extract led to the discovery and characterization of enzymes and coenzymes. The glycolytic pathway—the breakdown of glucose as a result of the fermentation process—was worked out in detail. The individual enzymes, their specific coenzymes, and the products formed at each step of the pathway were identified. As a rich source of the water-soluble B complex, yeast also was a principal contributor to early research in vitamins. Thus, many of the important concepts of biochemistry owe their origins to investigations in which yeast played a key role. The reemergence of yeast as an important organism in biochemical research is described in a review by de Robichon-Szulmajster and Surdin-Kerjan (1971).

The early period of yeast genetics was concerned mainly with establishing that yeast followed the rules of Mendelian inheritance. In a series of papers (see, e.g., Winge and Roberts 1952), Winge and his collaborators demonstrated the 2:2 segregation of certain enzymatic markers in four-spored asci from diploid cells. There was one important exception to orthodoxy. Lindegren and his colleagues reported departures from 2:2 segregation, i.e., 4:0, 3:1, 1:3, and 0:4, that occurred too frequently to be ignored. Lindegren (1949) interpreted these cases of irregular segregation as examples of "gene

[1]The strains of yeast now in use have been derived from many sources (see, e.g., Lindegren 1949). Claims to call them *Saccharomyces cerevisiae* come from their morphologies and the fact that they are interfertile with the species (for taxonomic considerations, see Lodder and Kreger-van Rij 1952).

conversion" after Winkler's (1930) theory of recombination bearing the same name and based on similar data cropping up at that time. Other explanations were put forward to explain these observations on more conventional grounds (see, e.g., Roman et al. 1951; Winge and Roberts 1954), until findings in *Neurospora* (Mitchell 1955) and in yeast (Roman 1956) provided unmistakable evidence of the essential correctness of the Lindegren observations.

Gene conversion is interpreted quite differently today than it was by Lindegren and Winkler, who thought of it as a mutational process. Now we think of it as being due to DNA heteroduplex formation and the subsequent repair of noncomplementary sites in the heteroduplex (Holliday 1964). Historically, it is a curious twist that gene conversion was originally proposed by Winkler to account for recombination in opposition to the then-emerging theory of recombination by the breakage and reunion of homologous chromosomes. Although Winkler was wrong in his interpretation of gene conversion and, therefore, in its application to recombination, it has turned out that gene conversion as we understand it today has provided the basis for a molecular theory of recombination that does in fact involve the breakage and reunion of chromosomes. The modern theory and its variations are discussed later in this volume.

The discovery of mating types in yeast (Lindegren and Lindegren 1943) was a technical advance that turned out to have an importance beyond its mere utility. Winge, in his experimental protocol, used spore-to-spore fusion to obtain the diplophase. Some couplings were successful, whereas others were not, for no apparent reason. By utilizing the vegetative cells derived from individual spores, the Lindegrens recognized that yeast had two mating types, which were designated **a** and α (Lindegren and Lindegren 1943). Cells of mating-type **a** mated only with cells of mating-type α. A new technique therefore became possible for crosses in which vegetative cells (not spores) of one mating type were mixed with vegetative cells of the other mating type. This meant that the parents of a cross could be retained for later testing and for subsequent crosses, if that proved to be desirable. The diploid zygotes that were formed in such a mixture could then be isolated with a micromanipulator or, as nutritional markers became available, they could be selected for on appropriate media. A refinement of the technique was the isolation of individual diploid cells from which clones were derived for sporulation and dissection. This improvement avoided the chief criticism of the mass-mating technique, namely, that spontaneous changes in either one of the two haploid parents could result in a heterogeneous mixture of zygotes and, therefore, tetrads obtained from the mixture did not come from a defined genetic source.

It immediately became evident that the strains of *Saccharomyces* used in genetic research belonged to two classes: the heterothallic class with its two mating types, **a** and α; and a homothallic class that was characterized by cell

fusion among the cells derived from a single spore and by the absence of mating type, or so it seemed. Spore-to-spore matings between heterothallic and homothallic strains produced a diplophase that in turn produced asci with two spores of the homothallic type and two spores of the heterothallic type (Winge and Roberts 1949). Thus, a single pair of alleles, designated D/d for diploidization (now called the *HO* gene for homothallism), is responsible for the homothallism/heterothallism difference. Segregation of the alleles in tetraploids showed that homothallism is dominant (D. C. Hawthorne, pers. comm.; later confirmed by Hopper and Hall 1975). Oeser (1962) and Hawthorne (1963b) further found that homothallism masked the presence of the **a** and α genes, and that in fact, the mating-type alleles were present in homothallic strains. A spore of mating-type **a** on germination gives rise to cells of the same mating type until a mutation to the opposite mating type occurs early in the clone's lineage. There follows cell fusion between the cells of opposite mating type to restore the diplophase of genotype $\mathbf{a}/\alpha$ and a loss of mating ability. Thus, the apparent failure to mate is due to the replacement of competent haploid cells with diploid cells. The same sequence of events occurs when an α spore germinates, but in this case, the mutation is to the **a** mating type with the subsequent restoration of the diplophase (Fig. 1).

A related observation, for which the stimulus arose from attempts to find alternative explanations for the irregular segregations found by Lindegren, was the discovery that polyploidy occurred in yeast (Lindegren and Lindegren 1951; Roman et al. 1951; Roman and Sands 1953). When a haploid culture of mating-type **a** was grown in nutrient broth, larger cells began to appear in the culture and, because of their superior growth rate, ultimately became dominant in the culture. The same was found to be the case with α cells. When the larger cells were isolated and analyzed, it was found that they were of two types: One type was heterozygous for mating type, was incapable of mating, and could be induced to sporulate; the other was capable of mating and was incapable of sporulation. The first type was not detectably different from an ordinary diploid and was undoubtedly due to mutation at the mating-type locus to the opposite mating type and subsequent fusion. Mating-type mutation was rare, occurring with a frequency of approximately 10^{-7} or less per cell generation. The second type, occurring with about equal frequency, was found to be diploid also, either $\mathbf{a}/\mathbf{a}$ from **a** cells or α/α from α cells, and these were able to mate but not to sporulate. Segregations typical of $\mathbf{a}/\mathbf{a}/\alpha/\alpha$ tetraploids were obtained from cells resulting from the mating of the putative $\mathbf{a}/\mathbf{a}$ and α/α diploids. Similarly, triploids could be constructed by crossing the diploid homozygote with a haploid cell of opposite mating type. The triploid gave a high frequency of inviable spores in tetrads, as would be expected from studies of triploidy in higher organisms. Disomics could be generated in this way, and these became useful in chromosome mapping (Mortimer and Hawthorne 1973).

When diploidization leading to homozygosis for mating type was found, it

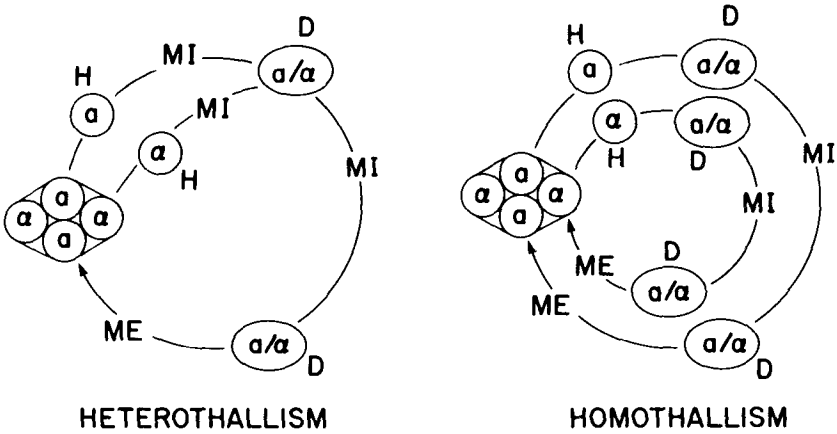

Figure 1 Schematic showing principal differences between heterothallism and homothallism. The individual spores are dissected from the ascus. In heterothallism, the haplophase (H) may exist more or less indefinitely, the cells multiplying by mitosis (MI). At any time when confronted by cells of opposite mating type, the diplophase (D) will be formed, and this in turn can be sustained indefinitely, again multiplying by mitosis, until conditions are such that the diploid cells undergo meiosis (ME) to produce the ascus and spores that are once again haploid. In homothallism, the cells of the haplophase are not stable. In the early divisions following spore germination, a genetic switch to the opposite mating type occurs in some of the cells; then mating occurs to produce the diplophase. The diplophase is stable and multiplies by mitosis. As in heterothallism, the diplophase can be interrupted at any time by inducing meiosis to restore the ascus.

became clear that diploidy was not a sufficient condition for sporulation, since the cells do not sporulate. The fact that they mate and **a**/α cells do not suggested that the genetic conditions for mating were antagonistic to those for sporulation. An additional key finding was that of Hawthorne (1963a), which suggested that the mating-type locus was separable into two parts, one responsible for **a** function and the other responsible for α function.

The complex control governed by the mating-type system was only vaguely hinted at in these early experiments. The genetic basis for a much more complicated pattern of controlling elements was worked out principally by Oshima and his collaborators (Harashima et al. 1974), and the interaction of these elements was postulated to account for changes of mating-type specificity. A further elaboration of the nature of these interactions led to a novel hypothesis of gene duplications, some distance removed from the mating-type locus and interacting with the latter to cause "switches" in mating type (Hicks et al. 1977). The "cassette" model of mating-type control and the pattern of that control are discussed by Herskowitz and Oshima (this volume).

In the development of an organism for use in genetic research, it is helpful

to know the number of chromosomes that are present and the distribution of genes on these chromosomes. Chromosome mapping became an important activity in yeast, since the chromosomes were difficult to see, and an estimate of the number of linkage groups was the best and most certain indicator of the number of chromosomes. We need not dwell on the controversies over chromosome number in the early days of yeast genetics. As the number of linkage groups increased, so did the number of chromosomes reported. Through the use of light microscopy, Tamaki (1965) estimated the haploid number to be 18. Today, with some 300 genes mapped, it is fairly well established that there are 17 chromosomes in the haploid set (R. K. Mortimer and D. Schild, pers. comm.). Byers and Goetsch (1975) counted synaptonemal complexes in meiotic cells of diploids and tetraploids; their observations were in general agreement with this number. The subject of mapping is discussed by Mortimer and Schild (this volume).

Ordinarily, the geneticist is not pleased to find that the organism he or she is working with has a large number of chromosomes. In this case, however, the finding that yeast also had a low nuclear DNA content—about 10^{10} daltons (Bicknell and Douglas 1970)—was an important factor in bringing yeast to the attention of molecular biologists. The average yeast chromosome was thus substantially smaller than the chromosome of *Escherichia coli*. Thus, although yeast is a eukaryotic organism possessing traits associated with such organisms, e.g., meiosis and the alternation of haplophase and diplophase, its chromosomes are sufficiently small to make them readily available for studies of replication and structure. Helpful for this purpose and others was the finding that the tough cell wall could be enzymatically removed (Eddy and Williamson 1957). In a key paper by Petes and Fangman (1972), the feasibility of using yeast for molecular studies of chromosomal DNA was demonstrated.

The fact that yeast can grow under either aerobic or anaerobic conditions renders it particularly suitable for studies of mitochondrial inheritance. In 1949, Ephrussi published the discovery that acridines induced, with high frequency, a class of mutants that he called "petite colonie," or simply petite. They were thus named because cells that were petite utilized glucose (or other fermentable carbon sources) less efficiently than did normal cells and therefore gave rise to colonies that were distinctly smaller than normal colonies. It was concluded from tetrad analysis that the petite phenotype was due to a cytoplasmic mutation. The consequence of the mutation was the simultaneous loss of several elements in the electron transport system, including cytochromes *a* and *b*. The mutation was stable, i.e., it did not revert to normal even under severe selective pressure. For routine diagnostic testing, advantage was taken of the fact that petites could not utilize carbon sources such as glycerol or ethyl alcohol for growth. The early history of cytoplasmic inheritance is given by Ephrussi (1953).

It was suspected that the mitochondrion was the site of the petite mutation.

Morphological and biochemical studies revealed irregularities in the structure of the mitochondrion and in its DNA content. However, an investigation of the genetics of this organelle awaited a technical breakthrough: the finding of mutations resistant to various antibiotics known to inhibit protein synthesis in bacteria, such as chloramphenicol and erythromycin (Wilkie et al. 1967). These were found to reside in mitochondrial DNA and formed the basis for early studies of transmission and recombination in cytoplasmic inheritance (described in Wilkie 1970). Slonimski, who earlier made important contributions to the biochemistry of the petite phenotype, immediately saw the power of the new technique, and he and his collaborators became the leading contributors to what has developed into a new and exciting research area in which *Saccharomyces* has been the organism of choice (Coen et al. 1970). The present status of mitochondrial genetics is given by Dujon (this volume).

Yeast has also played a distinctive role in the study of the control of cell division. By using time-lapse photography as a means of detecting arrest of cell division, Hartwell and his collaborators were able to accumulate some 150 temperature-sensitive mutants capable of growth at 22°C but not at 36°C. The mutants represent some 35 genes that control blocks in various stages of the cell-division cycle (Hartwell et al. 1974). Advantage was taken of the fact that yeast reproduces by budding. In the haploid strain used by Hartwell's group, the onset of budding was closely correlated in time with the onset of DNA replication. The size of the bud was a reliable indicator of the stage in the cell-division cycle of mother and daughter cells. More recent results of the cell-division cycle studies are discussed by Pringle and Hartwell (this volume).

Another area of research in which yeast has played an important part is in the elucidation of the action of supersuppressors. These, also demonstrated in bacteria, are nonsense suppressors that are allele-specific and not locus-specific (Hawthorne and Mortimer 1963). Three general classes of supersuppressors have been found in yeast, corresponding to the UAA, UAG, and UGA nonsense codons. The findings were taken as an illustration of the universality of the genetic code, since the same nonsense or termination codons were found in *E. coli*. Ultimately, the supersuppressors, of which a large variety was obtained at different loci by mutation, were shown to be altered tRNA genes whose products were now able to read the corresponding nonsense codons during the translation process. This subject is discussed in more detail by Sherman (1982).

There is one other subject that should be mentioned for its importance in propelling yeast as a major experimental eukaryotic organism. It also serves to point up again the flexibility of yeast as a facultative aerobe. After the amino acid sequence of iso-1-cytochrome *c* was worked out by Narita and Titani (1969), Sherman and his collaborators (1970) took the opportunity to investigate the question of colinearity between DNA and its protein product in a eukaryotic organism. Later studies embraced a variety of problems, such

as the dependence of recombination on gene distances of known nucleotide lengths and the control of protein synthesis, as well as other ancillary inquiries. Differences were indeed found, in relation to prokaryotic organisms, particularly as regards the mechanism of the control of gene action and the specificity of mutagens.

I have chosen topics that appear to be important in the early development of yeast as a potentially useful organism for investigations of certain types of fundamental problems. Not less important is the application of yeast in the investigation of problems in which other organisms also had a role to play, such as the regulation of enzyme biosynthesis, the structure and function of the cell surface, the control of transcription and translation, the mechanisms of macromolecular repair, the recent advances in recombinant DNA, and gene cloning.

Of special note in connection with gene cloning is the recent establishment of the technique for transformation in yeast (Hinnen et al. 1978). Oppenoorth (1960) was the first to report the achievement of transformation in this organism, but his paper was subject to criticism on grounds of experimental design, and efforts to repeat his results were unsuccessful. Hinnen et al. (1978) succeeded in transforming yeast by incorporating genetically marked yeast DNA into an *E. coli* plasmid, concentrating the DNA by passage through *E. coli*, and exposing yeast cells to the high concentration of specific DNA under conditions known to favor alterations in cell membranes. Subsequent to these observations, DNA fragments from yeast have been identified that are capable of autonomous, extrachromosomal replication when reintroduced into yeast by transformation. As a consequence, various yeast-transformation vectors have been constructed, and techniques have been developed that permit the isolation of a number of specific yeast genes by complementation of genetic lesions. Thus, through the wedding of yeast genetics and yeast transformation, one has the potential to isolate DNA corresponding to any yeast gene for which there exists a scorable phenotype for mutations in that gene. In addition, cloning strategies, such as cDNA cloning, probing with homologous sequences, etc., that are applicable to other organisms are also applicable to yeast. The techniques involved in cloning and transformation are discussed in detail by Botstein and Davis (1982).

Altogether, the subject matter of this volume shows quite clearly that *Saccharomyces* is for the most part a typical eukaryotic organism that offers the advantages of simplicity of organization and ease of handling for investigations involving classical techniques as well as techniques that are at the forefront of molecular biology.

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