CARL C. LINDEGREN
Southern Illinois University

Chapter 16

Genetics and Cytology of Saccharomyces

In the middle of the last century, Buchner ground up yeast cells and proved that the cell-free filtrate contained a substance capable of fermenting sugar. This experiment settled a heated controversy between Liebig and Pasteur concerning whether or not living structures were essential to fermentation. The substance responsible for the fermentation was called an enzyme, the word being derived from the Greek and meaning "in yeast." Since that time, yeast has been the organism of choice for experimenting in enzyme chemistry because of the abundant supply obtainable from breweries and from factories producing bakers' yeast. The biochemistry of fermentation has provided the foundation for our present understanding of the biochemistry of respiration and of muscular contraction—two of the fundamental problems which have intrigued biologists. It has led to an understanding of vitamins and through them to an understanding of chemotherapy.

BIOCHEMICAL DEFECTS AS GENE MARKERS

The work of Beadle and Tatum has popularized the generally accepted view that enzymes are derived somehow or other from genes. Their work initiated a new interest in biochemical genetics. They showed that the inactivation of a specific gene caused a deficiency which could be met by supplying a specific chemical. Vitamins, amino acids, purines, and pyrimidines were the substances chosen in this analysis. They used the fungus, Neurospora, because its life cycle had been thoroughly worked out by B. O. Dodge and because the Lindegrens had shown by genetical analysis that it contained conventional chromosomes on which genes, arranged in linear order, could be mapped by the standard procedures used in studying corn and the fruit fly.

YEAST GENETICS

Until 1935, yeasts were considered to be devoid of sex and, therefore, unsuitable for genetical analysis. At that time, Winge showed that the standard yeast cell carried two sets of chromosomes—one contributed from each parent—and was, therefore, a typical hybrid. The hybrid yeast cell produces four spores, each with a single set of chromosomes. Each of these spores is a sex cell. By fusing in pairs they can produce the standard (hybrid) yeast cell and complete the life cycle. In this laboratory it was shown that the spores are of two mating types, and that each spore can produce a culture each cell of which can act as a sex cell, like the original spore. Mass matings between two such spore-cultures result in the production of fusion cells, from which new hybrids are produced by budding.

This work made it possible to study the inheritance of biochemical deficiencies in the organism on which classical enzyme study is based, and to attack the problem of the relation of genes to enzymes in this fruitful material. We have related specific genes to several of the most thoroughly studied classical enzymes: sucrase, maltase, alpha methyl glucosidase, melibiase, and galactase.

The principal advantages of yeasts for biochemical genetics are:

(1) Yeast enzymes have been the subject of intensive biochemical study.

(2) Techniques for studying respiration and fermentation are based principally on work with yeast and thus especially adapted to this organism. Yeasts grow as free cells rather than as mycelial matts and, therefore, can be subdivided any number of times without injury, thus simplifying weighing and dilution of the cells.

(3) Large quantities of cells are available from industrial sources or can be grown cheaply and quickly and are easily stored in living condition.

(4) A variety of genes concerned with the differential utilization of numerous monoses as well as di- and poly-saccharides are available.

(5) A polyploid series of yeast cultures is now available: (*a*) haploid cells, each containing a single set of chromosomes, (*b*) diploid yeast cells, each containing the double number of chromosomes, (*c*) triploid, and (*d*) tetraploid cells (made available by our recent discovery of diploid gametes [Lindegren and Lindegren, 1951]).

(6) With free cells it is possible to study competition between genotypes and to observe the advantages or disadvantages in controlled environments. The populations involved are enormous and the life cycles short, so it is possible to simulate natural selection in the laboratory. Experiments of this type have enjoyed an enormous vogue with bacteria, but it has not been possible to distinguish gene-controlled variation from differentiation. For this reason, experiments with bacteria cannot be interpreted in terms of the comparison between gene-controlled and other types of inherited characteristics.

CHROMOSOMAL INHERITANCE

In our selected breeding stocks of Saccharomyces, irregular segregations do not occur very frequently. In maize or Drosophila a similar frequency of irregularity would not be detectable since tetrad analysis is not possible in these forms. Using regularly segregating stocks of Saccharomyces we have mapped four and possibly five chromosomes for genes controlling the fermentation of carbohydrates and the synthesis of various nutrilites (Fig. 16.1).

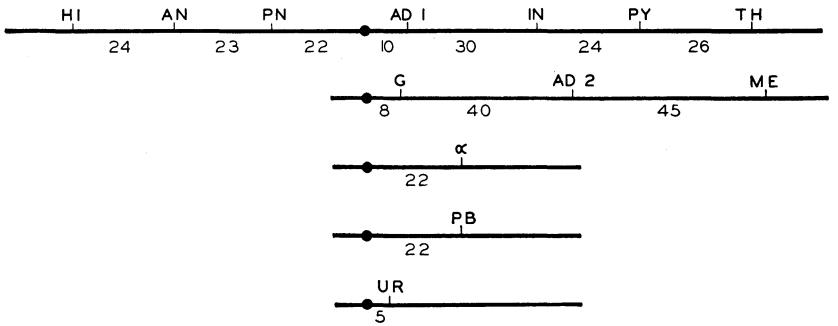

FIG. 16.1 Chromosome maps of Saccharomyces.

Chromosome I, PN (pantothenate), centromere, AD1 (adenine), IN (inositol), PY (pyridoxine), and TH (thiamin).

Chromosome II, centromere, G (galactose), AD2 (adenine), ME (melibiose).

Chromosome III, centromere, α (mating type).

Chromosome IV, centromere, PB (paraminobenzoic acid).

Chromosome V, centromere, UR (uracil).

Chromosomes IV and V may or may not be different; UR and PB have not been used in the same hybrid.

HI (histidine) and AN (anthranilic acid) are linked to each other (24 morgans) but have not yet been located on a chromosome.

DIRECT TETRAD ANALYSIS

The focal point in the life cycle is the reduction division, at which the chromosomes of a diploid cell are sorted out, and the haploid sex cells (such as sperm, eggs, pollen, or yeast spores) are produced. Each diploid parent cell divides twice to produce a tetrad of four haploid sexual nuclei. This process is substantially the same whether a single yeast cell produces four spores or a cell in the testis produces four sperm. In yeast, however, each of the four spores of a single tetrad can produce clones which are available for individual study, and the reduction division can be analyzed directly instead of by inference.

Many yeast hybrids have been produced by mating sex cells carrying chromosomes marked with biochemical mutant genes. The tetrads from these hybrids have been analyzed by growing clones from each of the four spores of a single ascus and classifying each of the spore-cultures. These experiments are *direct tests of the Mendelian theory.* They have shown that exceptions to the Mendelian theory occur more frequently than was hitherto supposed.

CURRENT STATUS OF IRREGULAR MENDELIAN SEGREGATION

Tetrad analysis of triploid and tetraploid yeasts has revealed that some of the irregular (not 2:2) segregations in hybrid asci arise from the fact that one or both of the parents is diploid (Lindegren and Lindegren, 1951; Roman, Hawthorne, and Douglas, 1951). Roman, Hawthorne, and Douglas have concluded that all irregular segregations in Saccharomyces arise from the segregation of triploid or tetraploid zygotes. We have recently completed the analysis of segregation in diploid hybrids heterozygous for both *MA/ma* and *MG/mg*. This analysis revealed that in many asci in which segregation of *MA/ma* was 2:2 (*MA MA ma ma*), segregation of *MG/mg* was 1:3 (*MG mg mg mg*). This finding excludes the possibility that the hybrid was either triploid or tetraploid since segregation of both genes would have been equally affected. The phenomenon has been explained as conversion of the *MG* gene to *mg* in the zygote. This conclusion is further supported by evidence indicating that both genes are in the same linkage group.

One hypothesis of the nature of the gene developed during the study of irregular segregation seems to have some merit. This is the proposal that the gene is a complex of many more or less loosely connected molecules rather than a single macromolecule. In this view, the gene is composed of a series of identical sites around the periphery of a more or less cylindrical chromosome. These sites may be extremely numerous since they are of molecular dimensions around the periphery of a thread easily visible under the microscope. At these sites identical agents responsible for the action of the gene are located.

GENE DIVISION

The concept of the gene as a *bracelet* of catalysts arranged on the outside of the chromosome simplifies the concept of gene reproduction. When one conceived of genes as macromolecules arranged like *beads on a string*, it was difficult to understand how all the genes on a chromosome could divide simultaneously. If, however, there are thousands of loci and chromogenes at the site of a single gene on the *outside* of an otherwise inert chromosome which is composed principally of *skeletal* material, any longitudinal splitting of the chromosome will partition two qualitatively equivalent parts which may or may not be quantitatively equivalent. The restoration of balance by *interdependence* of the autonomous organelles may make precise division unnecessary.

EXTRACHROMOSOMAL INHERITANCE

When a pure haplophase culture of red yeast (adenine dependent) is planted on an agar plate, both red and white colonies appear. When the white colonies are subcultured, only white colonies appear. The red cells when planted on a second plate continue to produce both red and white colonies. The white colonies are stable variants derived from red. Bacterial variations of this type are ordinarily called gene mutations, but bacteriologists have been unable to test their so-called mutations by breeding experiments except

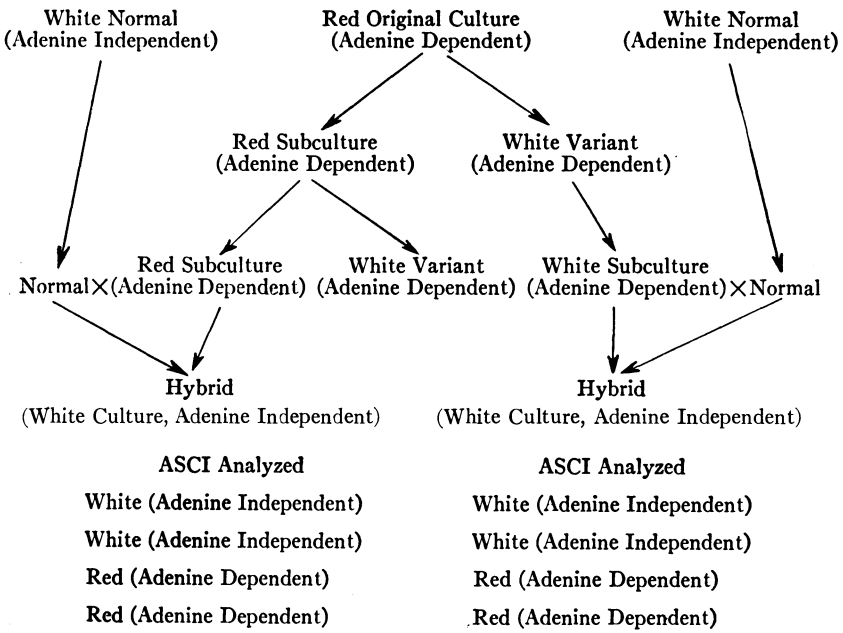

FIG. 16.2—Inheritance of pink versus white colony in Saccharomyces. The white mutants derived from pink produce pink offspring and are indistinguishable from the original pink genetically.

in a few cases. The white cultures have lost their color but they are still characteristically adenine dependent like their red progenitor. Breeding experiments (Fig. 16.2) have been carried out with the white yeast cultures derived from the red. When the derived white cultures were used as parents, they produced precisely the same kind of offspring as the original red culture from which they arose. This proves that the change from red to white did not affect a gene. The change from red to white may, therefore, be called a *differentiation* since it occurs without gene change.

The phenomenon of Dauermodifikation which was first described by Jollos (1934) has thus been confirmed in yeast genetics. The stable change from

red to white resembles the discontinuous variations which occur in the vegetative cycles of bacteria. Hybridization experiments have revealed that the origin of white cultures does not involve gene change. This phenomenon in yeast, called *depletion mutation*, is identical with Dauermodifikation in Paramecia. Since neither involves gene change, both are equivalent to differentiation.

It is not possible to study Dauermodifikationen using the classical objects of genetical research, maize and Drosophila, since each generation of these higher organisms is produced sexually—a process during which Dauermodifikationen revert to normal. The stable variants in vegetative cultures of yeast, which revert to normal (produce only normal offspring by sexual reproduction) have no parallel in maize and Drosophila. This points up a striking disadvantage of maize and Drosophila—that they cannot be propagated vegetatively. One cannot be certain that the characteristic variations in flies, which occur when they hatch on wet medium or are subjected to shock treatment, would be lost on vegetative cultures unless one were able to propagate the bent wings or other peculiarities asexually, possibly in tissue culture.

THE AUTONOMOUS ORGANELLES OF THE YEAST CELL

In addition to the chromosomes (Lindegren, 1949) there are other permanent structures in the yeast cell which never originate *de novo* (Lindegren, 1951). They have the same type of continuity in time as chromosomes but are less precisely partitioned than the latter.

The Cytoplasm

The cytoplasm is a limpid fluid which is transmitted to each daughter cell. It is rich in RNA but varies in basophily and contains the mitochondria, usually adhering to the surface of the centrosome or the nuclear vacuole.

The Mitochondria

The state of the mitochondria varies from highly refractile lipoidal structures, sharply defined from the cytoplasm to less refractile organelles with somewhat irregular boundaries.

The Centrosome

The centrosome is a solid and rigid structure which stains with acid fuchsin but does not stain with basic dyes. This highly basic organelle may contain some of the basic proteins which Caspersson and Mirsky have found in chromosomes. The centrosome is always attached to the nuclear vacuole and is the most rigid structure in the cell as revealed by its behavior following shrinkage of the cell. It never originates *de novo* and plays a leading part in budding, copulation, and meiosis.

The Centrochromatin

The centrochromatin is a basophilic, Feulgen-positive substance closely attached to the basic centrosome (probably by an acid-base reaction). Some portion of it is usually in contact with the nuclear vacuole. It is partitioned between the cells following budding by a direct division controlled by two tiny centrioles. In the resting cell it may assume a spherical form and cover most of the centrosome. In division it is usually present in the form of a long strand. The centrosome and centrochromatin have been identified with the nucleus by several workers, but this view has been criticized by Lindegren (1949), Lindegren and Rafalko (1950), and Rafalko and Lindegren (1951). The filament often bends on itself to assume a **V**- or **U**-shape. In some preparations it appears to be composed of numerous small particles, but this is due to poor fixation and is especially prevalent in preparations fixed with alkali. The view that the centrochromatin is a single filament external to the centrosome is supported by a multitude of observations on well-fixed cells. Centrochromatin is probably homologous to the heterochromatin of higher forms differing only in being carried on the centrosome rather than the chromosome.

The Nuclear Membrane and the Chromosomes

The nuclear vacuole contains the chromosomes and the nucleolus. The chromosomes are partitioned between mother and bud vacuole in a precise orderly manner without recourse to a spindle. The wall of the nuclear vacuole does not break down at any time in the life cycle; it is a permanent cellular structure.

The Cell Membrane and the Cell Wall

The cell membrane is a permanent cell structure. The cell wall appears to be formed *de novo* in the spores, but it may depend on the cell wall surrounding the ascus for its origin.

BUDDING

Figure 16.3–1 shows a cell in which the acidophilic centrosome attached to the nuclear vacuole is surrounded by the darkly staining cytoplasm. A band of basophilic centrochromatin is securely applied to the side of the centrosome and is also in contact with the nuclear vacuole. Greater differentiation often reveals a small centriole at each end of this band. The nuclear contents are unstained.

Figure 16.3–2 shows the first step in the process of budding. The centrosome produces a small conical process which forces its way through the cytoplasm and erupts into the new bud shown in Figure 16.3–3.

Figure 16.3–4. The nuclear vacuole sends out a long, slender process which follows the centrosome into the bud. Although the cell wall is not visible in these preparations it must be assumed that the cell wall never ruptures but is

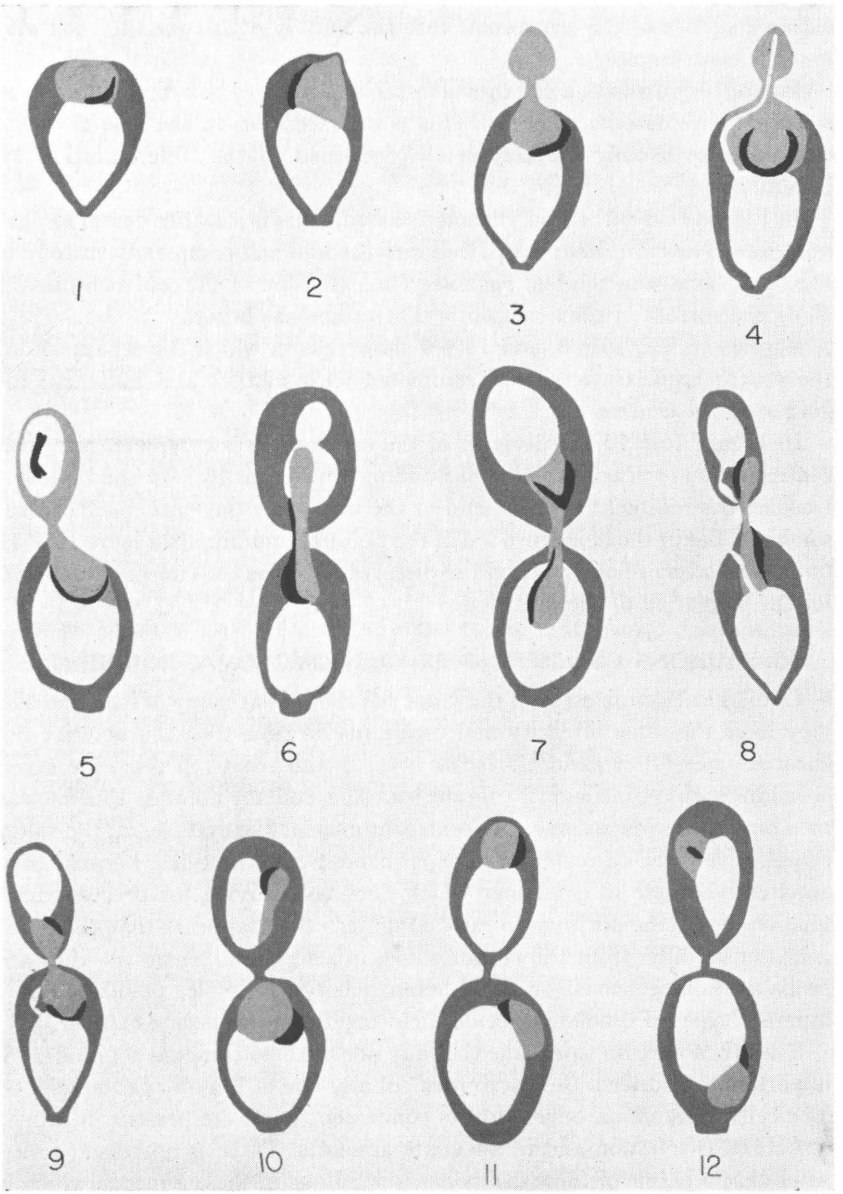

FIG. 16.3—Behavior of the centrosome and centrochromatin during budding.

extended to enclose the bud at all times. The vacuolar process follows the external surface of the centrosome into the bud, lying between the cell wall and the centrosome.

Figure 16.3–5 shows a cell in which the bud vacuole has received its two-stranded chromosome complex. This is an exception to the rule that the chromosomes usually are completely destained in the differentiation by iron alum.

In Figure 16.3–6 the bud vacuole is lobed. This is a rather common phenomenon. The cytoplasm has passed into the bud and completely surrounds the centrosome and the bud vacuole. The extension of the centrochromatin along the surface of the acidophilic centrosome has begun.

Figures 16.3–7, 16.3–8, and 16.3–9 show cells in which the separation of the centrochromatin has been completed with mother and bud held together by the centrosome.

In Figure 16.3–10 the division of the centrosome is complete, but both centrosomes are near the point of budding. In Figure 16.3–11 the bud centrosome has reached the distal end of the bud while the mother cell centrosome still lies in the neighborhood of the point of budding. In Figure 16.3–12 both centrosomes have reached the distal ends of the cells and are prepared for the formation of the next bud.

CONCLUSIONS CONCERNING EXTRACHROMOSOMAL INHERITANCE

Cytological examination of the yeast cell shows that many of its organelles may have the same integrity and continuity in time that characterize the chromosomes—they cannot arise *de novo*. In the yeast cell there are seven or eight such "continuous" organelles. The cell membrane, the nuclear membrane, the centrosome, the centrochromatin, the cytoplasm, the mitochondria, and the chromosomes are permanent cell structures. Because they apparently divide in a manner which does not provide for precise transmission of specific portions to each daughter cell, it appears that the other components differ from the chromosomes in a significant manner—they are probably homogeneous, or their heterogeneity is simple, possibly a few different types of dipolar molecules held together in a specific manner.

There is no reason to assume that any one of these components is of more importance, or directs the "activities" of any one of the other components. The cell can function only if all its component parts are present in proper structural correlation and in adequate amounts. There is no reason to assume that any one of these components is unique in the manner in which it reproduces itself. The present hypothesis proposes that they all reproduce by the simple accretion of molecules like those which they contain, and it is their association with each other in an adequate milieu which provides the molecules necessary for their increase in size. Each of the different organelles is rate limiting in growth. When any one is present in less than the minimal

amount, the other organelles cannot obtain the supply of molecules necessary for maintenance and increase until the amount of the deficient organelle has increased.

The chromosomes differ from the other permanent organelles in their high degree of linear heterogeneity. It is this characteristic which has given them the spurious appearance of "controlling" other cellular activities. Mutations with which we are familiar in the laboratory constitute defects or deletions in the extraordinarily heterogeneous chromosomes. The deficiency in the organism caused by the defect—the deletion of the contribution ordinarily made by the intact region of the chromosome—becomes apparent only because the rest of the chromosome produces sufficient materials to enable the defective cell to continue to grow in its absence, although in a manner different from that which was previously characteristic.

Any transmissible defect in a homogeneous structure like the cell wall, the cell membrane, the nuclear membrane, the centrosome, or the centrochromatin would result in total failure of the organism to survive and bring all vital activity to a halt. The survival of the defective mutants in their altered condition due to the defect in the chromosome (which has been called a mutant gene) has led to the view that genes are different from other cellular components since they can *reproduce variations in themselves*. This is an incorrect point of view. It is more proper to say that when a defect or deletion occurs in a small segment of a chromosome, the rest of the organism can carry on, albeit in a changed condition due to the absence of the contribution previously made by that region, now called the gene. This denies the importance of the ordinary mutations encountered in the laboratory as factors for progressive evolution, and implies that progress in evolution must occur in some other way.

It may be that progressive evolution occurs more frequently as the result of changes in the chromosomes than of other organelles. But the present hypothesis does not exclude the possibility that advances in evolution can occur by "progressive" changes in the composition of any one of the *eternal* organelles such as the nuclear membrane or the centrosome. The condition for the perpetuation of any change would be that the mutated organelle could be provided with the materials necessary for its continuance by the cell as a whole in its surrounding environment *at the time of its occurrence.* On this hypothesis, progressive changes in evolution are not confined to any single cellular component, but constitute a potential of every component of the cell. Although progressive changes of the different substances comprising the chromosome may not occur significantly more frequently than changes in the substances making up the other organelles, more changes may occur in the chromosomes *in toto* because a change in each individual component of the extraordinarily heterogeneous chromosome registers as a separate change.

In many types of organisms the chromosomes are always separated by the nuclear membrane from the cytoplasm. The mitochondria (like the chromosomes) are relatively non-homogeneous, but apparently the balance of their activities is not so critical since no specific devices appear to be required to limit their reproduction or activity. The cytoplasm is probably heterogeneous also, with every separate eternal component having the same continuity in time as the chromosomes. However, it comprises substances transmitted to the daughter cells in a manner which is apparently subject to control by the environment, and this may constitute the basis for differentiation. In the germ line, the *entire* cytoplasmic potential must be maintained. In fact, the main function of the germ line under this hypothesis would be to maintain an intact cytoplasm. The integrity of the chromosomes is usually provided for in either the somatic or the germinal tract. Defects in the extra chromosomal apparati are reconstituted in an outcross, thus differentiating so-called *cytoplasmic* from *genic* inheritance.