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Saccharomyces cerevisiae Cell Cycle

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INTRODUCTION

The yeast *Saccharomyces cerevisiae* is a single-celled organism capable of rapid division on defined medium. Each cell reproduces by budding and the bud grows in size throughout the cell cycle, thereby providing a morphological indicator of cycle progress. The organism possesses an elegant and extensively exploited genetic system. Cells of ploidy from haploid to octaploid undergo the same mitotic cycle so that recessive mutations affecting cycle progress can be detected in haploids and analyzed by complementation in diploids, and the effects of gene dosage can be examined in polyploids. Furthermore, the basic elements of cell structure, macromolecule synthesis, chromosome replication, and chromosome segregation in *S. cerevisiae* are extensively homologous to those in higher plant and animal cells. These facts taken together suggest that *S. cerevisiae* may be the most tractable of experimental organisms in current use for a combined genetic, physiological, and biochemical analysis of the mitotic cell division cycle in eukaryotes. This review constitutes an attempt to relate the potential of this organism for studies on cell division and to review our current meager understanding of this process in *S. cerevisiae*. Readers seeking a less parochial treatment of the cell cycle are referred to an excellent monograph that synthesizes observations from many organisms including prokaryotes and eukaryotes (114).

In considering the cell cycle as a whole one is impressed with the diversity of biological processes that must be coordinated to assure the ultimate goal of cellular reproduction. Mitchison appropriately stated "My thesis ... is that we can find the same processes of morphogenesis and periodic gene expression in the miniature system of the cell cycle and that differentiation can be found and investigated as properly here as in the whole developing organism" (115). I find it useful to consider the problems of morphogenesis and differentiation in the cell cycle in terms of three questions which are

admittedly neither comprehensive nor mutually exclusive:

How are the events of the cell cycle coordinated? How does the cell assure that any two discontinuous cell cycle events such as deoxyribonucleic acid (DNA) synthesis and nuclear division always occur in the proper order?

How is cell division controlled? How does the cell arrest the mitotic cell division cycle and commence upon an alternative developmental program such as sexual conjugation, meiosis, or stationary phase?

How is growth integrated with division? How does the cell accomplish the precise doubling of all of its macromolecular components between two consecutive cell cycles under conditions of balanced growth?

The material selected for the present review was chosen in large part because of its relevance to one or more of these questions. Although it will not be possible at the present time to provide satisfying answers to any of these questions, it will be possible during the course of this review to rephrase them. Whether that accomplishment represents an exercise in semantics or a measure of progress, the reader must decide.

The discontinuous events that occur once during each cell cycle constitute *landmarks* of cell cycle progress by which we can assess a cell's position. The landmarks of the *S. cerevisiae* cell cycle will be discussed in greater detail below, but it may be useful to consider their temporal order at this time (Fig. 1). For reasons that will become apparent later it is appropriate to consider the cycle as commencing with an unbudded cell in the G1 interval of the cycle. The nucleus contains a single-spindle plaque, a structure embedded in the nuclear membrane from which microtubules arise. Three events, whose precise temporal order has not been determined, mark the end of the G1 interval: spindle plaque duplication, the initiation of DNA synthesis, and the emergence of the bud. The DNA synthetic interval or S period constitutes about 25% of the cycle. The spindle plaques separate to form the complete spindle, and the bud grows in size throughout the remainder of the cycle. The end of G2 is marked by the migration of the nucleus to the neck of the cell where it undergoes the first stage of nuclear division, medial nuclear division, concomitant with the elongation of the spindle microtubules. The second stage of nuclear division, late nuclear division, is followed by cytokinesis, or cell membrane separation, which is followed in turn by cell wall separation. This event completes the cycle with the production of two unbudded cells. A diploid cell growing at the optimal temperature, 30 C, may complete a cycle in about 100 min. Although the literature on the *S. cerevisiae* cell cycle will be extensively referenced below, it is appropriate to acknowledge at this point that much of our current understanding of the yeast cell cycle is the result of pioneering research carried out for several years in the laboratories of H. O. Halvorson, C. F. Robinow, and D. H. Williamson.

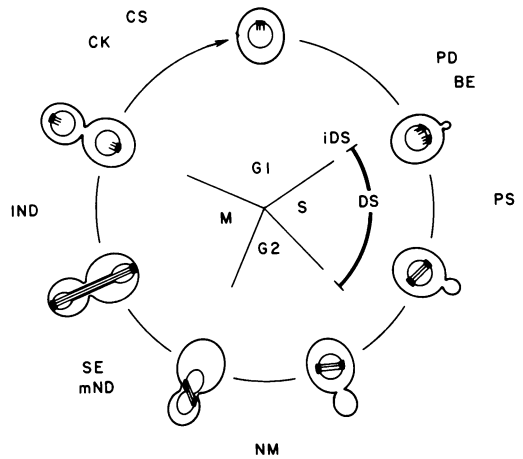

FIG. 1. *Landmarks of the S. cerevisiae cell division cycle. Abbreviations: PD, plaque duplication; BE, bud emergence; iDS, initiation of DNA synthesis; DS, DNA synthesis; PS, plaque separation; NM, nuclear migration; mND, medial nuclear division; SE, spindle elongation; lND, late nuclear division; CK, cytokinesis; CS, cell separation. Distance between events does not necessarily reflect interval of time between events.*

SYNCHRONOUS CULTURES

Assay of Landmarks

The experimental analysis of the cell cycle frequently requires synchronous cultures and a number of methods employing either induction or selection (114) have been devised to achieve partially synchronous division of *S. cerevisiae*. In such experiments, it is necessary to follow at least one landmark as a measure of the degree of synchrony, and more than one is preferable. A brief discussion of the synchrony techniques available for yeast can be usefully integrated with another methodological consideration, how each of the landmarks are assayed.

The most convenient landmark is bud emergence because it can be monitored by direct visual examination and there is essentially no subjective element involved in distinguishing an unbudded from a budded cell.

DNA synthesis has also been used as a measure of synchrony. However, because the S period occupies a significant but not easily determined portion of the cycle, this measurement is inherently less accurate than that of

bud emergence. DNA synthesis cannot be monitored in yeast by thymine or thymidine incorporation, possibly because the organism lacks thymidine kinase (59); however, it can be measured by incorporation of radioactive adenine (18, 187) or uracil (69) into alkali-resistant, acid-precipitable material. Recently mutants capable of incorporating thymidine 5'-monophosphate have been described (16, 89, 90, 182), and these may greatly facilitate DNA studies in yeast.

Nuclear division has been used to follow synchrony and is in principle a good marker, because during the brief interval between nuclear migration and medial nuclear division the nucleus is found in the neck of the cell and this stage can be used as a "mitotic index." Visualizing the position of the nucleus requires fixation of the cells and staining, usually with Giemsa (145).

Cytokinesis can be monitored by fixing cells with formaldehyde followed by the digestion of the wall with snail digestive juice (71). If the parent cell and bud come apart following this treatment, they are presumed to have completed cell membrane division or cytokinesis. The event measured empirically in this manner follows nuclear division and precedes cell separation, but there is no direct evidence that it coincides with cytoplasmic membrane division.

Most frequently cell division, measured either by particle count or by visual examination, has been used to monitor synchrony. When a particle-counting device is employed, it is usually necessary to sonically treat samples prior to counting in order to facilitate separation of cells that have completed physiological division, but that may otherwise remain adhered for several cell cycles (147). As an alternative to sonication, cells have been scored by visual examination in a hemocytometer by using the following convention. A particle with a single bud is scored as one cell and a particle containing two or three buds is scored as two cells (192). This convention is based upon the observation that under conditions of balanced growth, cell separation in one cycle precedes bud emergence in the next cycle (192). Of course, this method does not score the cell division event itself, but rather the immediately succeeding bud emergence event. Finally, it should be pointed out that some authors have employed a different convention by counting a unit with a bud as one cell until the bud reaches a certain size, whereupon the unit is counted as two cells. This latter procedure is not recommended because it is both arbitrary and subjective.

Other parameters of cell cycle progress and cell synchrony include particle volume distributions (64, 150, 168), the ratio of bud size to parent cell size (155), and the stepwise appearance of certain enzymes (65).

Synchrony once attained frequently decays rapidly. The parent cell always buds considerably before the daughter cell in haploid strains (19) as well as in diploids that are homozygous for mating type, and the same is probably true of diploid strains growing slowly on poor carbon sources. Hence, the longest maintenance of synchrony is to be expected with diploid or higher ploidy cells growing rapidly. It is quite a simple matter to determine whether or not a particular combination of strain and culture conditions produces synchronous budding of parent and daughter cell and is therefore conducive to the maintenance of synchrony. Because cell association is frequently maintained after mother and daughter have both budded, one can merely examine asynchronous cultures microscopically to see if such pairs have equal or unequal size buds.

Induction Synchrony

Any method that induces an asynchronous population to become synchronous is subject to the criticism that the inducing treatment may cause profound changes in cellular physiology and lead to aberrant behavior during the ensuing synchronous cycle. An advantage of induction synchrony, however, is the large yield of synchronous cells that can be produced by these methods.

One of the oldest and still most popular methods of achieving synchronous yeast cultures is that devised by Williamson and Scopes (185, 195) which involves shifting stationary-phase cells between growth medium and starvation medium coupled with the selection of the larger cells in the population. This technique takes advantage of the fact that stationary cultures of yeast produced by many types of nutritional deprivation (see section on growth integrated with division) are arrested synchronously with respect to cell cycle progress at a point between cell separation and the initiation of DNA synthesis. However, stationary-phase cells are usually quite heterogeneous in size, and the degree of synchrony in starting new cell cycles upon subculturing in fresh medium is markedly improved by selection of the larger cells in the population (184). The media shifts may induce some additional phasing of the cells (193). The method is capable of producing enormous quantities of highly synchronous cells, but suffers from the fact that two processes are occurring simultaneously, synchro-

nous division and synchronous emergence from stationary phase. The stepwise occurrence of any particular event may be a consequence of the latter as well as the former.

Any method that arrests cell division at a unique stage is potentially capable of giving rise to a synchronous culture. X rays (163), hydroxyurea (159), temperature-sensitive *cdc*-mutants (130), and the mating substances, α factor (18, 73) and *a* factor (L. Wilkinson and J. Pringle, submitted for publication), have all been used in this capacity with some degree of success.

Selection Synchrony

Selection synchrony involves selecting a small portion of an asynchronous population of cells on the basis of some physical attribute which changes during the cell cycle. The method suffers from the disadvantage that only a small percentage of the original population (usually less than 10%) can be used and, hence, has a much lower yield than induced synchrony techniques. One major advantage is that the technique does not require unbalanced growth and should, in theory, therefore cause fewer perturbations in the ensuing cycle. Furthermore, selection techniques can be adapted to examine events that occurred prior to the selection procedure during asynchronous growth, thus avoiding the perturbation problem altogether.

Two methods have been employed to achieve selection synchrony. One is the method of Mitchison and Vincent (116) which involves selecting the youngest cells by their slow rate of sedimentation in a sucrose gradient. This technique has been applied to *S. cerevisiae* by Tauro, Halvorson, and Carter (64, 168) who obtained a fraction of cells containing 80% unbudded cells which displayed partially synchronous growth upon subculturing. However, this method did cause physiological changes in the cells because a 1-h lag in the commencement of division was observed in an asynchronous culture derived by recombining all of the fractions from such a sucrose gradient (168). Perhaps a substance of lower osmolarity than sucrose would not produce this detrimental effect.

Sebastian, Carter, and Halvorson have used rate sedimentation on sucrose gradients in a zonal rotor to fractionate an asynchronous population of cells according to size (150) and, hence, according to age in the cell cycle. They were able to obtain synchronous cultures from samples removed from the top, the middle, or the bottom of the gradient. Because the entire cycle is displayed on the gradient, this method has two important advantages. First, all of the cells in the population can be used, and, second, subculturing of the cells is often unnecessary. Direct chemical measurements on the fractionated cells allowed an analysis of compositional changes in DNA and enzymes during the cell cycle, and pulse labeling with a radioactive precursor prior to fractionation would permit an analysis of synthesis during the cycle. It would be helpful for assessing the potential utility of this technique if additional parameters were monitored in the gradients, such as the presence or absence of buds, bud size, and the morphological stage of the nucleus.

A second method takes advantage of the observations by Mitchison (112) and Scopes and Williamson (147) that mass and volume do not increase coordinately during the yeast cell cycle, thus producing a density fluctuation. Upon banding an asynchronous culture isopycnically in a density gradient of low osmolarity, the cells are fractionated so that the lightest cells are near the end of the cycle and the densest cells are near the time of DNA synthesis (69, 183). Partially synchronous cultures can be obtained from either fraction.

This selection technique has also been adapted to avoid the necessity of subculturing the cells after fractionation. In one experiment an asynchronous culture of *S. cerevisiae* was pulse labeled with radioactive amino acids, and then the population of cells was isopycnically banded (155). By determining the radioactivity incorporated into ribosomal proteins isolated from the lightest or the densest cells, it was possible to follow the rate of synthesis of these proteins at two points in the cell cycle. Furthermore, by interspersing a chase period between the time of labeling and the time of isopycnic banding, synthesis at any time in the cycle could be examined.

CELL DIVISION CYCLE MUTANTS

Diagnostic Landmarks and Terminal Phenotypes

One of the strong advantages of *S. cerevisiae* as an experimental organism is its genetic system (for a review, see reference 123). This characteristic has been exploited for cell cycle analysis in the isolation of cell division cycle (*cdc*) mutants (75). In theory, a *cdc* gene product is necessary for one and only one of the discontinuous events of the cell cycle. Empirically, a conditional mutation in a *cdc* gene is recognized by the fact that, after a shift to the restrictive condition, all of the mutant cells from an asynchronous clone first exhibit defec-

tive behavior at the same landmark in the cell cycle. This landmark will be defined as the diagnostic landmark (the term initial defect has been applied to this parameter previously [74], but the word initial carries a misleading connotation). A consequence of the fact that all cells from an asynchronous population first arrest normal development at the same landmark is that nearly all of the mutant cells assume the same, albeit often aberrant, morphology (the *terminal phenotype*, previously termed the termination point [75]) after extended incubation at the restrictive temperature. It is this latter characteristic that has permitted the detection of *cdc* mutants (75, 76) from among a larger collection of temperature-sensitive mutants (68).

Cells carrying the *cdc* 13 mutation that were fixed and stained before and after incubation at the restrictive temperature are displayed in Fig. 2a and b, respectively. The diagnostic landmark for this mutant is medial nuclear division. The population of cells arrests after having completed bud emergence, DNA synthesis, and nuclear migration, but before completing medial nuclear division, late nuclear division, cytokinesis, or cell separation. Bud growth continues, but no new buds appear. Hence, the terminal phenotype is a cell with a large bud and a nucleus located in the cell neck. The identical morphologies of these cells are strikingly different from the originally asynchronous cell population and from other non *cdc* temperature-sensitive mutants even when the nucleus is not revealed by staining. The terminal phenotype for different *cdc* genes is often different, but is the same for all alleles of a particular gene, and this fact strongly suggests that each of the *cdc* genes does indeed perform an indispensible function in one and only one of the discontinuous cell cycle events (76).

First Cycle Arrest and Execution Points

cdc mutants can be classified into two categories depending upon the number of cell cycles that they complete after a shift to the restrictive temperature. In the first category are *cdc* mutants that traverse several cell cycles after a shift to the restrictive temperature. In the second category are mutants that behave as follows: those cells that are before a certain point in the cell cycle (*the execution point*) arrest development in the first cycle at the restrictive temperature and those cells that are after the execution point complete the first cycle, and arrest in the second cell cycle (75). Mutants in this second category are said to exhibit *first cycle arrest*, because a cell that commences the cycle at the restrictive temperature will arrest in the first cycle.

In several *cdc* genes there are representatives of alleles that do give first cycle arrest and other alleles that do not give first cycle arrest (76). Mutants carrying different alleles of the *cdc* 16 gene are displayed in Fig. 3 before and after incubation at the restrictive temperature. These two mutants both arrest with the same terminal phenotype as that displayed in Fig. 2, a cell with one large bud, but one allele permits several cycles of division before arrest at the restrictive temperature and the other allele causes arrest in the first or second cycle depending upon the position of the cell in the cycle at the time of the shift. Although there are several molecular mechanisms consistent with these two responses, the presence in a gene of alleles that do not exhibit first cycle arrest suggests that this gene product is normally present in excess. Hence, other alleles of this same gene that do exhibit first cycle arrest are probably thermolabile for the function and not for the synthesis of the gene product in question.

For those mutant strains that do exhibit first-cycle arrest the execution point can be determined by examining many cells by time-lapse photomicroscopy (75, 76). A mutant defective in gene *cdc* 13 before and several hours after a shift to the restrictive temperature is displayed in Fig. 4. The terminal phenotype of this mutant is a cell with a large bud. Cells that were early in the cell cycle at the permissive temperature, as evidenced by having no bud or a small bud, arrest at the restrictive temperature as one cell with a large bud. Cells that were later in the cell cycle, as evidenced by larger buds, arrest development after forming two cells each with a large bud. Therefore, the execution point for this mutant is passed during growth at the permissive temperature at a point in the cycle represented by the arrow in Fig. 4. From the fraction of cells before the execution point one can calculate the fraction of time in the cell cycle at which execution occurs after correcting for the age distribution of the population (76).

The execution point in some mutants considerably precedes the time of occurrence of the diagnostic landmark. The primary defect for such mutants must lie in some early, as yet undetected, process upon which the completion of the diagnostic landmark is dependent. For example, many of the nuclear division mutants exhibit execution points during the DNA replication period, and one surmises that processes which are necessary for chromosome segregation normally occur during chromosome

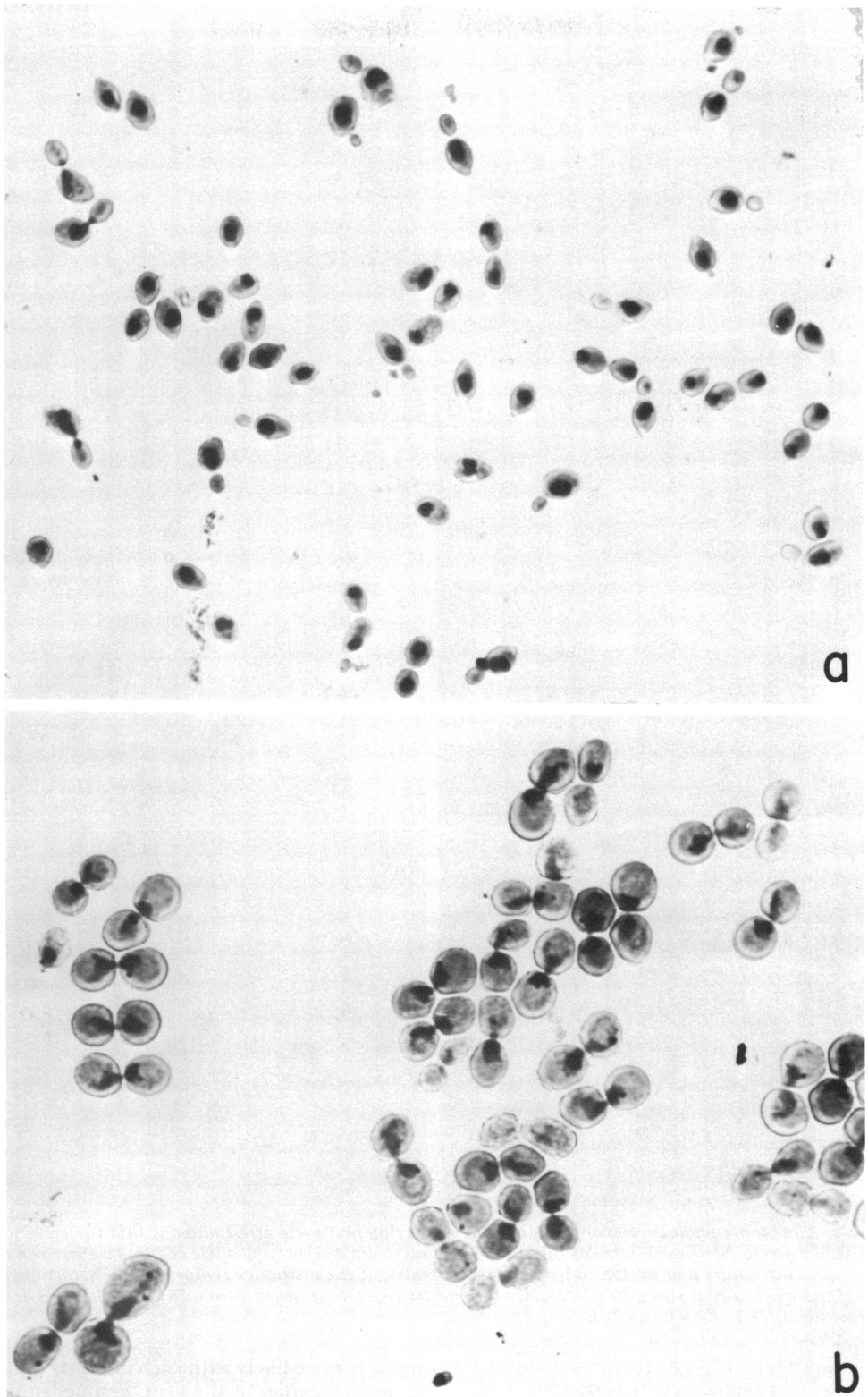

FIG. 2. *The terminal phenotype of cells defective in the cdc 13 gene. Cells were fixed and stained with Giemsa before (a) and 4 h after (b) a shift from the permissive to the restrictive temperature.*

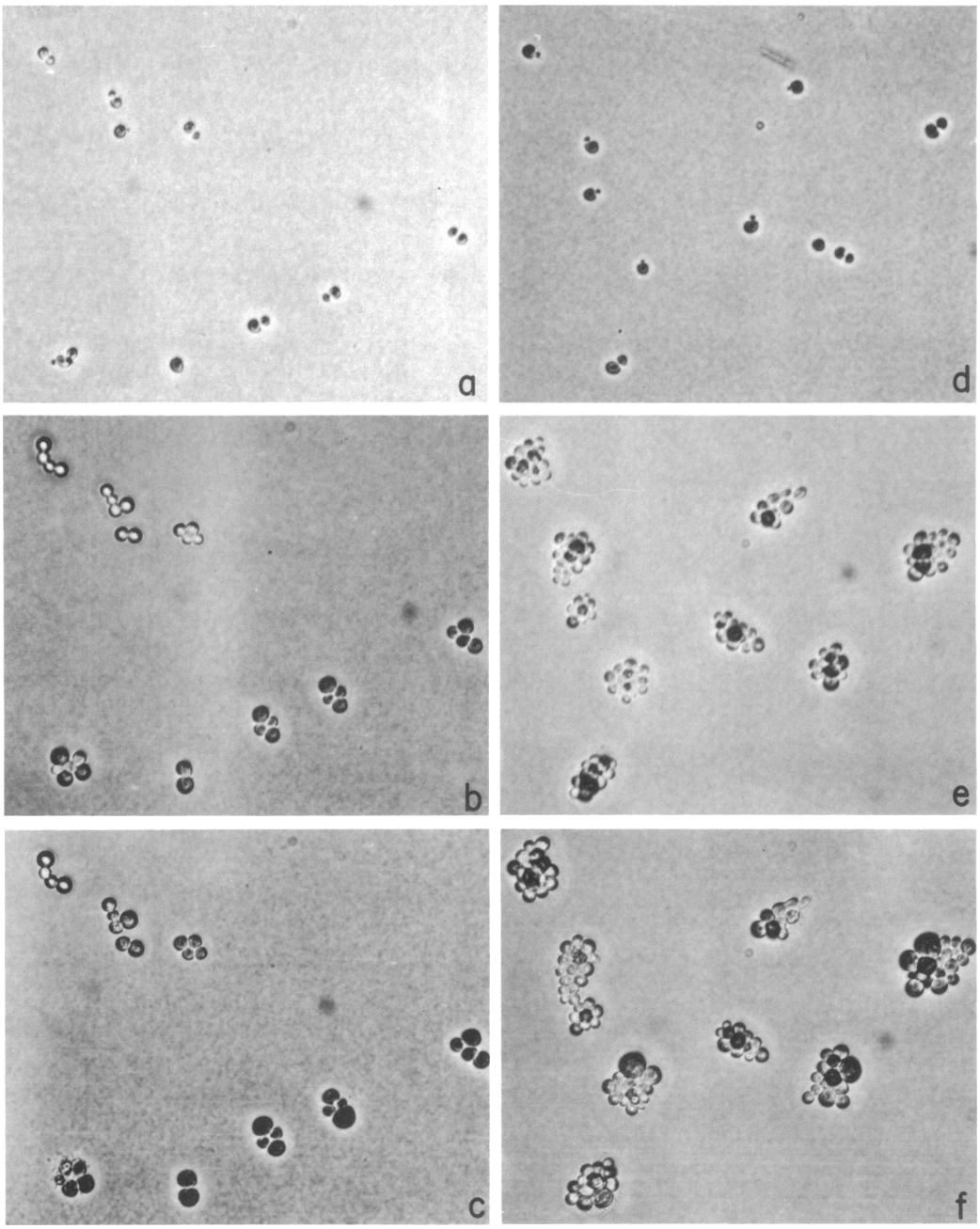

FIG. 3. *Time lapse photomicroscopy of a mutant displaying first cycle arrest and one that traverses several cycles before arrest. Strain 281 carrying allele cdc 16-1 and strain 486 carrying allele cdc 16-3 were grown at the permissive temperature and shifted to plates preequilibrated at the restrictive temperature. Photographs were taken of the same cells at successive intervals at the restrictive temperature. Strain 281, (a) 0 h; (b) 8 h; (c) 24 h; strain 486, (d) 0 h; (e) 8 h; (f) 24 h.*

replication (42). In other mutants such as the two with DNA replication defects, *cdc* 8 and 21, the execution point coincides with the time of completion of the diagnostic landmark (70, 73), and it is more likely with such mutants that the primary function of the gene product in question is to mediate an event closely associated with the diagnostic landmark.

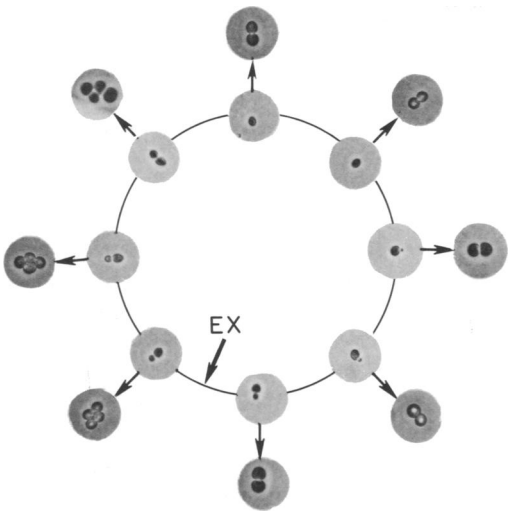

FIG. 4. *Determination by time lapse photomicroscopy of the execution point in a cdc mutant. Cells of strain 428D1 with a temperature-sensitive mutation in gene cdc 13 were grown at 23 C and shifted onto plates pre-equilibrated to 36 C. Cells were photographed at the time of the shift, and individual cells were cut from larger fields and arranged in order of bud size at the time of the shift (inner circle). The same fields were photographed after 6 h incubation at the restrictive temperature, and the cell(s) produced by each individual cell on the inner circle was cut out and arranged (outer circle). Each cell on the outer circle consists of a parent cell with a large bud attached. Cells early in the cycle (before EX) arrested development in the first cell cycle at the restrictive temperature and cells later in the cycle (after EX) finished the first cycle and arrested in the second cycle.*

The Mutant Collection

Examination of about 1,500 temperature-sensitive mutant clones by morphological criteria revealed the presence of 148 *cdc* mutants (76). Complementation studies demonstrated that these mutations define 32 complementation groups and that mutations in each of these groups are recessive. Genetic analysis suggested that 30 of these complementation groups are defined by single mutations in nuclear genes, and 14 of these genes were mapped on the yeast genetic map (76). (Recent results indicate that the single mutant representing *cdc* 22 carries two mutations, both of which are necessary for temperature sensitivity [L. H. Hartwell, unpublished data], and the mutant representing *cdc* 32 could not be analyzed because it produces inviable spores in crosses.) No evidence for the close clustering of functionally related genes was found in this study. A summary of the diagnostic landmarks, terminal phenotypes, and execution points of mutants with lesions in the *cdc* genes is presented in references 74 and 76. Mutant strains harboring these mutations may be obtained from the Yeast Stock Center, Dr. R. K. Mortimer, Donner Laboratory, University of California, Berkeley, Calif. 94720.

Role of cdc Genes in the Life Cycle

Although these mutations were isolated in haploid cells of *a* mating type during vegetative growth, subsequent analysis has demonstrated that most and perhaps all perform indispensible functions for the mitotic cycle at every stage of the life cycle. Life cycle stages that are basically distinct either in the origin of the cells or in the capabilities of the cells but which require a mitotic cycle include: vegetative growth in cells of *a*, α, and a/α mating type, cells emerging from stationary phase to vegetative growth, zygotes resulting from the fusion of two haploid cells, and spores germinating to vegetative growth (Fig. 5). Most of the *cdc* genes have been tested for their requirement in the mitotic cycle at each of these stages of the life cycle and with possibly only one exception all genes tested are required at all stages (76; Pringle, Maddox, Reid, and Hartwell, unpublished data).

Perhaps of greater interest is the question of whether the genes that mediate mitosis also perform indispensible functions for meiosis. The same *cdc* mutations have been tested by Giora Simchen for their role in the meiotic cycle (157). Of 20 genes that were tested, 13 (*cdc* 2, 4, 5, 7–9, 13, 14, 16, 17, 20, 23, and 28) appear to be essential for meiosis, because diploid cells homozygous for these mutations were drastically impaired in their ability to sporulate at the restrictive temperature and, in some cases, even at the permissive temperature.

COMPONENT PROCESSES OF THE CELL CYCLE

This section will review some of the current information available on the cytological, genetical, and biochemical processes that underlie the landmarks of the cell cycle. The reader is referred to the authoritative review by Matile et al. (108) for a more comprehensive discussion of yeast cytology.

Bud Emergence

The future daughter cell appears early in the cell cycle as a discrete morphological entity, the bud. The site at which the bud arises becomes a channel (the neck) connecting parent and daughter cell through which the nucleus and other organelles pass into the daughter cell, in which the nucleus divides and at which cytokinesis and cell wall separation take place.

Early in the cell cycle, prior to the emergence

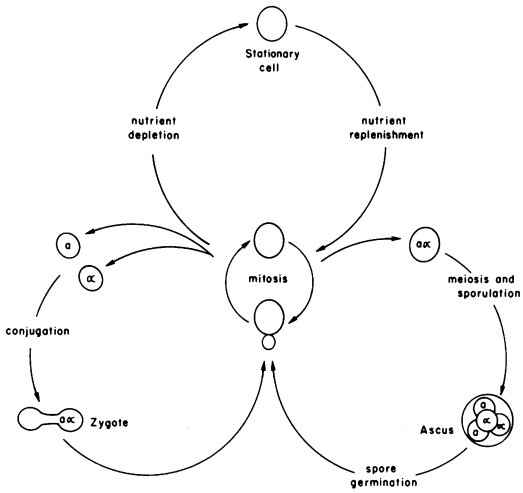

FIG. 5. *The life cycle of S. cerevisiae emphasizing the relationship of the mitotic cycle to the other developmental pathways; a and α refer to mating type alleles; unlabelled cells may be a, α, or aα. Mitotic haploid cells of mating type a or α may enter stationary phase under conditions of nutrient depletion or may fuse with haploid cells of opposite mating type to create a diploid zygote which in turn produces aα diploid mitotic cells. Mitotic aα diploid cells may enter stationary phase under conditions of nutrient depletion or may undergo meiosis and sporulation in a medium containing acetate and lacking a nitrogen source. The resulting haploid a and α spores will germinate to produce haploid mitotic cells when nutrients are replenished. Not shown on the figure are other pathways of development: haploid stationary cells or spores may fuse to form a zygote without passing through the mitotic cycle and diploid stationary cells may undergo meiosis and sporulation without the intermediacy of a mitotic cycle.*

of the bud, a stainable circular rim which is probably a precursor of the bud scar appears on the parent cell (78). The scars which appear on mother and daughter cells after cell separation contain chitin. Hayashibe and Katohda have claimed that the chitin is synthesized prior to the budding event and hence is temporally and probably spacially associated with the appearance of this circular rim (78). This interpretation has been challenged by Cabib et al. (25), who suggest an inadequacy of the method employed by Hayashibe and Katohda to measure chitin and who support the view that chitin is synthesized later in the cell cycle near the time of cell separation (see below). If the latter interpretation is correct, then the chitin would have to be added to the rim later in the cycle as it matured into the bud scar.

Small membrane-bound vesicles accumulate prior to bud emergence, and they are found localized at the site of emergence of the young bud (119; Fig. 8b, d). The plasmalemma of the young bud contains small circular invaginations when observed by freeze-fracture electron microscopy, and it has been suggested that the invaginations arise by the fusion of these vesicles with the plasmalemma of the new bud (119).

Within the cytoplasm, adjacent to the cytoplasmic membrane, a ring of fibrous material has recently been detected and been shown to be present in the youngest bud detectable and to remain in the neck throughout the growth of the bud (B. Byers, personal communication). The ultimate fate of this structure at the time of cell separation is unknown.

The suggestion that the bud emerges explosively as a naked protoplast due to the localized rupture of the cell wall (131) appears to be wrong in detail because even the smallest buds contain a thick cell wall (107, 109, 154). Nevertheless, the basic idea inherent in this proposal that the force behind bud emergence may be the hydrostatic pressure of the cell acting on a localized weakening of the wall is still a viable possibility and may account for the observation that the first few buds on a new parent cell occur in the region of maximal wall curvature (51).

It has been suggested that the double-plaque configuration of the spindle plaque may play a causative role in the budding process since cells with very small buds aways contain a double-spindle plaque (20). Furthermore, the double plaque is found closely associated with and oriented in the direction of the new bud with the extranuclear microtubules extending into the bud (Fig. 8d; 20). Observations on two *cdc* mutants support the contention that the double plaque has a role in bud emergence. Cultures containing the *cdc* 1 mutation arrest with some of the cells containing tiny buds and these cells also have the double-spindle plaque configuration while other cells arrest without buds and these cells have a single-spindle plaque (20). Cells carrying a mutation in *cdc* 4 arrest in the double-plaque configuration and although they do not initiate DNA synthesis, they do undergo several cycles of bud emergence terminating development with as many as five buds on a single cell (20, 70).

The phenotype of mutants defective in *cdc* 4 also suggests that some type of clock controls the bud emergence event because successive budding events at the restrictive temperature maintain a periodicity of about one cell cycle time (70). This clock runs in the absence of DNA synthesis, nuclear division, cytokinesis, or cell separation, events which do not occur in this mutant at the restrictive temperature.

The *cdc* 24 gene product is probably inti-

mately involved in bud emergence because mutations in this gene block budding, but not DNA synthesis or nuclear division, and because the execution point is precisely at the time of bud emergence (74). In populations of cells carrying the *cdc* 1 lesion some of the cells arrest unbudded and others form tiny buds (71, 75); although the cells synthesize DNA, they do not complete nuclear division, and macromolecule synthesis is rapidly arrested (71). It is difficult to attribute to this gene product a role in one known cell cycle event, especially in view of the properties of the *cdc* 24 mutants. Mutations in *cdc* genes 19, 22, 25, 29, and 32 also prevent bud emergence (76), but they have not been well characterized either because the available alleles do not show first-cycle arrest or because the genetic situation is complicated.

Bud Growth

The bud grows in size throughout the cell cycle (112), and this fact greatly facilitates cell cycle studies with budding yeast because the relative size of the bud and parent cell provides a marker by which one can order individual cells within the cycle (187). The new daughter cell may continue to grow between the end of one cell cycle (cell separation) and the beginning of the next (bud emergence), but the extent of this growth is markedly dependent upon the culture conditions. Mitchison (112) measured individual cells during the course of the cell cycle and found that the mother cell maintained a constant volume, whereas Johnson (92), who measured a population of cells and ordered individuals according to bud size, concluded that the mother cell may undergo as much as a 50% cyclic variation in volume over the course of the cycle. The parent cell increases slowly in volume over the course of many generations, and it has been suggested that this increase may be due to the accumulation of bud scar material (102, 125).

The yeast wall is composed of glucan, mannan, and protein with small amounts of chitin. The bud wall is not derived from the material of the mother cell wall but is newly synthesized (32, 174). The glucan and mannan components of the wall are synthesized continuously throughout the cell cycle (156). Three groups have investigated the topology of wall growth during the division cycle. The site of addition of new glucose residues to the glucan component of the bud wall was determined by autoradiography of cells pulse labeled in [^{3}H]glucose and extracted to remove all nonglucan material (94). New glucan was added at the tip of the bud distal to the parent cell. 2-Deoxyglucose induces lysis of growing yeast cells at this same site possibly by inhibiting the synthesis of glucan without preventing glucan breakdown at the growing point in the wall (93).

The appearance of newly synthesized mannan on the cell wall was studied by labeling old mannan with fluorescein-conjugated concanavalin A, followed by growth for an interval in the absence of concanavalin A conjugate (173, 174). The fluorescence was found to be uniformly distributed over the parent cell and the region of the bud proximal to the parent cell. The portion of the bud distal to the parent cell was unlabeled, indicating that the newly synthesized mannan was added at the tip of the bud distal to the parent cell.

The simple picture of cell wall growth at the tip of the bud is confused by a third investigation using fluorescein-labeled antibody which arrived at a different conclusion (32). Newly synthesized wall antigen was found to be laid down on the new bud in the region proximal to the parent cell. It seems that there are only two ways to reconcile these observations. Either there are strain differences in the mode of wall synthesis or the unidentified antigen(s) investigated in the third study represents a third component of the wall that is added to the new bud wall at a point 180° removed on the bud surface from the site of addition of the glucan and mannan components.

The addition of an extracellular enzyme, invertase, to the wall has also been studied by using fluorescent antibody and found to be deposited only in newly synthesized bud walls (175). The reported results were not of sufficient resolution to decide between addition at the tip of the growing bud distal to the parent cell or at the neck region, proximal to the parent cell.

Cytokinesis and Cell Separation

When the mature bud separates from the parent cell at the conclusion of the cell cycle (cell separation), a permanent birth scar remains on the surface of the bud and a permanent bud scar is left on the parent cell (1, 5, 6). The bud and birth scars can be observed by fluorescence microscopy following staining with primulin (Fig. 6; 165, 166) or brightener (78), and the number of generations that a particular cell has undergone can be determined by counting the number of bud scars (9). The bud scar has the appearance of a crater on the surface of the parent cell with a raised, circular rim that is composed in part of chitin (3, 21).

An elegant biochemical and cytological analysis of the cell separation or septation process has been undertaken by Cabib and co-workers

(for a review see reference 25). Cell separation has been divided into two cytologically distinguishable stages: the formation of the primary septum, which is composed of chitin, and the addition to this of a secondary septum which is composed of glucan (Fig. 7; 25). The chitin is synthesized during a discrete interval of the cell cycle (22), sometime between bud emergence and cell separation, although the precise timing of this synthesis relative to other cell cycle events is not obvious from the published data. The suggestion that the chitin is actually synthesized prior to bud emergence (78) is disputed by Cabib et al. on technical grounds (25) as discussed previously.

A particulate enzyme preparation capable of chitin synthesis has been described by Cabib and co-workers, and the chitin synthetase is found in cell lysates as an inactive zymogen that can be converted to active enzyme by incubation with trypsin or a vesicle-bound activator found in yeast cells (22, 23, 96). The activator, which is a proteolytic enzyme (77), is in turn subject to inhibition by a heat-stable protein (22). A model invoking these three elements has been proposed to account for the localized deposition of chitin in the bud scar rim (22, 24, 25).

Four gene products that are necessary for cell separation (*cdc* 3, *cdc* 10, *cdc* 11, and *cdc* 12) have been identified by mutation (71). These mutants do not complete the process of cytokinesis or cell membrane separation (71) based upon an empirical test; however, the relationship between the point of arrest in these mutants and the two stages of septation defined by Cabib et al. (25) is at present unknown. First-cycle arrest is displayed by some of the mutants in three of these genes, and the execution points for all three are early in the cell cycle considerably before cytokinesis itself. In fact, two exhibit execution points near the time of bud emergence. It would be interesting to examine these mutants for defects in formation of the circular rim that precedes bud emergence or in chitin synthesis.

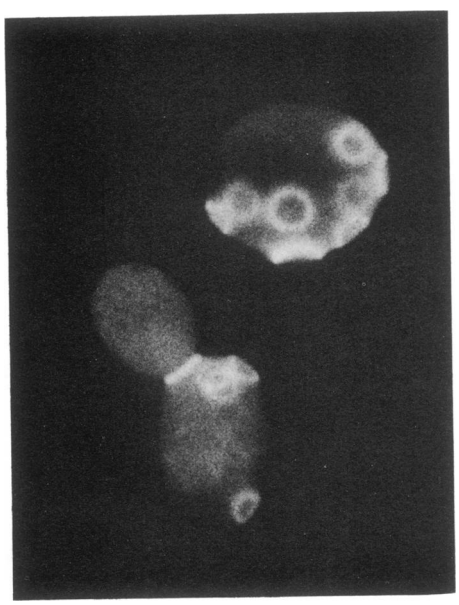

FIG. 6. *The bud scars of a diploid strain of S. cerevisiae. Note the presence of a stained rim on the neck of the budded cell. This photograph was kindly provided by Streiblova and Beran. It appeared previously in Beran (9), and is reproduced with permission of the authors and publisher.*

The claim that "protein disulfide reductase" is a cellular division enzyme in yeasts (132) deserves comment. This conclusion stems from the observation that a morphologic variant of *Candida albicans* isolated in 1940 (106) was later found to have a lower activity of protein disulfide reductase than its parent strain (132). The comparison of two strains separately cultured for 16 years is of questionable validity but, even granting that the biochemical difference arose at the time of the morphological difference, the two changes may both be pleiotropic manifestations of some other primary lesion. Until it can be demonstrated that a single genetic alteration in the structural gene for the protein, disulfide reductase, results in filamentous growth, the conclusion that this enzyme plays a role in division is unwarranted.

Nuclear Structure

The nucleus of the yeast cell is surrounded by a pair of double-unit membranes, the nuclear envelope, which remains intact throughout the mitotic cycle (108). The nuclear envelope is studded with circular pores approximately 80 to 90 nm in diameter at a density of 1 to 1.5 per square micrometer (121) that are filled with granules smaller than ribosomes (145).

A portion of the nucleus stains differentially, has a crescent shape during part of the mitotic cycle, and is believed to be the nucleolus (145). Evidence has been presented that the crescent region is the site of synthesis of ribosomal ribonucleic acid (RNA) (162).

During part of the mitotic cycle two disks are present in the nuclear membrane. The cross section of the disks appear by electron microscopy as bands of material forming a structure about 150 nm in length and several hundred nanometers wide. These structures have been

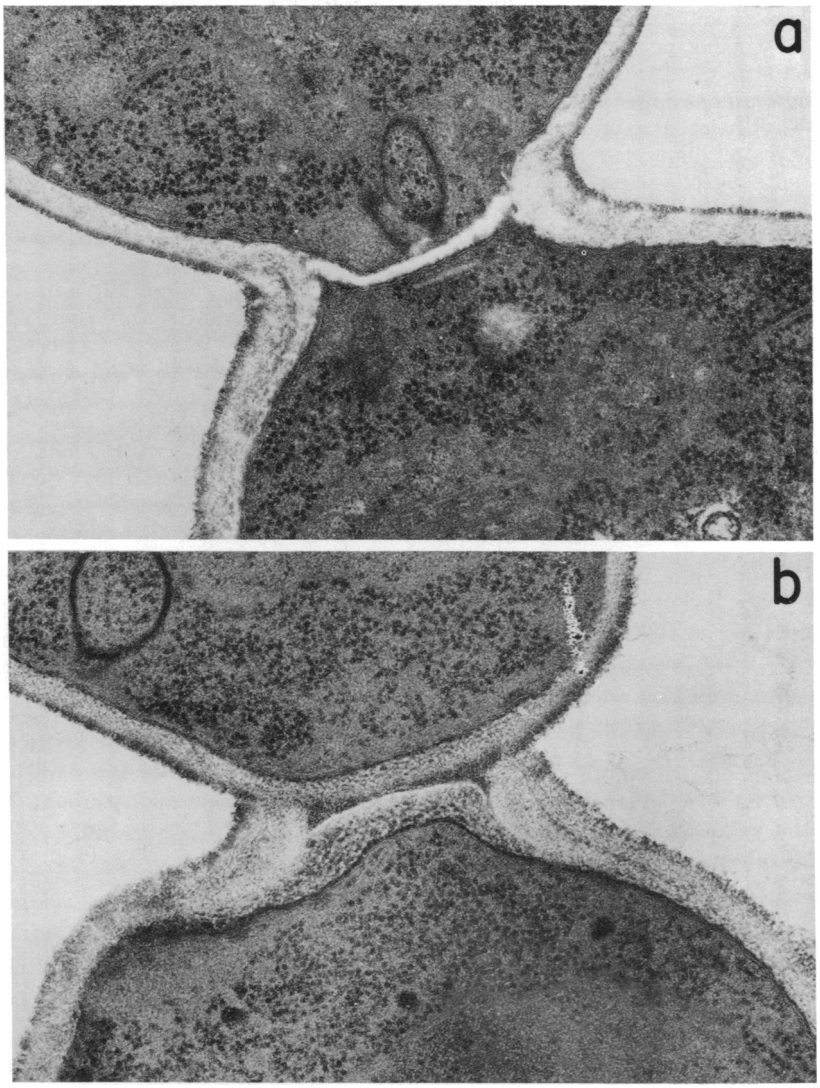

FIG. 7. *Electron microscopy of the primary (a) and secondary (b) septa of S. cerevisiae strain 316. Figure 7a was kindly provided by E. Cabib and Fig. 7b is from Cabib, Ulane, and Bowers (Fig. 1g, ref. 25) with permission of the authors and the publisher.*

termed centriolar plaques or spindle plaques and are the sites of origin of hollow microtubules 15 to 18 nm in diameter (20, 108, 117, 118, 145). One bundle of approximately 15 microtubules forms a straight continuous fiber connecting the two spindle plaques (117, 118, 145). Other short microtubules radiate in a cone shape from the spindle plaque into the nucleus, and others extend from the spindle plaque out into the cytoplasm (20, 117, 145). The fine structure of the microtubules and their subunits has been described (120).

Under most conditions of fixation and staining both for light and electron microscopy, the chromatin appears diffuse throughout the nucleus, and the existence of condensed chromosomes at any stage of the life cycle is not generally accepted (108, 121, 145). Although some authors have concluded on the basis of these negative results that yeast cells do not possess condensed chromosomes, the extremely small size of such chromosomes if they did exist would make them difficult to resolve by light microscopy, and the notorious problems associated with the fixation of yeast cells for electron microscopy make negative results by

this technique equally unconvincing. Indeed, Williamson, by using permanganate fixation, has described structures which he suggested to be condensed chromosomes (188), and Tamaki (167) and Fischer et al. (50) have reported seeing condensed chromosomes visible by light microscopy, although these reports are not considered to be definitive (108). Chromatin fibers from *S. cerevisiae* have been reported to be similar in structure to those from higher eukaryotes in that they have a diameter of about 17.5 nm (58). Proteins have been extracted from yeast chromatin and have been termed histones because they are basic and because they appear to influence the accessibility of the DNA for transcription and the structure of DNA as measured by thermal denaturation (176, 179). However, the relationship of these proteins to the histones of higher eukaryotes and their role in vivo are unknown.

Spindle plaque cycle

The cytology of the spindle plaque has been studied during the mitotic cycle by Moens and Rapport (117) and in *cdc* mutants by Byers and Goetsch (20). An unbudded cell early in the cell cycle contains a single, dense spindle plaque lying in the nuclear envelope (Fig. 8a). Both intra- and extranuclear microtubules originate from it (20, 117, 145). At about the time of bud emergence the spindle plaque duplicates, resulting in two plaques lying side by side and connected by a short bridge (Fig. 8b; 20, 117). This double-plaque configuration is usually found in close proximity to the site of budding. The extranuclear microtubules emanating from the outer surface extend into the new bud and, as discussed earlier, it has been suggested that the double-plaque configuration plays a causative role in the budding process (20). The *cdc* 28 mediated step is the earliest known gene-controlled event in the cell cycle (see section on events of cell cycle coordination), and mutations in this gene specifically prevent plaque duplication (20).

Next, the two spindle plaques rapidly migrate to opposite sides of the nucleus while remaining within the nuclear membrane, and a continuous straight fiber of microtubules of length about 0.8 μm connects them (Fig. 8c). This structure has been termed the complete spindle (20, 117). The *cdc* 4-mediated step is the second known step in the pathway of gene-controlled events, and the expression of this gene is necessary for the separation of the two spindle plaques (20).

Some time after DNA replication the nucleus migrates into the neck of the bud, and throughout this process the two plaques maintain a relatively constant distance of separation of about 0.8 μm (20, 117). It has been emphasized that the force for nuclear migration and for the initial stage of nuclear elongation is probably not supplied by the complete spindle because its orientation is random with respect to the long axis of the cell and because the spindle often does not extend the length of the nucleus (20, 145).

After nuclear migration and prior to the completion of nuclear division the complete spindle abruptly elongates in the long axis of the cell to about 6 to 8 μm as does the entire nucleus (Fig. 8e; 20, 117, 145). The two spindle plaques are now found at opposite ends of the elongated nucleus, and it is not unlikely that the growth of the spindle provides the force for this final stage of nuclear elongation. It is tempting to imagine that chromosome segregation occurs during the spindle elongation, but there is no evidence to support this idea. The final spindle elongation is blocked in mutants defective in the initiation of DNA synthesis (*cdc* 7), DNA synthesis (*cdc* 8 and 21), and medial nuclear division (*cdc* 2, 6, 9, 13, 16, 17, 20, 21, and 23; reference 20). Some of the latter group of mutants might well be defective in processes directly involved in chromosome segregation and nuclear division such as the spindle elongation step. However all of the mutants have an apparently normal complete spindle and, hence, there is no evidence that any are defective in microtubule assembly (B. Byers and L. Goetsch, personal communication).

Vesicles of endoplasmic reticulum accumulate in the bud neck as the nuclear membrane and cytoplasmic membranes pinch in two (145). Mutants defective in late nuclear division (*cdc* 5, 14, and 15) arrest at the elongated spindle stage and could be defective in one of these terminal steps of nuclear division (20).

Nuclear DNA Components

The total amount of DNA in a haploid yeast cell has been reported by various workers to be between 1.2 and 1.4 $\times 10^{10}$ daltons (33, 48, 133, 148, 194). About 90% of this DNA bands in $CsCl_2$ gradients at a density of about 1.699 g/cm^3 (127), and most of this main band DNA is contained in the nucleus because it is retained in isolated nuclei (12, 40).

Renaturation studies with whole-cell DNA indicate that the bulk of the cellular DNA has a kinetic complexity equivalent to a genome size of 9.2 $\times 10^9$ daltons (13), a value which is in reasonably good agreement with the total DNA

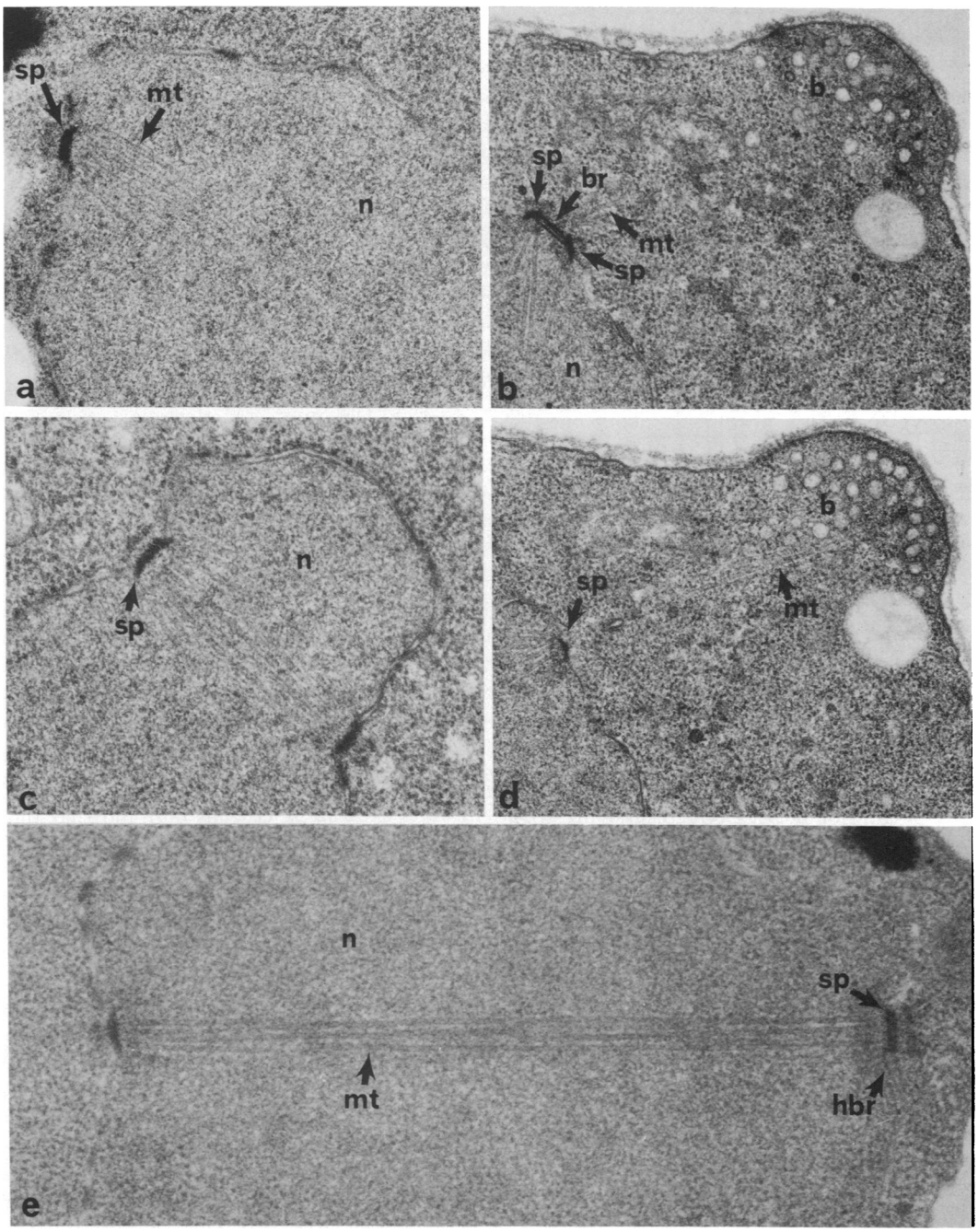

FIG. 8. *Electron microscopy of spindle plaques and associated structures in haploid strain A364A fixed during vegetative growth. (a) The single plaque of an unbudded cell (×43,000). (b,d) The double plaque at early budding (b). Extranuclear microtubules project toward the vesicle-filled early bud. Three serial sections away (d), these microtubules enter the bud (both ×32,000). (c) The short complete spindle of a budded cell shortly after separation of the spindle plaques (×48,000). (e) The long complete spindle of a cell with a large bud (×40,000). Symbols: sp, spindle plaque; mt, microtubules; n, nucleus; b, bud; hbr, half-bridge; br, bridge. These figures are from Byers and Goetsch (20) with permission of the authors and publisher.*

content of the haploid cell. Although no evidence of a repetitive DNA fraction was seen in this study, other data suggested the presence of 11% repetitive DNA with a kinetic complexity of about 2×10^7 daltons in nuclear preparations from *Saccharomyces carlsbergenesis* (31). The repetitive DNA might be due in part to ribosomal DNA and/or 2-μm circular DNA (see below).

Genetic studies have identified 17 independently segregating groups of centromere-associated genes in diploid cells, and a statistical assessment of this data suggested that this number may represent the complete chromosome complement (124). If a haploid cell contains 17 chromosomes the average chromosome would have a molecular weight of about 5 to 6×10^8 of DNA, an amount only about one-fifth that of the *Escherichia coli* chromosome (26). This small size probably accounts for the inability of cytologists to reproducibly visualize individual chromosomes in the yeast nucleus but, on the other hand, their small size has facilitated physical studies of intact yeast nuclear DNA.

Sedimentation studies reveal that the bulk of the cellular DNA sediments as large molecules with an average molecular weight number of about 6×10^8 (14, 138). In one study the distribution of molecular weights was found to be broad, ranging from 5×10^7 to 1.4×10^8 (138), whereas in another it was narrow, ranging from 4×10^8 to 6×10^8 (14). The average molecular weight number of DNA in these investigations is in striking agreement with the expected average molecular weight of DNA per chromosome and suggests that each chromosome may contain a single DNA molecule. However, since the equations that relate sedimentation velocity of DNA molecules to molecular weights have not been validated with molecules this large, the sedimentation data alone does not prove this important point.

Direct visualization of yeast DNA molecules before and after velocity sedimentation revealed that the molecules are linear and range in contour length from 50 to 355 μm (excluding mitochondrial DNA and 2-μm circular DNA) with an average value of 165 μm (137, 140). The average length corresponds to a molecular weight of 3.8×10^8. Because any artifactual breakage of these long molecules would lead to an underestimate of their true size these data are interpreted to demonstrate that the yeast chromosomes each contain a single, linear DNA molecule that is continuous through the centromere (137, 140).

The availability of yeast strains that are monosomic or disomic for one or a few chromosomes provides a method for associating the genetic linkage groups with specific-size DNA molecules by the cosedimentation of differentially labeled aneuploid and euploid DNA preparations (14, 49, 55, 140). Data obtained by this approach suggest that the linkage group designated as chromosome I is contained on a DNA molecule of about 4×10^8 daltons (49) and that chromosome VIII is associated with a molecule of about 4 to 5×10^8 daltons (14, 140). These correlations of changes in sedimentation profile with alterations in ploidy for certain nuclear genes demonstrate that the large DNA molecules are indeed the repository of the nuclear genetic information.

A satellite DNA band of a density of 1.705 g/cm^3 (γ band) is produced from nuclear DNA when the large molecules are sheared to molecular weights of 3×10^7 or less, but is not observed if the nuclear DNA molecules are greater than 8×10^7 daltons (40). Gamma-band DNA accounts for 11% of the total nuclear DNA and hybridizes with 18 and 25*S* ribosomal RNA (40, 43) and contains the 5*S* ribosomal RNA cistrons (146) as well. Because there are about 140 ribosomal RNA cistrons in the haploid genome of *S. cerevisiae* (43, 52, 144, 149) that are each transcribed into a molecule of 2.5×10^6 daltons (178), it can be calculated that about one-half of the γ band DNA is accounted for by the ribosomal RNA cistrons (40). Furthermore, the shear data suggests that these are clustered in units of 10 to 32 cistrons (40) and that the individual units are separated from one another by DNA of main-band density (40, 44). The majority of the ribosomal cistrons appear to be located on chromosome I (95, 135), based upon the fact that strains monosomic for chromosome I have a lower percentage of rDNA.

Initiation of Nuclear DNA Synthesis

In synchronous yeast cultures the majority of the cellular DNA replicates early in the cell cycle at about the time of bud emergence (134, 186, 192). However, because results with synchronous cultures are always suspect, a more definitive investigation is that of Williamson who showed by an autoradiographic approach that in unperturbed, asynchronous populations most of the cellular DNA (and therefore the nuclear DNA) replicates during an interval comprising approximately one-quarter of the cell cycle beginning at about the time of bud emergence (187).

Events that are required for the initiation of DNA synthesis (that is, events that constitute a precondition for the onset of synthesis), but are

not required for DNA replication (elongation) itself, should satisfy the following criterion. All cells that have entered the DNA synthetic period will have completed the event in question and, hence, they will be able to complete the full round of DNA synthesis after inhibition of the initiation event is imposed. Because the DNA synthetic period occupies only about 25% of the cell cycle, the imposition of an initiation block in an asynchronous culture would permit a DNA increase of only about 15% (80). In a synchronous culture if the inhibition of initiation is imposed just after completion of the sensitive event then nearly a complete round of DNA synthesis should occur.

Evidence of this type has been presented suggesting that α factor (18, 81) and a factor (L. Wilkinson and J. Pringle, submitted for publication), the mating pheromones (see below), cycloheximide and anisomycin (inhibitors of protein synthesis; 80, 160, 190), and lesions in the *cdc* 28, *cdc* 4, and *cdc* 7 genes (73, 80) prevent the initiation but not the completion of DNA synthesis. However, this type of datum is not sufficient to distinguish a true initiation block from a leaky replication block, a replication block with a delayed onset of expression, or a specific block in the early portion of DNA synthesis.

Such a distinction can, however, be made by a direct examination of DNA molecules for replication structures. As will be pointed out below, DNA molecules in the process of replication contain "bubbles" and "forks" that can be visualized by electron microscopy (130, 140). When the DNA obtained from a replication mutant (*cdc* 8) that had been incubated at the restrictive temperature for an interval sufficient to accumulate all of the cells at the block was examined, a large fraction of the molecules contained multiple small bubbles (T. D. Petes and C. S. Newlon, personal communication). This result is interpreted as being due to the slow, "leaky" replication of DNA in essentially all of the cells. However, when the DNA from the purported initiation of DNA synthesis mutant (*cdc* 7) was examined, very few molecules had bubbles. Nevertheless, nuclear DNA synthesis in the *cdc* 7 mutant at the restrictive temperature was even more leaky than that in the *cdc* 8 mutant. This result is interpreted to mean that most of the cells with the *cdc* 7 mutation are arrested prior to the actual beginning of chromosome replication. Leakiness of DNA synthesis in this mutant must be due to an occasional cell that leaks through the initiation block and then rapidly replicates its entire genome so that only a small fraction of the molecules from the population contain bubbles at any one time. Because the other purported initiation blocks, α factor, a factor, protein synthesis inhibitors, and mutations in *cdc* 28 and *cdc* 4, have been shown to be steps that precede the *cdc* 7-mediated step in the cell cycle (81), these results provide strong confirmation of the suggestion that all of these agents act on preconditions for DNA replication.

The finding that inhibitors of protein synthesis impose blocks specifically in the initiation of DNA synthesis suggests that *S. cerevisiae* unlike most eukaryotes is capable of replicating its genome in the absence of protein synthesis. DNA synthesis in the absence of protein synthesis apparently leads to the complete duplication of all chromosomes since cells that have replicated their DNA under these conditions can subsequently complete nuclear division (160) and cell division (80) without further DNA synthesis. Hence, all of the proteins needed both for the initiation and the replication of the 200 replicons (140) in the yeast genome are present at the onset of the S period. It is not clear whether these proteins synchronously initiate all replicons at the beginning of S or whether they merely accumulate at the beginning of S and are later used to mediate the asynchronous initiation of replicons throughout the S period.

Nuclear DNA Synthesis

The structure of replicating DNA molecules from *S. cerevisiae* has been examined (130, 140). DNA molecules which contained terminal forks and/or internal bubbles were obtained from synchronous cultures in the S phase with high frequency but not in those obtained from G1 cells (Fig. 9). Furthermore, in DNA obtained from cells following a shift from isotopically dense to light medium, molecules with these structures were highly enriched among those that were intermediate in density between the fully dense and fully hybrid peaks. Both of these correlations strongly suggest that forked and bubbled structures are replication intermediates. The terminal forks are assumed to arise by internal initiation of replication that had progressed by replication to the molecular terminus or by terminal initiation sites that had progressed internally. The bubbles are presumed to arise by internal initiation of replication. A high proportion of these molecules contained multiple initiation sites and the center-to-center distances between these sites ranged from 3 to 86 μm with clusters at 15 to 20 and 30 to 35 μm. In this respect, the chromosomes of *S. cerevisiae* closely resemble the chromosomes of

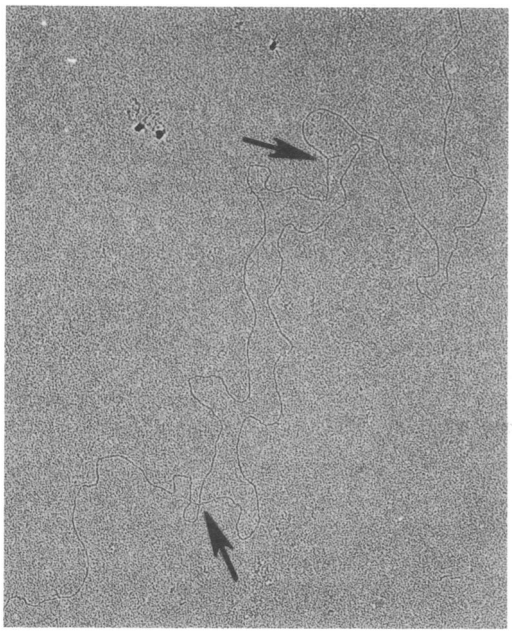

FIG. 9. *An electron micrograph of a replicating DNA molecule displaying a "bubble." The arrows indicate the limits of the bubble; kindly provided by C. Newlon and W. Fangman.*

animal somatic cells which also have multiple internal sites of replication initiation spaced at intervals of 15 to 60 μm (27, 85) in contrast to the chromosome of *E. coli* which contains a single initiation site that serves a 1,400-μm replicon (26).

In synchronous cultures the proportion of rDNA to total DNA remains constant throughout the S period (54). This result suggests that the replication of the rDNA is not restricted to a small portion of the S period. However, this conclusion is only valid if the length of the S period in the individual cell was nearly as long as the length of the S period in the synchronized culture, an assumption which is reasonable in this experiment, but not proven.

Two known gene functions, *cdc* 8 and *cdc* 21, are required for DNA replication (70, 73). Both have execution points considerably after bud emergence and are probably required therefore throughout the DNA synthetic period. Indeed, cells carrying these mutations cease ongoing DNA synthesis abruptly following a shift from permissive to restrictive temperature (70, 73). One of these mutants, *cdc* 8, has been found to be temperature-sensitive for DNA synthesis in a permeabilized cell system that incorporates deoxynucleotide triphosphates into DNA (79) and, hence, this mutant is probably not defective for precursor synthesis. The other mutant has not been tested.

Mutants with a diagnostic defect in medial nuclear division arrest with the same terminal phenotype as those with diagnostic defects in DNA replication. However, the former synthesize DNA before arrest at medial nuclear division, whereas the latter do not. Furthermore, many of the medial nuclear division mutants have execution points around the time of DNA synthesis. These facts suggest the possibility that some or all of the medial nuclear division mutants might be defective in proteins that are required for the completion of chromosome duplication, but not for the polymerization of deoxyribonucleotides. The DNA ligase enzyme of *E. coli* is an example of such a protein (136).

Other specific inhibitors of DNA synthesis in *S. cerevisiae* include hydroxyurea (159), Trenimon (88), and fluorodeoxyuridine (48). Cells that are arrested by these inhibitors assume the same terminal phenotype as do cells carrying defects in gene *cdc* 8 or *cdc* 21.

Repair and recombination of DNA molecules may be either restricted to the DNA synthetic period or induced during the S period as evidenced by two observations. The capacity to undergo X ray-induced mitotic recombination is periodic in synchronous yeast cultures with a peak at about the time of DNA synthesis initiation (48), and survival to high doses of X rays is much greater in budding yeast than in unbudded cells (7).

Mitochondrial DNA

Reviews on mitochondrial DNA and mitochondrial genetics have appeared recently (15, 103), and these areas will be only briefly and selectively reviewed here.

In addition to nuclear DNA, the yeast cell contains mitochondrial DNA that constitutes 5 to 20% of the total cellular DNA and differs in density (1.683) and base composition (17% G+C) from nuclear DNA (10, 110). Although the size and topology of the mitochondrial DNA molecule is still a subject of controversy (see reference 15), recent gentle lysis and handling procedures have permitted the isolation of a majority of the mitochondrial DNA molecules from sucrose gradients as linear structures with a length of about 21 μm (56, 137). Direct visualization of lysed mitochondria revealed some closed, circular molecules of about 25 μm in length, and it has been suggested that this structure represents the native molecule (83).

The renaturation kinetics of mitochondrial DNA is reported to be compatible with a minimum genome equivalent to 25 μm of DNA (83), but an anomalous behavior of mitochondrial DNA in renaturation experiments suggests caution in interpreting these results (31).

Irreversible changes in the mitochondrial DNA occur spontaneously with high frequency resulting in respiratory deficient (petite) yeast and can be induced by many agents in close to 100% of the cells in a population. The mitochondrial DNA from petites frequently displays an altered density and base composition (10 11 110, 126) and may be entirely missing in some petites (36, 56, 127-129).

The ratio of mitochondrial to nuclear DNA is fairly constant under a variety of growth conditions (53, 189), and some control mechanism probably exists to coordinate their relative rates of synthesis. Although the replication of mitochondrial DNA has been reported to occur within a brief portion of the cell cycle, immediately following nuclear DNA replication in synchronous cultures of *S. lactis* (161), similar studies with *S. cerevisiae* suggest a continuous replication of mitochondria DNA throughout the cell cycle (191).

One possible model for achieving a constant relationship between the two DNA components would be for the duplication of one to be dependent upon the duplication of the other. This simple model appears to be ruled out, however, since conditions are known that permit the synthesis of either nuclear or mitochondrial DNA while preventing that of the other. Thus, nuclear DNA replication occurs in the absence of mitochondrial DNA synthesis, because yeast strains lacking mitochondrial DNA are viable (56, 128). Conversely, mitochondrial DNA synthesis continues under conditions that prevent nuclear DNA synthesis. When cytoplasmic protein synthesis is prevented by cycloheximide, the net synthesis of mitochondrial DNA continues in the absence of nuclear DNA synthesis for about one generation time, approximately doubling in amount (60). Even more striking are results obtained in the presence of α factor where net accmulation of mitochondrial DNA continued at a normal rate in the absence of nuclear DNA synthesis for at least two generations undergoing a fourfold increase in amount (139). In certain mutants that are defective in the initiation of nuclear DNA replication, mitochondrial DNA synthesis proceeds uninhibited at the restrictive temperature (38, C. S. Newlon and W. L. Fangman, personal communication).

Two gene products that are required for nuclear DNA replication (*cdc* 8 and *cdc* 21) are also essential for mitochondrial DNA synthesis (C. S. Newlon and W. L. Fangman, personal communication). However, the three genes that are required for the initiation of nuclear DNA synthesis are not necessary for mitochondrial DNA synthesis (38, C. S. Newlon and W. L. Fangman, personal communication). Perhaps other, as yet unidentified, gene products are specifically involved in initiation of mitochondrial DNA synthesis.

In view of the dependence of the initiation of replicons in yeast (80, 160, 190), in animal cells (84), and in bacterial cells (100) upon protein synthesis, the apparent insensitivity of mitochondrial DNA synthesis to the inhibition of either cytoplasmic or mitochondrial protein synthesis is quite interesting. In the presence of 200 μg of chloramphenicol per ml, a specific inhibitor of mitochondrial protein synthesis, yeast cells continued mitochondrial DNA synthesis at a normal rate for at least two generations (60). Although the level of chloramphenicol used in these experiments was sufficient to inhibit respiratory activity, the degree of inhibition of mitochondrial protein synthesis was not measured. However, a lack of dependence of mitochondrial DNA replication upon mitochondrial protein synthesis can also be reasoned from the fact that petites with grossly altered mitochondrial DNA are able to replicate this DNA, although they must not be capable of synthesizing an active mitochondrial protein synthetic system (103).

Two-Micron Circular DNA

About 1 to 5% of the total yeast cell DNA is present as 2-μm closed, circular DNA molecules with a density similar to that of the main band nuclear DNA (2, 61, 158). The renaturation kinetics of isolated 2-μm circles are consistent with their size, and this result suggests that all of the molecules are identical in base sequence (4). The cellular location of this DNA fraction is unclear at present, but the isolation of 2-μm circles free of both nuclear and mitochondrial DNA in association with a membrane-containing component suggests the possibility of a distinct organelle location (34). Mechanisms must exist to coordinate the synthesis of 2-μm DNA with that of nuclear and mitochondrial DNA, but this question has not yet been examined. Because of the ease with which these molecules can be isolated and characterized, they should be very useful in investigations of

the molecular details of DNA replication in yeast.

Macromolecule synthesis

Total cell mass increases continuously throughout the cell cycle (19, 147, 168). Although some experiments suggested a periodic increase in total cell RNA (184, 187), it is now generally accepted that both protein and RNA accumulate continuously throughout the cell cycle (37, 57, 69, 150, 183, 184). Synthesis of the two stable ribosomal RNAs and total transfer RNA have each been examined and also found to occur throughout the cell cycle (170). The resolution in these experiments was not sufficient, however, to rule out the possibility of a brief cessation of RNA and/or protein synthesis during mitosis and, in view of the documentation of this phenomenon in animal cells (142), it would be of interest to examine this possibility by an autoradiographic approach.

Kuenzi and Fiechter have investigated the carbohydrate composition of the yeast cell during the cell cycle (98, 99). In cells synchronized by glucose starvation and refeeding, the glucan and mannan contents per milligram of dry weight remained relatively constant, but the glycogen and trehalose contents decreased abruptly at the time of budding (98). In cells synchronized by selection from a growing population of unbudded cells by sedimentation in a density gradient the contents of all four carbohydrates were found to remain relatively constant throughout the cell cycle (99). Kuenzi and Fiechter suggest that glycogen and trehalose may provide a source of energy for the budding cycle under the special conditions of glucose limitation (99).

The phenomenon of periodic or discontinuous enzyme synthesis during the cell cycle has been documented in many organisms and is potentially of great significance to our understanding of the cell division cycle (reviewed in references 65, 113). For a large number of enzymes that have been examined, total activity was observed to double abruptly (step synthesis) at a specific time in synchronous cultures, whereas for other enzymes an abrupt increase followed shortly by a decrease (pulse synthesis) was found. The former pattern is thought to signify the restricted synthesis of a stable enzyme during a discrete interval of the cell cycle, whereas the latter is explained by the same hypothesis for an unstable enzyme. Periodic enzyme synthesis has been extensively studied in *S. cerevisiae* by Halvorson and his colleagues (65).

Step synthesis is apparently quite general in *S. cerevisiae*, having been documented for 33 different enzymes (65). Five different permease activities also exhibit a periodic doubling (29). No example of continuous enzyme synthesis during the cell cycle of *Saccharomyces* has been reported, although such examples have been reported in other organisms including the yeast *Schizosaccharomyces pombe* (113). However, the synthesis of the ribosomal proteins appear to occur continuously during the cell cycle of *S. cerevisiae* (155). It has been suggested that some examples of continuous enzyme synthesis may in reality be the summation of several patterns of discontinuous synthesis by each of several non-allelic genes that code for proteins with the same function or activity (168).

A number of observations limit the possible interpretations of this phenomenon. Although almost all of the experiments have been done with cells synchronized by one of two rather harsh procedures, either by induced synchrony using shifts from starvation to growth medium as in the procedure of Williamson and Scopes (195) or selection synchrony utilizing sucrose gradients to select the small cells in the population (168), the hypothesis that the observations are artifacts of the synchronization techniques appears to be ruled out on several counts. First, periodic synthesis has been observed for many different enzymes. Second, although the two synchronization techniques are harsh, they are quite different and yet the step time of a particular enzyme is nearly the same regardless of the synchronization regime (168). Third, the result is reproducible in successive cycles in the same experiment (39, 57, 169). Finally, the phenomenon has been reproduced for three enzymes by the technique of Sebastian et al. (150), which does not require presynchronization of the culture.

Any model that invokes the activity of the enzyme itself in the production of repressor or metabolism of inducer to control the step in enzyme activity appears to be incompatible with observations for certain enzymes in yeast. Thus α-glucosidase, β-galactosidase, and β-glucosidase display step synthesis at the same time in the cell cycle in synchronous cultures synthesizing the enzyme at the basal rate and at a highly induced rate (30, 63). The enzyme uridine 5'-diphosphate galactose-4-epimerase is reported to undergo step synthesis at the same time in the cell cycle in cultures that are induced as well as in cultures producing the enzyme constitutively (65). Finally, any one of several non-allelic genes (124) endow the cell

carrying the dominant allele with the capacity to produce the same enzyme, α-glucosidase (67). The patterns of α-glucosidase synthesis in cells carrying more than one of these genes has been interpreted to be the result of several distinct steps of enzyme synthesis occurring at different times in the cell cycle, with each step being the result of synthesis from one of the non-allelic genes (168). However, it is not clear that these non-allelic genes are the structural genes for the enzyme(s) and, in fact, at least one, MAL6, is apparently not structural but regulatory (171).

Halvorson and his colleagues have discussed several models (65) to explain these phenomena, but favor a sequential chromosome transcription model. "In its most explicit form this model assumes that the order of genes on the chromosome determines the program for transcription and, thus, subsequent translation during the cell cycle. Thus, there is a linear relationship between the *time* of ordered enzyme appearance and the *position* of genes along the chromosome" (65). As pointed out by the authors, in order for this model to account for the observations it is also necessary that the messenger RNA of yeast be metabolically unstable, and this appears to be the case (86, 177). In support of this model is the finding that enzymes from closely linked genes, β-galactosidase and β-glucosidase in *Saccharomyces lactis* (66), and aspartokinase, phosphoribosyl-adenosine 5'-triphosphate-pyrophosphorylase, and threonine deaminase on chromosome V of *S. cerevisiae* (169), exhibit steps at about the same time in the cell cycle, whereas enzymes from genes that are unlinked exhibit steps as early as 0.1 and as late as 0.8 in the cell cycle (169). Although the sequential transcription model has strong appeal, it is important to note that there is no evidence that the steps in enzyme activity are correlated with the time of transcription of the relevant gene or the translation of this transcript as would be required by the model.

Apparently strong confirmation of the sequential transcription model was provided by the observation that a change in the map position of two genes was correlated with a change in step times of the products of these genes (39). The genes gal_1 and lys_2 are reportedly closely linked on the same side of the centromere of chromosome II in the *S. cerevisiae* strain S288C (122), whereas they are reportedly far apart on opposite sides of the centromere in strain 61009 (87). In synchronous cultures with strain S288C, Cox and Gilbert (39) found that the step times for the enzyme products of these genes, galactokinase and α-aminoadipic acid reductase occurred at about 0.5 and 0.3, respectively, in the cell cycle, whereas in strain 61009 they occurred at about 0.7 and 0.2, respectively. Thus the greater separation of the two structural genes appeared to be correlated with a greater separation in step times for the synthesis of their gene products. Unfortunately, Cox and Gilbert did not attempt to confirm the different mapping results previously reported from two different labs. In order to generate the purported map differences two inversions would be required. It is conceivable that the reported difference is due to a mapping error in one of the laboratories, and before accepting the correlated change in time of enzyme synthesis as support for the linear transcription model, it is imperative that the map difference be confirmed in a single laboratory.

If the sequential transcription model is correct it appears that some genes may be exceptions to the rule of discontinuous transcription. The synthesis of some, if not all, of the ribosomal proteins occurs continuously during the cell cycle (155). However, one could accommodate this observation with the sequential transcription model in one of two ways. First, each ribosomal protein may be encoded by several non-allelic genes with different times of expression. This hypothesis seems unlikely because at least one ribosomal protein, that conferring cycloheximide resistance to the 60S ribosomal subunit, is encoded by a single gene, cyr_2 (35). Second, the messenger RNAs for the ribosomal proteins may be exceptionally stable.

The recent finding that ornithine transaminase in *S. cerevisiae* and β-galactosidase in *S. lactis* are inducible at all times in the cell cycle (151) is incompatible with any model that restricts transcription potential within the cell cycle, but is not necessarily incompatible with ordered transcription of the genome under fully induced or fully repressed conditions.

HOW ARE THE EVENTS OF THE CELL CYCLE COORDINATED?

Dependent Pathways of Landmarks

Most of the cell cycle landmarks must occur in the proper order to ensure the production of two viable daughter cells. How is this fixed order achieved? A partial answer to this question comes from the observation that many landmarks in the cell cycle are dependent for their occurrence upon the prior completion of

earlier landmarks. Such cases of dependence dictate a fixed sequence of events because no late landmark can occur until all early landmarks upon which it is dependent have been completed.

The sequences of dependent events can be mapped with the use of specific cell cycle blocks (74). When the occurrence of one landmark is specifically prevented by an inhibitor or temperature-sensitive mutation, some of the landmarks that normally follow are also prevented, whereas others are not. Those that do not occur are dependent upon the completion of the inhibited landmark, whereas those that do occur are not dependent upon such completion. Of course, the assumption inherent in this argument is that the specific inhibitor or mutation directly interferes with only one landmark. The extensive genetic and physiological characterization of the *cdc* mutants (76) lends support to the validity of this assumption for these temperature-sensitive mutants, and it is a reasonable assumption for some inhibitors as well. The data currently available with a variety of cell cycle inhibitors and *cdc* mutants was reviewed recently (74). With this data one can construct the pathways of dependent events shown in Fig. 10. Evidence for some of the relationships shown in Fig. 10 is stronger than for others, and it is not unlikely that alterations in this model will be made as more data become available. The model is presented more as an inducement to further experimentation rather than as the final word, and the interested reader is advised to consult Hartwell et al. (74) and Byers and Goetsch (20) for the detailed arguments that support each dependent step.

The landmarks of the cycle are apparently ordered into two dependent pathways. One pathway is comprised of the following landmarks: plaque duplication, plaque separation, initiation of DNA synthesis, DNA synthesis, medial nuclear division, late nuclear division and spindle elongation, cytokinesis, and cell separation. The other pathway is composed of the following landmarks: bud emergence, nuclear migration, cytokinesis, and cell separation. Only the inner pathway of events must be completed in one cycle in order for the cell to commence upon a second cycle. If this model is correct, then the order of events in each of the two separate pathways is assured. Furthermore, the integration of the two pathways would be accomplished by the facts that both pathways diverge from a common landmark, plaque duplication, and that both converge upon a common landmark, cytokinesis.

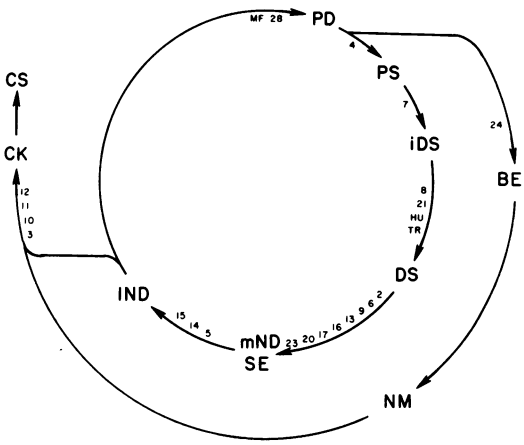

FIG. 10. *Dependent pathways of events in the cell cycle. Abbreviations are the same as those used in Fig. 1. Small numbers refer to cdc genes that are required for the particular step in question and small letters designate inhibitors that block the step. MF, mating factor; HU, hydroxyurea; TR, Trenimon.*

Events on different pathways are independent of one another. For example, DNA synthesis and nuclear division occur in the absence of bud emergence in mutants defective in gene *cdc* 24 at the restrictive temperature and, conversely, bud emergence and nuclear migration occur in the absence of DNA synthesis in mutants defective in *cdc* and 21 at the restrictive temperature (74). It would not be surprising, therefore, if landmarks that belong to different pathways exhibited a reversal of order under two different sets of conditions. Indeed, the order of the events nuclear migration and nuclear division does appear to be variable under some conditions (143). In this context it is interesting to note that the landmarks are so distributed between the two pathways that such an occurrence would not result in the production of inviable daughter cells, providing that both pathways were eventually completed.

Dependent Pathways of Gene Expression

The demonstration that order is achieved in the cell cycle by a dependence of late events upon early events raises another question. What is the basis of this dependence? At the next level of analysis this question can be approached by inquiring whether the steps mediated by the *cdc* gene products are organized into dependent pathways. In theory, two-gene-mediated steps may be related to one another in one of four possible ways as shown in Fig. 11. They may be related in one of two dependent sequences, with

step A being dependent upon the prior completion of step B or vice versa. They may be independent with either step A or step B being capable of completion in the absence of the completion of the other step, or they may be interdependent so that neither step can occur without the concomitant occurrence of the other.

Two rationales have been used to distinguish between these four possible relationships (81). One is applicable to two steps for which reversible conditional blocks are available that can be independently imposed. This method involves shifting cells from one restrictive condition to the other and it is capable of distinguishing all four possibilities (81, 91). A second method is applicable to two blocks that need not be reversible and that cannot be independently imposed. For two-gene-mediated steps it requires a comparison of the phenotypes of the single-gene mutants with that of a mutant strain defective in both genes. The double mutant approach is capable of resolving three of the four possible relationships. For a discussion of these methods and their limitations the reader is referred to Hereford and Hartwell (81).

By using this approach the sequence of gene-mediated steps diagrammed in Fig. 12 was determined. The steps are organized into a dependent sequence. Although the data are not extensive, they do suggest that the reason for the dependent pathways of landmarks (Fig. 11) is because the expression of the gene products that mediate these landmarks is also organized into dependent sequences. For example, nuclear division cannot take place until after the replication of DNA because the gene products that mediate nuclear division cannot express themselves until after the gene products that mediate nuclear DNA synthesis. More work of this type with additional inhibitors and mutants will permit the construction of a detailed map of gene expression and a more comprehensive test of this conclusion.

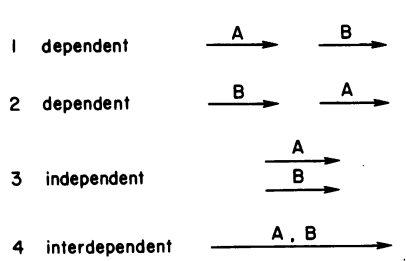

FIG. 11. *Four possible relationships between two gene-mediated steps. Two steps may be related in a dependent sequence (1 or 2), in which case the first step must occur before the second. They may be independent (3), in which case either step can occur in the absence of the other, or they may be interdependent (4), where both steps must occur concomitantly.*

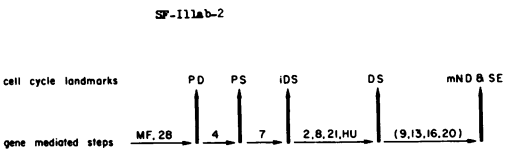

FIG. 12. *Dependent pathway of gene-mediated steps in the cell cycle. Succeeding steps are related in a dependent sequence (as in Fig. 11) of given order. Numbers refer to cdc genes and letters to inhibitors (HU, hydorxyurea; MF, mating factor) or to landmarks (as in Fig. 1). Designations over the same arrow are interdependent (as in Fig. 11) unless they appear in parentheses, in which case order has not been determined.*

We can now rephrase the question that open this section as follows: what is the molecular basis of the dependence of gene expression in the cell cycle? Although we do not have an answer to this question, it may be useful to consider two broad categories of possibilities. The dependence might exist at the level of the synthesis of a gene product or at the level of its functional expression. For example, the fact that *cdc* 7 must express itself before *cdc* 8 (Fig. 12) might be due to the fact that *cdc* 7 product is required for the synthesis of *cdc* 8 product (i.e., *cdc* 7 product is a positive regulator of the synthesis of *cdc* 8 product) or because the *cdc* 7 product is required for the function of *cdc* 8 product (perhaps they are sequential steps in a pathway so that the product produced by the action of *cdc* 7 product is the substrate for *cdc* 8 product). A resolution between these two possibilities is hampered by the fact that we do not know the molecular basis of the temperature-sensitive lesion in either gene. If we can determine whether the mutant gene products are thermolabile for their synthesis or for their function, then it may be possible to distinguish these possibilities.

The extensive data suggesting that many, if not all, proteins are synthesized periodically during the cell cycle (65) offers a plausible and testable explanation for the dependent pathways of landmarks and gene expression. Taken together the data suggest a cascading system of positive regulation where the function of early gene products turns on the synthesis of later gene products whose function in turn induces the synthesis of still other gene products, etc. If this model is valid, then the arrest of the cell cycle with an inhibitor or with a *cdc* mutation might arrest the program of sequential protein

synthesis or prevent the synthesis of *cdc* gene products that function later in the cell cycle.

Indeed, cells arrested in DNA synthesis by hydroxyurea do not acquire the capacity to complete nuclear division, cell division, or subsequent rounds of budding in the absence of further protein synthesis (159, 160), suggesting that some of the proteins required for each of these processes cannot be synthesized until DNA replication is completed. However, cells that are arrested in cell cycle progress by X-ray treatment continue to exhibit pulse synthesis of DNA polymerase (47), and cells arrested at DNA synthesis by Trenimon continue to synthesize dihydrofolate reductase although the normal step pattern of synthesis is altered (88). These results demonstrate that experimental rationales exist for advancing an answer to the question, but much more data is needed before general conclusions are warranted.

HOW IS CELL DIVISION CONTROLLED?

In the transition from mitotic cell division to the three alternative stages of the yeast life cycle—stationary phase, sexual conjugation, and meiosis—the mitotic cell division cycle is arrested and an alternative developmental program is undertaken (see Fig. 5). Do yeast cells arrest the cell cycle at a specific stage before commencing on one of the alternatives, and if so is such arrest obligatory? A definitive answer to these questions is not yet available for all three situations, but all evidence points to a restriction of developmental potential to the G1 interval of the cell cycle and suggests that once a cell has passed a critical point in G1, it is committed to completion of the mitotic cell cycle before it can commence on one of the three alternative programs. The most complete data available concern the transition from the mitotic cell cycle to sexual conjugation.

Transition from Mitosis to Conjugation

The relationship between the mitotic cycle of haploids and the sexual cycle may be relatively simple. Bücking-Throm et al. (18) proposed that the two haploid cells synchronize their cell cycles prior to cell fusion and zygote formation. Furthermore, they proposed that this synchronization is achieved in part through the agency of the constitutively produced α factor by cells of α mating type. In this model, the α factor specifically arrests a cells as unbudded, mononucleate cells prior to the initiation of DNA synthesis (i.e., in the G1 interval of the cell cycle). Some reciprocal mechanism for arresting the α cell at the same point in the cycle was foreseen, and evidence is now at hand that an analogous a factor exists. Considerable evidence supports this model, but some points remain unproven.

First, let us consider the proposition that a and α cells are at the same point in the cell cycle at the time of cell fusion. Because the resulting zygote undergoes nuclear fusion followed by a diploid mitotic cycle, it is clear that at some point before or after fusion of the two haploid cells their mitotic cell cycles must become synchronized. It has been known for some time that cell fusion can occur between unbudded yeast spores (196) or stationary-phase yeast cells (17) without the intervention of a budding cycle. Unbudded cells isolated from log cultures also undergo cell fusion without prior budding (152). These results demonstrate that cells in the unbudded portion of the cell cycle can undergo fusion, but they do not permit any conclusion about cells in the rest of the cycle. A more critical test of the proposition was provided by a kinetic study of zygote morphology after the mixing of two asynchronous cultures of haploid cells (72). The first zygotes that were formed were unbudded and binucleate and, hence, resulted from the fusion of two unbudded, mononucleate, haploid cells. This result suggests that cell fusion preferentially occurs among cells in the unbudded portion of the cell cycle, but does not rule out the possibility of slower or less frequent conjugation between budded cells.

The most convincing evidence on this point comes from an ultrastructural study of conjugation by Byers and Goetsch (20). In a mass mating induced by mixing two asynchronous populations they found that each of the two haploid nuclei contained a single nuclear plaque in all mating pairs that had not yet completed nuclear fusion. This result demonstrates that the two haploid nuclei are synchronized at the single-plaque stage at the time of cell fusion. This conclusion is supported by the finding of Sena et al. (153) that newly formed zygotes undergo DNA replication during the first budding cycle. Because plaque duplication is a prerequisite for DNA replicaton and bud emergence in the cell cycle, all of the results collectively support the proposition that cell fusion normally occurs at the unbudded portion of the cell cycle prior to plaque duplication and the initiation of DNA synthesis.

Studies by Reid (personal communication) suggest that this portion of the cell cycle is not only the preferred period for cell fusion, but that conjugation is restricted to this interval. He

arrested haploid *a* or *α* cells at various positions in the cell cycle by shifting temperature-sensitive *cdc* mutants to the restrictive temperature and then challenged them for mating with cells of opposite mating type. Cells arrested at the *cdc* 28 block retained the ability to mate, and this result is consistent with those reported above, for this mutant arrests as an unbudded cell with a single nuclear plaque at a stage prior to the initiation of DNA synthesis (20, 73). Mutants arrested at all subsequent stages of the cell cycle exhibited drastically reduced mating propensity, suggesting that conjugation is not possible at other positions in the cell cycle. Mutants with a block in cytokinesis exhibited a low but significant ability to mate, and it is interesting that these mutants continue to traverse the nuclear mitotic cycle at the restrictive temperature (71). Two arguments suggested that the inability of cells blocked at most stages of the cell cycle to mate was not an artifact of their being "sick." First, if the cells were shifted back to the permissive temperature after incubation at the restrictive temperature they rapidly regained the ability to mate (presumably due to traverse of the cell cycle and arrival at G1). Second, synchronous cultures of a mutant with a very late block in the cycle lost the ability to mate at the restrictive temperature at the time of bud emergence, long before reaching the point of cell cycle arrest.

Much circumstantial evidence suggests that *α* factor and the recently described *a* factor are the agents responsible for the synchronization of the haploid cell cycles prior to cell fusion, but proof is lacking. The existence of mating type-specific diffusible substances was first evidenced by the finding of Levi (101) that *a* cells which were near but not physically touching *α* cells underwent morphological changes. The *a* cells elongated and enlarged, a process described by Levi as putting out "copulatory processes." Cells with these processes are commonly referred to as schmoos (104). The substance responsible for this phenomenon (*α* factor) is produced constitutively only by cells of *α* mating type (*α* or *αα*), and only cells of *a* mating type respond to it (*a* or *aa*) (45). Cells that carry at least one allele of both mating types (*aα*, *aαα*, *aaα*) neither produce nor respond to *α* factor (45). The *α* factor has been extensively purified, has a molecular weight of about 1,400, and contains a number of common amino acids as well as cupric ions (46).

When *α* factor is added to cells of *a* mating type, cell division and nuclear DNA synthesis are arrested, but protein and RNA synthesis continue (172); consequently the cells enlarge and elongate. The cell population becomes synchronously arrested as unbudded mononucleate cells that have not initiated DNA synthesis (18, 81), and each cell has a single nuclear plaque (20). Furthermore, the site of arrest has been specifically mapped within the sequence of three gene functions that lead to the initiation of DNA synthesis, and the *α* factor sensitive step is the same as that mediated by the *cdc* 28 gene product, the first step in the sequence (Fig. 12; 81).

Recently a factor produced constitutively by cells of *a* mating type, *a* factor, has been discovered by the fact that it occasionally causes shape changes in cells of *α* mating type (104). This substance specifically arrests *α* cells as unbudded, mononucleate cells prior to the initiation of DNA synthesis (L. Wilkinson and J. Pringle, submitted for publication). The *a* factor arrested cells have a single nuclear plaque (B. Byers, personal communication) and, again, the site of arrest is at the *cdc* 28 mediated step (L. Wilkinson and J. Pringle, submitted for publication). *a* factor has no effect upon cells of *a* mating type or upon *a*/*α* diploid cells (L. Wilkinson and J. Pringle, submitted for publication). Nothing is known of the chemistry of *a* factor. The relationship, if any, of these two factors to substances that are reported to cause mating-type-specific cell expansion (197) is unknown.

The observations reported above strongly implicate *α* and *a* factors in the synchronization of the haploid cell cycles prior to cell fusion. The factors have the appropriate specificity of production and response with respect to the mating type alleles. The factors arrest cells at precisely the point in the cell cycle at which conjugating cells are blocked. Finally, a high proportion of sterile mutants either fail to produce factor or respond to factor, or both (104, 105).

Nevertheless, caution has been suggested in accepting the proposed roles for these factors in the mating response (152) because the addition of *α* factor to mass matings can actually delay zygote formation (T. R. Manney, personal communication; 152). This observation is perhaps not too surprising because an excess of what is surely an impure preparation of only one of the factors is being added to cells that already contain both factors in their appropriate biological concentrations. However, under one set of rather artificial conditions *α* factor actually promotes mating. The mating of several temperature-sensitive *cdc* mutants at the restrictive temperature is dramatically enhanced by

arresting the cells with α factor, presumably because the factor arrests the cell at the appropriate position in the cell cycle and prevents the cell from blocking at an inappropriate position (B. Reid, personal communication). Nevertheless, the proposed role for these factors can only be accepted when sterile mutants are found that do not produce the appropriate factor and whose ability to mate can be restored by adding purified factor.

Transition from Mitosis to Stationary Phase

A unified picture of how yeast cells control the cell division cycle at the onset of nutritional deprivation is beginning to emerge. Prototrophic yeast cells upon encountering nutritional conditions that are unsatisfactory for growth appear to arrest cell division at its onset in the G1 portion of the cell division cycle.

Beam et al. (7). noted that yeast cells placed in starvation medium containing only glucose and phosphate buffer accumulated as unbudded cells. Williamson and Scopes (185, 195) exploited the same fact in devising their synchronization scheme and demonstrated that stationary-phase cells that have exhausted a rich medium were not only unbudded, but were arrested prior to DNA synthesis in the cell cycle.

A systematic investigation of the site of cell cycle arrest after various defined states of nutritional deprivation was carried out by Pringle and Maddox (personal communication). They showed that if a prototrophic yeast strain is starved of carbon and energy source including glucose, glycerol, acetate, lactate, or ethanol the population of cells arrested with 97% or more of the cells unbudded. The cultures were lightly sonicated before scoring for budded and unbudded cells to disrupt clumps of cells. Starvation for ammonia, sulfate, phosphate, biotin, or potassium also elicited arrest within the unbudded portion of the cell cycle. The only obligate nutritional requirement of the prototrophic strain that failed to produce uniform arrest at the unbudded stage was magnesium.

The question of strain specificity in this response was examined by testing 14 unrelated haploid strains and 12 unrelated diploid strains after stationary-phase arrest in rich medium. All strains displayed a high percentage of unbudded cells in stationary phase, the mean for the haploid strains being 95% and the mean for the diploid strains being 97%.

The site of cell cycle arrest after nutritional deprivation was investigated with respect to the sequence of gene-mediated steps shown in Fig. 12. Cells carrying a *cdc* mutation were arrested by nutritional deprivation at the permissive temperature and then placed back into complete medium at the restrictive temperature. The emergence of the cells was followed by time lapse photomicroscopy. If a cell completed the particular *cdc* gene-mediated step before nutritional arrest that cell should be capable of completing one cell cycle at the restrictive temperature and would arrest in the second cell cycle. If the cell had not completed the step, it should have arrested in the first cell cycle. The inhibitors, α factor and hydroxyurea, were tested in an analogous manner. The conclusion from these experiments was that the cells were arrested at or prior to the step that is sensitive to α factor in cells of *a* mating type and is mediated by the *cdc* 28 gene product.

These observations must mean that the *cdc* 28-mediated step or some prior step in the cell cycle is uniquely sensitive to nutritional deprivation. At some time in the growth of the culture, cells that are before the *cdc* 28 step are unable to complete it, whereas cells that are after this step continue through the entire cell cycle to complete cell separation. The response of yeast cells to nutritional deprivation by arresting cell cycle progress in G1 may be a control mechanism that has evolved to cope with times of nutritional inadequacy. Perhaps cells in G1 can retain viability under resting conditions for longer periods than cells arrested in other parts of the cycle, but this idea has not been tested experimentally.

G1 arrest is not, however, a necessary consequence of all types of growth limitation, because most ways of artificially interfering with yeast growth do not lead to G1 arrest. Starvation of auxotrophs for required amino acids or purines or pyrimidines did not in general lead to G1 arrest (J. Pringle and R. Maddox, personal communication). Furthermore, of 2,000 temperature-sensitive mutants which are blocked in a presumably random sample of diverse, albeit unknown, metabolic processes, no more than 6 showed G1 arrest (76).

Transition from Mitosis to Meiosis

The landmarks of the meiotic cycle in yeast include DNA replication (41), genetic recombination, first and second nuclear divisions, and spore wall formation (117). Although recombination does occur during the mitotic cell cycle, its frequency is orders of magnitude higher in the meiotic cycle. Bud emergence does not occur during meiosis, and the first meiotic nuclear segregation differs from mitosis in that

homologous chromosome pairs are segregated. The second nuclear segregation has the same genetic consequence as mitosis, and both nuclear divisions involve the duplication and segregation of spindle plaques, although the meiotic plaque is cytologically distinct from the mitotic plaque (117). The first and second chromosome segregations occur within a single membrane-bound nucleus, which at the completion of the second division pinches off four nuclei (117). Cytokinesis and cell wall separation are quite distinct in meiosis, being accomplished by the growth of the spore wall around the nuclear membrane (117).

Despite some basic differences, the molecular events that underlie many of the landmarks in meiosis and mitosis must be fundamentally similar, because the same gene products mediate these processes in both the meiotic and mitotic cycles. Thirteen out of twenty *cdc* genes that were tested were found to be essential for meiosis and sporulation (157). These essential genes include mutants with diagnostic defects in plaque duplication, plaque separation, initiation of DNA synthesis, DNA synthesis, medial nuclear division, and late nuclear division of the mitotic cell cycle. The stage of arrest of these mutants in the meiotic cycle has not yet been investigated.

Of the seven genes that did not appear to be essential for meiosis, because diploid cells homozygous for mutations in these genes were able to sporulate at the restrictive temperature, the diagnostic landmark for one (*cdc* 24) is bud emergence and for three (*cdc* 3, 10, and 11) is cytokinesis. Because sporulation does not require bud emergence and because cytokinesis appears to be accomplished during meiosis by an entirely different process than during mitosis, this result is not unexpected.

With this introduction we can pose the question of whether there is a unique point in the mitotic cell cycle from which a cell enters the meiotic program. It is clear that cells can begin the meiotic process from the G1 interval of the mitotic cycle because stationary-phase cells undergo meiosis without the intervention of a mitosis (41). However, can cells which have passed the G1 interval of the mitotic cell cycle commence upon a meiotic cycle without completing the mitotic cycle first? The answer to this question must be no for cells that have progressed to the point of chromosome segregation (perhaps late nuclear division), because the first meiotic nuclear division is reductional and the mitotic nuclear division is not. However, it is conceivable that cells between G1 and nuclear division in the mitotic cycle would be able to change their program to bring about a reductional division, especially in view of the fact that DNA synthesis and nuclear division are, in part, mediated by the same gene products.

Two studies have been reported in which synchronous mitotic cells were removed from culture and challenged to undertake meiosis by a shift of medium at various times during the mitotic cell cycle. Haber and Halvorson (62) found that cells in the budded portion of the mitotic cycle could sporulate with high frequency, whereas unbudded cells sporulated with lower frequency. Milne found that the parent cell was able to sporulate with high efficiency at all stages of the mitotic cell cycle, but that the bud produced spores only if it was relatively large at the time of the medium shift (111). Both studies suggest that cells can undertake meiosis from most stages of the mitotic cycle, and the different behavior of the unbudded cells in the two experiments can probably be attributed to differences in strain or presporulation culture conditions, or both.

However, in the context of the present discussion, the important question is not answered merely by the observation that cells from most stages of the mitotic cycle eventually sporulated, but we must ask whether or not they completed the mitotic cycle before undergoing meiosis. Milne (111) showed that some of the unbudded cells at the time of the medium shift proceeded to form small buds, and the proportion of such cells was that expected from those cells which were within 10 min of budding in the mitotic cycle. All of the cells that acquired or had small or large buds at the time of the medium shift proceeded to complete mitosis and cytokinesis despite the fact that the bud did not grow appreciably in the meiotic medium. All of the unbudded cells did not appear to pass through a mitotic nuclear division. These results suggest that under the conditions of this experiment those cells which were within 10 min of the bud emergence event completed the mitotic cycle before undertaking the meiotic cycle, whereas those unbudded cells which were before this point in the mitotic cycle proceeded directly to meiosis. Although confirmation of this observation with other strains and culture conditions is highly desirable, the results do suggest that the transition from mitosis to meiosis is controlled within the G1 interval of the cell cycle. Perhaps further experiments with *cdc* mutants will permit a more rigorous test of this idea and a greater resolution of the point of control.

HOW IS GROWTH INTEGRATED WITH DIVISION?

Although some heterogeneity in the size of cells that are at the same stage of the cell cycle does exist (92), on the average all of the cell's macromolecular components are precisely doubled once each cycle under conditions of balanced growth. Very little hard information is available to suggest how this coordination might be achieved, and even the qualitative data that is at hand relates only to the grossest of parameters such as size or mass. Nevertheless, a few observations suggest the outline of a model for the integration of growth and division that may at least serve to stimulate further experimentation.

Growth and division might be coordinated in one of two fundamentally different ways. First, it might be that one or more successive increment of growth require the completion of one or more successive cell cycle landmark. In this model cell growth beyond a certain size could not occur until a specific landmark had been passed. This mechanism could serve to maintain cell size only if progress past the landmarks were rate limiting and not growth. Second, it might be that the passage of one or more landmarks requires growth beyond some specific size or sizes. This mechanism could serve to maintain cell size only if growth were rate limiting and not the passage of landmarks. Of course, a combination of the two models is also possible. Let us consider a few observations on the growth of yeast cells with respect to cell division in the context of these possibilities.

It does not appear that growth is dependent upon the completion of any particular landmark in the cell cycle. Although careful quantitation is lacking, it is quite evident from visual inspection that cells arrested at all of the landmarks in the cell cycle identified by the *cdc* mutants continue to grow in size after the time of arrest and that the arrested cells attain sizes many-fold in excess of the normal size range after several cycle times at the restrictive temperature (42). The increase in size is accompanied by accumulation of macromolecules because the *cdc* mutants continue to synthesize stable RNA species at an uninhibited rate for at least one or two cycles after arrest (42, 70, 71, 73). Furthermore, yeast cells arrested prior to plaque duplication by the α or *a* mating factors (172; L. Wilkinson and J. Pringle, submitted for publication), at DNA synthesis by hydroxyurea (159) or Trenimon (88), and at nuclear division by X-ray treatment (82, 164) continue to increase dry mass and accumulate protein and RNA, eventually becoming several times larger than untreated cells. Although quantitation of macromolecule synthesis and size increase in the *cdc* mutants is essential before firm conclusions can be drawn, it appears that growth is not dependent upon the completion of plaque duplication, plaque separation, bud emergence, initiation of DNA synthesis, DNA replication, medial or late nuclear division, spindle elongation, cytokinesis, or cell separation.

Conversely, most of the cell cycle events can be completed without extensive growth. Although stationary-phase yeast cells are uniformly arrested in the G1 interval of the cell cycle, they are very heterogeneous in size. Some cells are of normal size, some are extremely small (193), and the average cell contains less mass, protein, and RNA than the average growing cell (141). Most of the decrease in average size occurs during the last cell cycle or two during the approach to stationary phase (141). We may conclude that as the medium becomes deficient for growth, the cells are able to complete the last division or two without the normal amount of growth. This same phenomenon has been documented for cells that cease growth due to ammonia, sulfate, or phosphate limitation (J. Pringle and R. Maddox, personal communication). The origin of the small cells under conditions of nutrient limitation was well documented for one particular situation (111). Synchronous cultures of cells growing in glucose-containing, rich medium were shifted to medium containing only acetate. Most unbudded cells did not bud after the shift. Budded cells, even those with the smallest buds, completed both nuclear division and cytokinesis. However, the buds did not grow perceptibly in size so that cells with small buds produced one normal-size cell (the parent cell) and one very tiny cell (the bud), and it is not unlikely that the size heterogeneity in most stationary-phase populations arises in the same way. Indeed this hypothesis could explain the bimodal size distribution of cells observed in stationary cultures (194). This result suggests that all stages of the cell cycle after bud emergence can be completed in the absence of the normal amount of growth. Furthermore, if we combine the observation that the last cycle upon approach to stationary phase is not accompanied by much growth with the observation that stationary-phase cells are arrested at or prior to the *cdc* 28-mediated step, it is reasonable to conclude that all steps after the *cdc* 28 step can be completed in the absence of a normal amount of growth.

There is evidence that growth is required for some early event in the cell cycle. Williamson

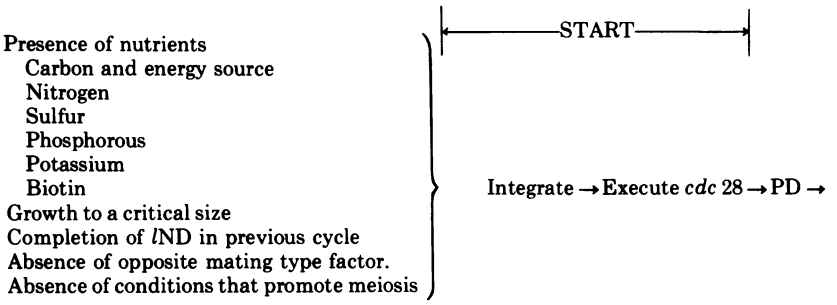

FIG. 13. *Requirements for "start" in the cell cycle.*

and Scopes (192) noted that the small cells in stationary phase populations took longer to bud in fresh medium than the larger cells. A quantitative study of this phenomenon by time lapse photomicroscopy (J. Pringle, personal communication) demonstrated that an inverse relationship exists between the size of a cell and the length of time before the onset of budding in fresh medium. Because the small cells grow during the interval before budding this observation can best be explained by the hypothesis that some early event in the cycle prior to bud emergence requires the attainment of a certain cell size for completion. The deficit of the small cells was made up by the time of budding, for the interval between first and second budding cycles was found to be the same for the originally small and originally large cells.

Another observation that is consistent with a unique requirement for growth at some early step in the cell cycle is provided by studies of von Meyenburg (181) and Beck and von Meyenburg (8). They found that when the growth rate and doubling time of *S. cerevisiae* cultures were varied by limiting the supply of glucose in a chemostat, almost all of the increased cell cycle time at slower growth rates was accounted for by an increase in the length of time spent in the unbudded portion of the cell cycle, whereas the length of time spent in the budded portion was almost unaffected by the growth rate.

THE CONCEPT OF "START"

The results reviewed in the previous sections suggest that the control of cell division may be achieved in the G1 portion of the yeast cell cycle, in particular at those steps that precede and include the step mediated by the *cdc* 28 gene product. Haploid cells appear to be able to mate only at this point in the cycle, and the same restriction may hold for diploid cells embarking upon the meiotic program. Cells that are nutritionally deprived of carbon and energy, ammonia, sulfate, phosphate, potassium, or biotin arrest here, and there appears to be a unique requirement for growth in this early portion of the cycle.

We have coined the term "start" for those events in early G1 that culminate in the execution of the *cdc* 28-mediated step (75). Before this step, we view the yeast cell as undifferentiated in the sense that it is capable of undertaking several alternative developmental programs, but after this step it appears to be committed to the mitotic cell cycle. We reason that many inputs are integrated by the cell at this unique point of control (Fig. 13) and that only if all are sufficient does the cell commit itself to a new cell cycle.

This concept obviously requires further experimental testing, but it does provide an appealing unity to some of the diverse questions of regulation posed at the outset of this review. If these speculations are substantiated we will be able to rephrase these questions in the following ways: how do the mating factors, the pathways of carbon, nitrogen, sulfur, phosphorous, potassium, and biotin assimilation, and the processes of macromolecule synthesis communicate with the *cdc* 28 gene product, and how are all of these diverse inputs integrated at the "start" event? Recalling that the regulation of cellular proliferation (28), the integration of growth and division (97), and the control of cellular differentiation (180) occur within the G1 interval of the mammalian cell cycle, it would not be unreasonable to expect that answers to these questions would have import beyond the boundaries of mycology.

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