REVIEW

CELL DIVISION FROM A GENETIC PERSPECTIVE

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Recently, a number of laboratories have begun to study mutant cells that are defective in specific stages of the eukaryotic cell cycle. The long-range goals of this work are to identify the genes that code for division-related proteins, to define the roles that these gene products play and to investigate the hierarchies of order that assure their coordinated activity. It is my intent in this brief review to discuss the strategies employed in this genetic approach and to enumerate some of the new conclusions that have come to light. A recent review on the genetics of meiosis (2) complements this review on mitosis.

MUTANTS

Mutations that inactivate gene products essential for division would be lethal. Cell cycle mutants must be therefore either temperature-sensitive or suppressor-sensitive conditional mutants in order to be useful for study. Most reports to date have utilized the former, but the availability of temperature-sensitive nonsense suppressors (46) should permit study of the latter as well.

Although any mutation that blocks cell division could be considered a cell-cycle mutation, the term will be reserved here for mutations that lead to defects in, or failure of, a stage-specific event (or landmark) of the cell cycle, such as DNA replication, or nuclear division, events which normally occur but once each cycle. This definition of a cell cycle mutant excludes mutations in genes whose products control the continuous processes of growth and metabolism and permits the stage-specific mutations to be distinguished empirically: an asynchronous population of mutant cells will become arrested at one cell cycle landmark after incubation at the restrictive condition for that mutation, whereas mutants with defects in one of the continuously required functions will arrest at the restrictive temperature with cells at a variety of positions in the cell cycle.

Classes of mutants may be distinguished from one another and the roles of their products delimited by determining the stage-specific event at which they arrest. It is convenient to have a designation for the first landmark of the cell cycle that is blocked in a particular mutant, and I shall call it the *diagnostic landmark* for that mutant. Mutants of *Saccharomyces cerevisiae* have been identified that have diagnostic landmarks at spindle pole body (SPB) duplication, SPB separation, initiation of DNA synthesis, DNA replication, each of two stages of nuclear division, cytokinesis, and bud emergence (8, 9, 25). In *Schizosaccharomyces pombe,* diagnostic landmarks have been localized to DNA replication, nuclear division, and two stages of cell plate formation (42). Mutants with diagnostic landmarks at DNA replication (44), at least two stages of nuclear division (38, 44), and septation (38) have been detected in *Aspergillus nidulans;* some of the nuclear division mutants arrest with condensed chromosomes characteristic of a metaphase block (39, 44). *Tetrahymena pyriformis* mutants with diagnostic landmarks at two stages of cell division, formation of the fisson zone and constriction of the fission zone have been found (17). The work with *Ustilago maydis* mutants has concentrated upon lesions affecting DNA replication and recombination (58); a mutant with a thermolabile DNA polymerase has been described (33). In both *Physarum polycephalum* (19, 61) and *Chlamydo-*

monas reinhardtii (30), mutants have been isolated and studied that may be cell cycle mutants, but their characterization has not progressed far enough to identify the diagnostic landmarks. A number of mutants have been described for vertebrate animal cells (reviewed in reference 4); their properties suggest diagnostic landmarks in G1 (7, 12, 35, 49, 50, 51, 56), at DNA synthesis (50, 52, 54), at mitosis (60), or at cytokinesis (28), although not all have been rigorously demonstrated to achieve synchronous arrest from an initially asynchronous culture.

Mutants with specific effects on mitosis are also being studied systematically and elegantly in *Drosophila melanogaster* (reference 1; B. Baker, A. Carpenter, and P. Ripoll, personal communication). Although most of the mutations that have been studied to date appear primarily to affect genetic recombination and/or DNA repair, the *Drosophila* studies merit discussion because the methodology is applicable to a large variety of mitotic lesions and because the information already attained is relevant to several topics considered below. The types of mutations utilized in the *Drosophila* studies and, consequently, the experimental rationales used to extract information from them are distinctly different from those of the other organisms reviewed here. Whereas other investigators have concentrated specifically upon mutants displaying an absolute block at some stage of the mitotic cycle, the *Drosophila* work employs mutants having less deleterious mitotic defects which do not prevent the extensive cell division that is essential for the construction of an adult fly.[1] In order to study such mutants, fly embryos homozygous for the mitotic mutation in question and heterozygous for various recessive, cell autonomous genes that affect body color or bristle shape are constructed by appropriate genetic crosses. The influence of the mutation upon mitosis is revealed through analysis of the number, size, and distribution of patches of mutant tissue on the surface of the fly, patches which result from abnormalities in chromosome behavior during development of the integument. It is possible to detect and distinguish by these measurements mitotic chromosome loss, nondisjunction, recombination, mutation, or deletion, because each type of aberration produces a unique pattern of spots. Mutations at 13 loci, originally isolated for their effects upon meiosis, have been examined. Six of them produce mitotic chromosome instability, and at least three of these are also abnormally sensitive to mutagenic agents, suggesting defects in DNA repair. Four influence the frequency of chromosome nondisjunction and/or chromosome loss.

The mutants studied to date represent only initial forays into the genetics of cell division, yet they demonstrate that stage-specific mutants can be found for each landmark. Hundreds of cell cycle genes will probably be found in any species, if and when systematic and exhaustive searches are conducted.

ORDER

One of the impressive characteristics of cells is the reproducibility with which they achieve the complicated task of division. Although potentially lethal pitfalls exist at every stage of the cell cycle, cells accomplish with only rare mistakes the faithful replication of each gene, packaging of every chromosome, disjunction of sister chromatids, and distribution of nuclei and other organelles to daughter cells. The numerous biochemical and morphological events in each of these processes are undoubtedly rigorously ordered with respect to one another. Studies of mutants are revealing some of the underlying events responsible for this order.

Temporal Order

Conditional mutants lend themselves to an investigation of the temporal order of events because a single gene product can be inactivated at any time during the division cycle simply by shifting cells from the permissive to the restrictive condition. With temperature-sensitive mutants one can determine the point (termed the execution or transition point) in the cell cycle at which the temperature-sensitive event has been completed since, before this point, cells are incapable of dividing upon a shift to restrictive temperature, but after this point they are capable of division (Fig. 1). It should, however, be noted that in the absence of additional biochemical information about the defective gene product, the molecular basis of the execution point is ambiguous because proteins can be thermolabile in at least two different ways: for synthesis or for function. If the

[1] It should be noted that strategies exist for studying the effects on development of mutations that completely arrest cell division (48), although none of the mutants studied to date is known to produce a stage-specific arrest.

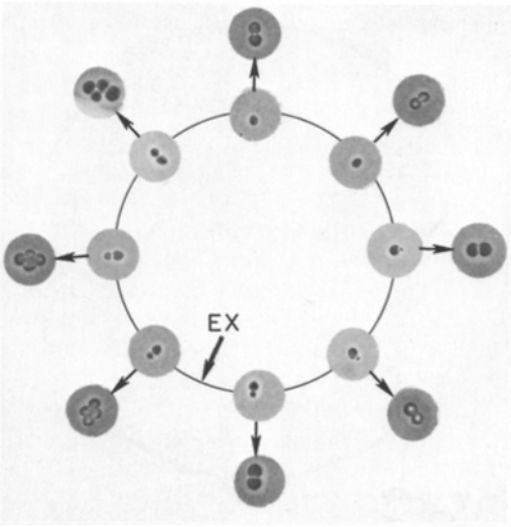

FIGURE 1 Determination by time-lapse photomicroscopy of the execution point in a temperature-sensitive *cdc* mutant of the budding yeast, *Saccharomyces cerevisiae* (23). Cells growing at the permissive temperature were shifted to the restrictive temperature and photographed at the time of the shift (cells on the inner circle), and the same cells were photographed after 6 h at the restrictive temperature (cells on the outer circle). Cells were cut from photographs taken at the time of the shift and arranged on the inner circle in order of bud size, a measure of position in the cell cycle. Each cell on the outer circle developed from the corresponding cell on the inner circle and is composed of a parent cell with a large bud. Cells early in the cycle (before the execution point, *EX*) arrested development in the first cell cycle at the restrictive temperature, and each cell later in the cell cycle (after *EX*) finished the first cycle and arrested in the second cycle.

lesion arrests *synthesis* of new gene product but does not affect the function of that extant at the time of the shift, then the execution point defines the point in the cycle where sufficient product accumulates to accomplish division. If, on the other hand, the lesion arrests *function* of the gene product, then the execution point defines the time when its function for division is completed.

Execution points have been measured for a number of temperature-sensitive cell division cycle (*cdc*) mutants in *Saccharomyces cerevisiae, Schizosaccharomyces pombe, Aspergillus nidulans,* and *Tetrahymena pyriformis*. Often, the execution point closely coincides with the time of completion of the diagnostic landmark. For example, mutations in either of two *cdc,* genes, 8 and 21, of *S. cerevisiae* result in an immediate cessation of DNA replication upon a shift to the restrictive temperature; both mutants have execution points near the end of the DNA synthetic period (20, 26). The coincident occurrence of execution point and diagnostic landmark, found for these and many other mutants of *S. cerevisiae* (23), also obtains for 10 of 13 *cdc* genes of *Schizosaccharomyces pombe* (42), for 7 of 9 mutants in *Aspergillus nidulans* (45), and for all 3 of the fully penetrant mutants of *Tetrahymena pyriformis* (J. Frankel, personal communication). This result implies that the thermolabile gene product in each of these mutants is synthesized or functions at the time of occurrence of the diagnostic landmark and suggests that the gene product in question plays a role intrinsic to this stage-specific event. In such cases, a biochemical analysis of the mutants for enzymes thought to be involved in this landmark is in order. Indeed, the *cdc* 21 *S. cerevisiae* mutant mentioned above has been shown to be defective in thymidylate synthetase (5).

In some cases, however, the execution point for a particular mutant occurs considerably before the mutant cell exhibits any observable defect in its progress through the cell cycle. For example, mutants of *S. cerevisiae* were found with execution points early in the cell cycle, at about the time of budding, but they display no obvious abnormality at the restrictive temperature until cytokinesis (21). They may be defective in the synthesis of a protein that is made early and used later in the cell cycle, or they may be defective in some early, here-to-fore unrecognized, cell cycle event whose completion is prerequisite for cytokinesis. The latter proved to be true. Recent work demonstrated that a ring of microfilaments, normally formed early in the cycle within the cytoplasmic membrane at the neck between parent cell and bud (10), is not made in these mutants at the restrictive temperature (11). Thus, the failure of coincidence between execution point and occurrence of the diagnostic landmark suggests that a search for new cell cycle landmarks might be fruitful, and the execution point localizes the interval in which to search. Mutants with execution points that significantly precede the diagnostic landmark have been observed for 3 out of 13 *cdc* genes of *S. pombe* (42) and 2 out of 9 *cdc* genes of *A. nidulans* (45).

Execution points of mutants can be informative in yet another way. When one examines several alleles of the same gene, one frequently finds that mutants fall into two classes: those having alleles

with execution points that can be mapped to the cycle in progress at the time of the temperature shift (first cycle arrest) and those that do not arrest division until two or more divisions after the shift (multicycle arrest). In genes that exhibit alleles of both types, it is likely that the former are temperature-sensitive for function and the latter for synthesis. The fact that the latter are capable of completing several divisions at the restrictive temperature implies that the gene product in question is normally present in excess and suggests that its synthesis cannot be a controlling influence on the course of division. Multicycle arrest mutants were found in 14 of the 35 *cdc* genes identified in *Saccharomyces cerevisiae* (26), and would probably have been found in other eukaryotes as well but were discarded as leaky in the *S. pombe* search (42) and were selected against in the *A. nidulans* screen (44).

Causal Order

Another type of order is evidenced from a catalogue of landmarks completed and not completed in mutants at the restrictive temperature. Each mutant as it attempts to pass through the cell cycle at the restrictive temperature completes all early landmarks normally and then fails at its diagnostic landmark. Of interest is the fate of landmarks that would have occurred in a normal cell cycle subsequent to the diagnostic landmark. Some of these may occur in a particular mutant, and others may not. The pattern observed for all of the mutants permits the derivation of pathways of dependent or causally related landmarks in the cycle. Pathways derived for *S. cerevisiae* and *S. pombe* are shown in Fig. 2. In these two yeasts as well as in mammalian cells, cell division is dependent upon nuclear division which in turn is dependent upon DNA replication. Interestingly, in all three organisms the initiation of a second nuclear cycle consisting of DNA replication and nuclear division does not require cytokinesis of the preceding cycle but does require the completion of some stages of nuclear division in the preceding cycle (25, 38, 42, 44). The *Tetrahymena pyriformis* cycle is ordered similarly, in that micronuclear cycles and macronuclear DNA replication are not dependent upon cell division of the previous cycle, but differently in that macronuclear division is dependent upon prior fission zone formation (17); that is, in the *Tetrahymena* cell division mutants, many micronuclei accumulate but the single macronucleus may endoreduplicate its DNA without dividing.

Sequential Gene Product Expression

The dependent pathways of landmarks that can be defined by mutant phenotypes must be determined by dependent sequences in the function or synthesis of specific gene products. It is of considerable interest to inquire therefore whether the gene-controlled steps that are identified by the thermolabile cell cycle mutations are organized into causal sequences. Two strategies exist for determining these sequences.

The less precise method (double mutants) requires a comparison of single with double mutant phenotypes (29). It is necessary that both of the two mutations display first cycle arrest and that the phenotypes of the two mutants be distinguishable. If two mutants are defective in sequentially dependent steps, then those double mutant cells that begin a cycle at the restrictive temperature should assume the phenotype characteristic of the first of these steps. If the double mutant phenotype is distinct from either single mutant, we can conclude that the two steps are in independent pathways. Double mutants have been used to decipher the most probable dependent sequence for cell cycle mutants of *S. cerevisiae* and to identify independent pathways (Fig. 4 and references 25 and 29). Double mutants between various cell division mutants of *T. pyriformis* have phenotypic characteristics of the two contributing single mutants, implying that the steps function independently of one another (18).

A more precise method (reciprocal shifts) for mapping sequential pathways of gene product expression (Fig. 3) is capable of distinguishing unambiguously the four possible relationships between two steps (29, 32); these four are: the two dependent sequences; the independent pathways; and an interdependent pathway in which the two gene products must express themselves concomitantly. The reciprocal shift method involves shifting cells from one condition that produces a stage-specific block, i.e., the restrictive temperature for a temperature-sensitive mutant, to another, i.e., the presence of an inhibitor like hydroxyurea, and determining whether or not cell division ensues. This method has two advantages over the double mutant procedure. Not only is it more discriminating, but it is not limited to mutants with different phenotypes. The application of this method is

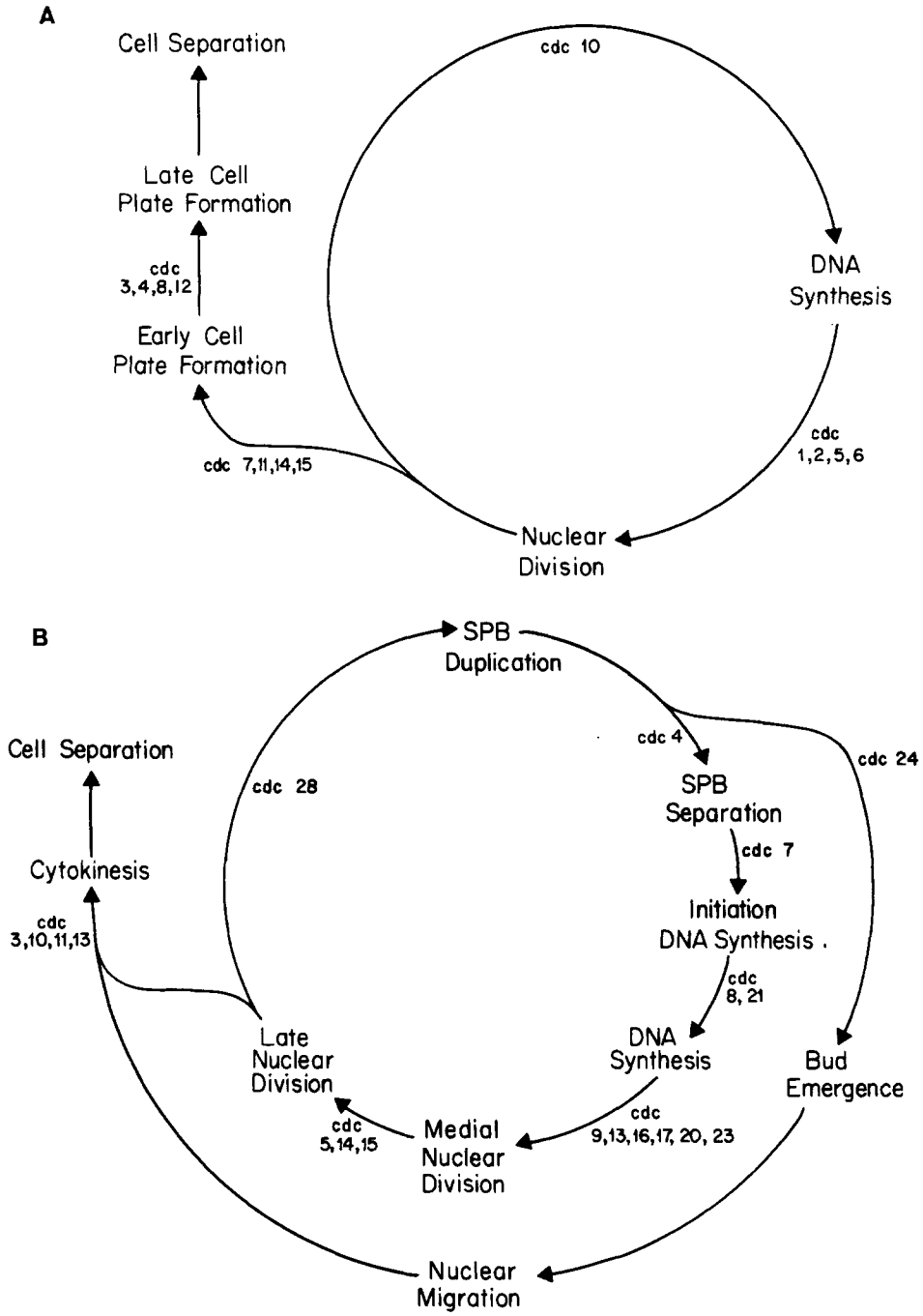

FIGURE 2 Dependent pathway of landmarks in the cell cycles of *Schizosaccharomyces pombe* (Fig. A, reference 42) and *Saccharomyces cerevisiae* (Fig. B, reference 25) derived from mutant phenotypes. *Cdc* gene designations are placed immediately preceding their diagnostic landmark. The diagram relates to mutant phenotypes as follows: Upon a shift to the restrictive temperature, mutant cells arrest synchronously at the position designated by the *cdc* number; all events flowing from this point do not occur while all other events do.

more demanding, however, in that it requires that the two stage-specific blocks under comparison be reversible and have different restrictive conditions. It is therefore not applicable to the ordering of two heat-sensitive mutants but can be used to order a set of heat-sensitive mutants with respect to the arrest point of either a stage-specific inhibitor (29) or a cold-sensitive mutation (32). A pathway deduced from a combination of the two methods for the *Saccharomyces cerevisiae* cell cycle is shown in Fig. 4. One step, that controlled by the *cdc* 28 gene product, initiates the cycle; the pathway then bifurcates into two independent pathways that later converge before cell division. The pathways established by either method necessarily incorporate any ambiguity that exists about the nature of the mutation employed. That is, if it is not known whether the mutations block synthesis or function of the mutant gene products, one cannot know whether an observed dependence is over the synthesis or function of these products. Many new questions are raised by the elucidation of such maps. One would like to know how the dependence at each step is established.

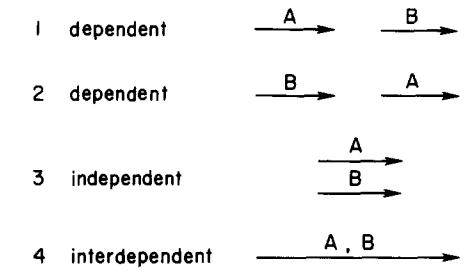

FIGURE 3 Four possible relationships between two gene-mediated steps. Two steps may be related in a dependent sequence (*1* or *2*), in which case the first step must occur before the second. They may be independent (*3*), in which case either step can occur in the absence of the other, or they may be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

DEVELOPMENT

The pathways deduced for the mitotic cycle provide a map within which the origins of other developmental programs may be located, and the mutants provide the means to achieve this task.

Mitotic Cycles in Different Cell Types

Many distinct cell types exist in metazoans and in many eukaryotic microorganisms at different stages of their life cycle. It is of interest to know whether different types of cells execute the same genetic program for their mitotic cell cycles.

S. cerevisiae exists as several recognizably different cell types each of which undergo mitosis,

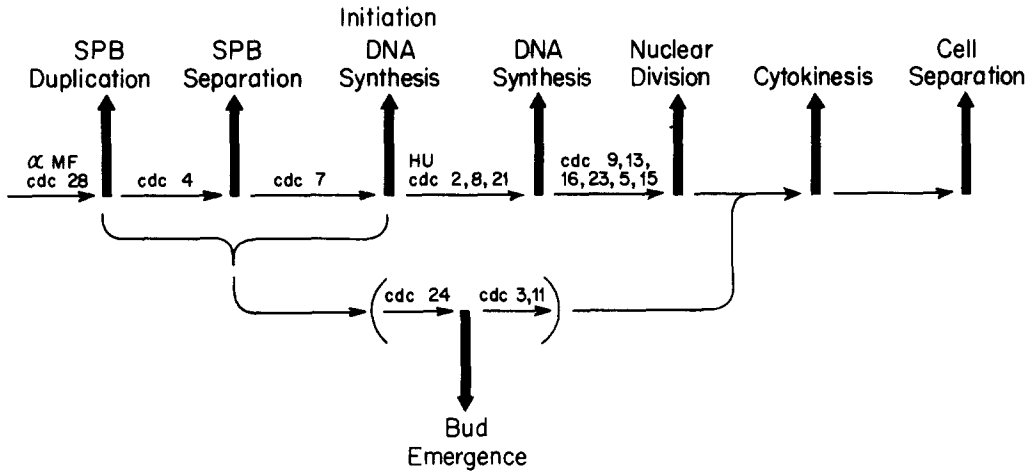

FIGURE 4 Summary of dependent relationships between gene-controlled steps in the *S. cerevisiae* cell cycle taken from reference 24. Succeeding steps are related in a dependent sequence (as defined in Fig. 3) of given order. The relationships between arrows or symbols enclosed within parentheses are undefined. Completion of steps *cdc* 24, 3, and 11 are known to be dependent on the α-mating factor-sensitive step and independent of the hydroxyurea-sensitive step; however, their relationship to steps mediated by *cdc* 4 and 7 is unknown. Designation: numbers, *cdc* genes; α, mating factor; HU, hydroxyurea; and SPB, spindle pole body.

haploid cells of mating type *a* or α, nonmating diploid cells (a/α), zygotes, and germinating spores. The *cdc* mutants described above, originally isolated in haploid *a*, were employed in appropriate genetic crosses to determine whether the same genes control mitosis in all of these cell types. They do (26 and unpublished results).

The use of genes that are expressed cell autonomously in *Drosophila* as signals of mitotic abnormalities permits a comparison of the kind and frequency of abnormality between different tissues. Most of the mutants studied so far appear to produce the same (mutant-specific) derangements in chromosome behavior in all tissues examined with two notable exceptions. Two mutants increase mitotic recombination dramatically in abdominal tissue but not in that of the wing tissue (B. Baker, A. Carpenter, and P. Ripoll, personal communication). The reason for this interesting tissue specificity is unclear but several possibilities exist. This gene might function only in certain tissues or its substrate might exist in different states in different tissues. Further possibilities arise from the fact that the abdominal and wing tissues proliferate at different times during development; in this context, it is interesting that the size range of the somatic spots produced by one of these mutants is such as to locate its time of action to a time when the cells susceptible to this mutation are arrested in the G2 state.

Relationship Between Mitotic and Meiotic Cycles

In meiosis, events similar to those in mitosis occur but achieve a different outcome. DNA synthesis is followed by extensive recombination involving a structure unique to meiotic cells, the synaptonemal complex, and chromosome segregation occurs twice. The meiotic segregations of chromosomes differ from the mitotic in that, in the first segregation, homologous centromeres separate and, in the second, segregation is not preceded by DNA replication. Furthermore, cytokinesis and cell division in meiotic *S. cerevisiae* cells is accomplished by the growth of new wall material within the cytoplasm of the mother cell, in contrast to the case in mitotic cells (37). It is of considerable interest to determine whether the cell calls upon an entirely new genetic program during meiosis or merely alters the mitotic one.

This question was answered for *S. cerevisiae* by testing the temperature-sensitive mitotic *cdc* mutants to see whether they were able to complete meiosis at the restrictive temperature. Nearly all of the genes that are known to control nuclear events during mitosis—spindle pole body duplication and segregation, DNA replication, chromosome segregation—were found to be essential for meiosis as well (53). Three of the genes that control the parallel mitotic pathway leading to budding and cytokinesis were found not to be essential for meiosis. Clearly, the meiotic program is achieved through modification of the mitotic one. It will be of interest to determine the pathway of *cdc* gene function during meiosis since some of the unique meiotic outcomes might be achieved merely by changing the order of mitotic gene expression. Genes that function uniquely in *S. cerevisiae* meiosis have also been identified (14). It should be possible to locate the positions of the meiosis-specific functions within the altered mitotic program.

Similar conclusions arise from a comparison of the effects of mutations upon meiosis and mitosis in *Drosophila*. Furthermore, since the *Drosophila* mutants are of a fundamentally different type, producing alterations in chromosome behavior rather than the absolute blocks in mitosis and meiosis characteristic of the *S. cerevisiae* mutants, the two investigations together lead us to understand that most gene products are shared between mitosis and meiosis. Of seven mutants originally recognized by their depression of recombination in female *Drosophila* meiotic cells, six increased the frequency of chromosome instability in mitotic cells (reference 1; B. Baker, A. Carpenter, and P. Ripoll, personal communication). Interestingly, none of the six decreased the frequency of mitotic recombination, suggesting either that these gene products function in different ways in mitosis and meiosis or that their function is not intrinsic to recombination. Of six mutants originally detected for the abnormalities they produced in meiotic chromosome segregation, four affected mitotic chromosome behavior as well.

Relationship Between Conjugation and Mitosis

Conjugation between *S. cerevisiae* cells of opposite mating type constitutes yet a third program in the *S. cerevisiae* life cycle. Cell agglutination is followed by cell fusion and nuclear fusion. The resulting zygote then embarks upon a mitotic cell cycle which employs the program outlined above

(Fig. 4). The two haploid cell cycles become synchronized by mating hormones before cell fusion, each mating type constitutively producing a hormone that is a stage-specific, cell cycle inhibitor for the cell of opposite mating type (6, 22, 62). With the *cdc* mutants, it was possible to locate the site of synchronization in both mating types witin the pathway of gene function at the step mediated by *cdc* 28 product (29, 62).

The synchronization of opposite mating types is of obvious value, if not necessity, when the conjoined elements are to embark upon a mitotic program. However, it is not obvious that a particular site in the cycle would be better than another, and hence it was of interest to determine whether conjugation was restricted to the *cdc* 28 step or merely facilitated at this step. Under normal conditions, the mating hormones assure synchronization of the cells at this point and preclude challenge at other steps. When *cdc* mutants were presynchronized at various steps in the cycle by incubation at the restrictive temperature and then challenged to mate, it was found that mating is restricted to the *cdc* 28 step in the cycle (47). The steps which are unique to the mating program are currently being identified by the isolation and study of sterile mutants (36).

Coordination of Growth and Division

During the cell cycle a continuous accumulation of macromolecules, protein, RNA, and polysaccharides accompanies the program of stage-specific events so that, over innumerable generations, growth and division remain coordinated. That controls exist to assure the coordination of growth and division has been elegantly demonstrated with *S. pombe* (15). The filamentous *S. pombe* cells growing exponentially divide at a distribution of lengths with a mean of 13.5 μm. Measurements of individual cells followed in time-lapse photomicrographs over several generations demonstrated that cells which divide at a size smaller or larger than the mean in one generation divide at the mean size in the next generation; that is, deviations from the mean size are corrected within one generation.

Genetic studies with two yeasts, *S. cerevisiae* and *S. pombe,* have contributed new information on the coordination of growth and division, and here again the mitotic gene program provided a pathway within which to locate the control point and the mutants provided implements to investigate its nature.

When growth is slowed in *S. cerevisiae* by limitation of carbon or energy source or by a direct limitation of protein synthesis, division is coordinately slowed as in other organisms. The lengthening of the division program occurs almost exclusively in the G1 interval of the cell cycle (3, 55, 59), as in mammalian cells and many other eukaryotes, and has been localized in *S. cerevisiae* to the G1 interval preceding the *cdc* 28 step (27, 31). Numerous observations relating growth and division in *S. cerevisiae,* including response of the cell to nutritional starvation (57), nutrient limitation under steady state conditions (55, 59), and the peculiar cell cycle kinetics manifested in the production of unequal cells at division (27), can be accounted for by the hypothesis that the attainment of a critical cell size is a prerequisite for completion of the *cdc* 28 step (27, 34). It was proposed that growth, that is the doubling of cell mass (rather than completion of the stage-specific program of cell cycle events), is normally rate limiting for *S. cerevisiae* cells and hence that growth and division are coordinated in each cycle because the stage-specific program pauses at the *cdc* 28 step until growth (or the accumulation of some substance whose amount is proportional to cell mass) catches up (34). There appear to be no feedback controls in the opposite direction, from division to growth, since mutants arrested at most[2] steps in the cell cycle grow unabated and achieve masses several times the norm at the restrictive temperature (34). It is of considerable interest that the attainment of integral values of mass increase appears to be requisite for the initiation of chromosome replication in *Escherichia coli* (13) and, as will be discussed below, for *S. pombe* as well.

Unlike *S. cerevisiae, S. pombe* exhibits a short G1 interval in its cell cycle under most conditions of exponential growth, and it is unlikely therefore that the G1 interval can serve as a time in which growth and division are synchronized in this organism as it does in *S. cerevisiae.* A number of observations have suggested that the G2 interval satisfies this function in *S. pombe* under most conditions because attainment of a specific size is necessary for the completion of nuclear division (16, 40). Firstly, mutations in one of two genes, *wee* 1 and *wee* 2, lead to mutant cells that are

[2] The only exceptions are G1 arrest mutants whose primary defect may be in some aspect of growth rather than in a stage-specific cell cycle event (34).

smaller than normal (reference 40; unpublished work quoted in reference 41). *wee* 1-50 is temperature-sensitive and, upon a shift from 25°C (normal size) to 35°C (small size), the first stage-specific event to occur at a smaller than normal size is nuclear division (40). Secondly, wild type *S. pombe* cells are larger at division in a medium supporting a rapid growth rate than in one supporting a slow growth rate; shifts between the two media again reveal that the first event to occur at the size characteristic of the new medium is nuclear division (16). Strong support for the model of size coordination at nuclear division is provided by the finding that several alleles at the *wee* 2 locus are temperature-sensitive *cdc* mutants that arrest at nuclear division when shifted to the restrictive temperature while another mutation that maps to the same locus (Nurse and Thurioux quoted in reference 41) is not a temperature-sensitive lethal but rather produces cells that are smaller than normal at all temperatures. Thus, the *wee* 2^+ gene product is essential for nuclear division and is involved in the coordination of size and division.

S. pombe appears to have a second cycle position near the initiation of DNA synthesis that monitors cell size under certain conditions but is cryptic under conditions of exponential growth. When wild type *S. pombe* cells are starved of a nitrogen source or sporulated, the resulting cells are arrested in G1 and are much smaller than those found in an exponential population (43). Furthermore, the *wee* 1 mutant acquires a longer G1 interval in its cell cycle when it is growing at the restrictive temperature, at which it is smaller (40). All three cell types, starved wild type cells, spores, or mutant cells at the restrictive temperature, grow to about the same size before initiating DNA synthesis (43). Since this size is smaller than any wild type cell found in an exponential culture, these workers argue that *S. pombe* does have a critical size requirement for the initiation of DNA synthesis but that it is cryptic in exponentially growing cells since they are never small enough to express it. Consequently, exponential wild-type cells have no G1 and rely upon the second mechanism for coordinating growth and division at nuclear division as discussed above.

Many eukaryotic cells exhibit a highly variable G1 interval such as that of *S. cerevisiae* while others exhibit little or no G1, like *S. pombe*. Perhaps, the hypothesis of two sites for coordinating growth and division, one of which exists in some organisms (*S. cerevisiae*) and both of which exist in others (*S. pombe*), will be sufficient to account for the various eukaryotic cell cycles.

SUMMARY

A novel view of the eukaryotic cell cycle is taking form as genetic strategies borrowed from investigations of microbial gene regulation and bacteriophage morphogenesis are being applied to the process of cell division. It is a genetic construct in which mutational lesions identify the primary events, thermolabile gene products reveal temporal order, mutant phenotypes yield pathways of causality, and regulatory events are localized within sequences of gene controlled steps.

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