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Genetic Control of the Cell Division Cycle in Yeast

A model to account for the order of cell cycle events is deduced from the phenotypes of yeast mutants.

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Mitotic cell division in eukaryotes is accomplished through a highly reproducible temporal sequence of events that is common to almost all higher organisms. An interval of time, *G1*, separates the previous cell division from the initiation of DNA synthesis. Chromosome replication is accomplished during the DNA synthetic period, *S*, which typically occupies about a third of the cell cycle. Another interval of time, *G2*, separates the completion of DNA synthesis from prophase, the beginning of mitosis, *M*. A dramatic sequence of changes in chromosome structure and of chromosome movement characterizes the brief mitotic period that results in the precise separation of sister chromatids to daughter nuclei. Mitosis is followed by cytokinesis, the partitioning of the cytoplasm into two daughter cells with separate plasma membranes. In some organisms the cycle is completed by cell wall separation.

Each of these events occurs during the cell division cycle of the yeast, *Saccharomyces cerevisiae (1)* (Fig. 1). However, two features which distinguish the cell cycle of *S. cerevisiae* from most other eukaryotes are particularly useful for an analysis of the gene functions that control the cell division cycle. First, the fact that both haploid and diploid cells undergo mitosis permits the isolation of recessive mutations in haploids and their analysis by complementation in diploids. Second, the daughter cell is recognizable at an early stage of the cell cycle as a bud on the surface of the parent cell. Since the ratio of bud size to parent cell size increases progressively during the cycle, this ratio pro-

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vides a visual marker of the position of the cell in the cycle.

We have taken advantage of these features of the *S. cerevisiae* cell cycle in the isolation and characterization of 150 temperature-sensitive mutants of the cell division cycle (*cdc* mutants). These mutants are temperature-sensitive in the sense that they are unable to reproduce at 36°C (the restrictive temperature) but do grow normally at 23°C (the permissive temperature); the parent strain from which they were derived reproduces at both temperatures. These mutations define 32 genes, each of whose products plays an essential role in the successful completion of one event in the mitotic cycle (*2*). Although our genetic dissection of the cell cycle is in its early stages, the phenotypes of the mutants already examined provide information on the interdependence of events in the cycle. We shall discuss the conclusions that can be derived from the mutant phenotypes in the context of the following question: How are the events bud emergence, initiation of DNA synthesis, DNA synthesis, nuclear migration, nuclear division, cytokinesis, and cell separation coordinated in the yeast cell cycle so that their sequence is fixed? While it is not necessarily the case that all events in the cell cycle are ordered relative to one another in a fixed sequence, it is reasonable to assume that these events are, since their proper order is essential for the production of two viable daughter cells.

It has been pointed out by Mitchison that two possible mechanisms exist for ordering a fixed sequence of cell cycle events relative to one another (*3*). First, there may be a direct causal connection between one event and the next. In this case, it would be necessary for the earlier event in the cycle to be completed before the later event could occur. For example, the "product" of the earlier event might provide the "substrate" for the later event, as in a biochemical pathway, or the completion of the earlier event might activate the occurrence of the later event. We shall refer to this model as the "dependent pathway model" (Fig. 2).

A second possibility is that there is not a direct causal connection between two events, but that they are ordered by signals from some master timing mechanism. In this model it would not be necessary for the earlier event to be completed before the later event could occur, although the two events would normally occur in the proper order because of the activity of the timer. This model has appeared frequently in the literature in one guise or another, and two specific ideas have been presented concerning a possible timing mechanism. One invokes the accumulation of a specific division protein (*4*) and another a temporal sequence of genetic transcriptions (*5*). We shall refer to this model as the "independent pathways model" (Fig. 2).

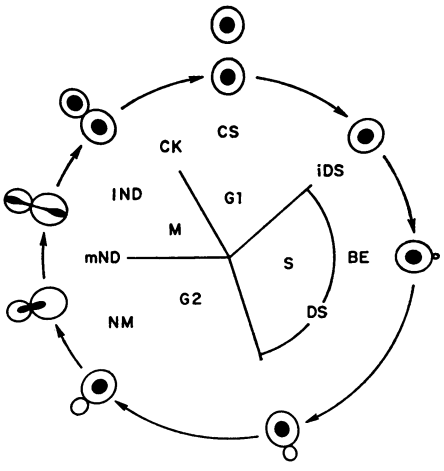

Fig. 1. The sequence of events in the cell division cycle of yeast: *iDS*, initiation of DNA synthesis; *BE*, bud emergence; *DS*, DNA synthesis; *NM*, nuclear migration; *mND*, medical nuclear division; *lND*, late nuclear division; *CK*, cytokinesis; *CS*, cell separation. Other abbreviations: *G1*, time interval between previous cytokinesis and initiation of DNA synthesis; *S*, period of DNA synthesis; *G2*, time between DNA synthesis and onset of mitosis; and *M*, the period of mitosis.

It is important to note that these two possible models relate, strictly speaking, to the dependence or independence of events in the cell cycle taken two at a time. It is quite possible that the cell cycle is controlled by a combination of the two models, with some events related to one another in a dependent pathway and others in independent pathways.

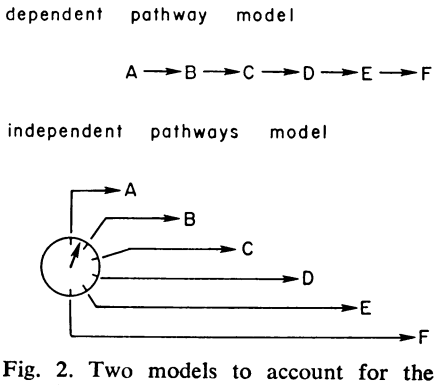

Fig. 2. Two models to account for the ordering of cell cycle events.

It should be possible to distinguish between these fundamentally different models by specifically inhibiting one and only one event of the cell cycle. If an event is dependent upon the prior occurrence of an earlier event, a specific block of the earlier event should prevent the occurrence of the later event. If, on the other hand, the two events are independent of one another, then a specific block of the earlier event should not prevent the occurrence of the subsequent event. Indeed, studies employing inhibitors that act specifically on one event of the cycle, such as DNA synthesis or mitosis, have already provided some information on the interdependence of cell cycle events. However, the temperature-sensitive *cdc* mutants of *S. cerevisiae* permit more detailed conclusions, both because of the greater number of specific cell cycle blocks in a single organism and because of the greater assurance that a single gene defect directly affects one and only one event in the cell cycle.

Mutations Affecting the Cell Cycle

Cell division cycle mutants of *S. cerevisiae* were detected among a collection of temperature-sensitive mutants by looking for mutants in which development was arrested at the restrictive temperature at a specific stage in the cell cycle, as evidenced by the cellular and nuclear morphology (*6*). The phenotype of each mutant class is described in Table 1 by the sequence of events that occurs in a cell when it is shifted from the permissive temperature to the restrictive temperature at the beginning of the cell division cycle (that is, at cell separation, see *CS* in Fig. 1). The initial defect in a mutant is defined as the first cell cycle event (among those which can presently be monitored) that fails to take place at the restrictive temperature. The events for which initial defects have been found in mutants include the initiation of DNA synthesis, bud emergence, DNA synthesis, medial nuclear division, late nuclear division, cytokinesis, and cell separation. Information on the interdependence of steps in the cell division cycle is obtained by observing which events in the first cell cycle at the restrictive temperature occur or do not occur after arrest at the initial defect.

Two dependent pathways in the cycle. The model of the cell division cycle presented in Fig. 3 can be derived from the phenotypes of the mutants (Table 1) by the following reasoning. First, let us compare the phenotypes of these mutants with the predictions of the dependent pathway model. Working backwards through the cell cycle we see that this model is adequate for the sequence: cell separation, cytokinesis, late nuclear division, medial nuclear division, DNA synthesis, and the initiation of DNA synthesis. A mutant with an initial defect in any one of these six processes fails to complete any of the other events in this group which normally occurs later in the cycle. The simplest explanation of these observations is that these six events comprise a dependent pathway in which the completion of each event is a necessary prerequisite for the occurrence of the immediate succeeding event (Fig. 3).

In contrast, although bud emergence and DNA synthesis normally occur at about the same time in the cell cycle, they must be on separate pathways (Fig. 3) since they are independent of one another. Mutants defective in the initiation of DNA synthesis (*cdc* 4 and *cdc* 7) or in DNA synthesis (*cdc* 8 and *cdc* 21) undergo bud emergence, and mutants defective in bud emergence (*cdc* 24) undergo DNA synthesis. Furthermore, inhibitors that block DNA synthesis (hydroxyurea and trenimon) do not inhibit bud emergence (*7*).

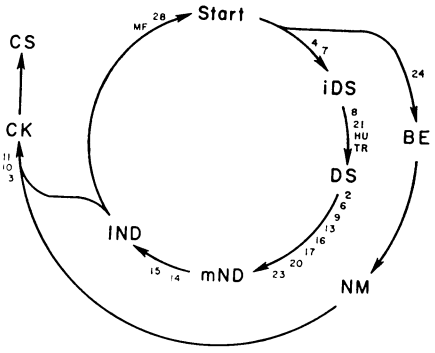

Fig. 3. The circuitry of the yeast cell cycle. Events connected by an arrow are proposed to be related such that the distal event is dependent for its occurrence upon the prior completion of the proximal event. The abbreviations are the same as in Fig. 1. Numbers refer to *cdc* genes that are required for progress from one event to the next; *HU* and *TR* refer to the DNA synthesis inhibitors hydroxyurea and trenimon, respectively; *MF* refers to the mating factor, α factor.

Although we do not have mutants with initial defects in nuclear migration, it is apparent that this event, like the bud emergence event, occurs in all mutants defective in the initiation of DNA synthesis and in DNA synthesis (Table 1). Nuclear migration also occurs when DNA synthesis is inhibited with hydroxyurea or trenimon (*7, 8*). Nuclear migration is therefore independent of initiation of DNA synthesis and DNA synthesis. Furthermore, since the nucleus normally migrates into the neck between the bud and parent cell, it seems reasonable to suppose that nuclear migration is dependent upon bud emergence. We propose, therefore, that nuclear migration is an event on the same pathway as bud emergence and subsequent to it on this pathway (Fig. 3).

Finally, medial and late nuclear division are completed in the mutant defective in bud emergence (*cdc* 24) but neither cytokinesis nor cell separation occurs in this mutant. These observations suggest that the separate pathway that leads to bud emergence and nuclear migration joins the first pathway at the event of cytokinesis (Fig. 3). Thus, cytokinesis and cell separation are dependent upon bud emergence as well as upon nuclear division.

A common step controls both pathways. Although bud emergence is not necessary for the initiation of DNA synthesis, and vice versa, the product of gene *cdc* 28 is required for both processes (Table 1). Furthermore, the mating factor produced by cells of mating type α (α factor) blocks both bud emergence and the initiation of DNA synthesis in cells of mating type *a* (*9, 10*). One hypothesis to explain these observations is that the two pathways leading, respectively, to bud emergence and to initiation of DNA synthesis diverge from a common pathway, and that both the *cdc* 28 gene product and the α factor sensitive step are elements of this common pathway.

A prediction of this hypothesis is that the α factor sensitive step and the step mediated by the *cdc* 28 gene product should precede and be required for the *cdc* 4 and *cdc* 7 mediated steps that lead to the initiation of DNA synthesis and for the *cdc* 24 mediated step that leads to bud emergence. Both of these predictions have been confirmed (*11*). We assume, therefore, that the *cdc* 28 gene product and the α factor sensitive step mediate some early event or events in the cell cycle that are necessary prerequisites for both of the dependent pathways described above.

Table 1. Summary of mutant phenotypes. Cells were shifted from 23° to 36°C at the time of cell separation. Abbreviations are as in Fig. 1. A minus sign indicates that an event does not occur, a plus indicates that the event occurs once, and a double plus indicates that the event occurs more than once.

*cdc**	Initial defect	Events completed at restrictive temperature								
		BE	iDS	DS	NM	mND	lND	CK	CS	Reference
28	Start	–	–	–	?	–	–	–	–	(*18*)
24	BE	–	++	++	?	++	++	–	–	(*2*)
4	iDS	++	–	–	+	–	–	–	–	(*16, 18*)
7	iDS	+	–	–	+	–	–	–	–	(*18*)
8	DS	+	+	–	+	–	–	–	–	(*16, 18*)
21	DS	+	+	–	+	–	–	–	–	(*18*)
2	mND	+	+	+	+	–	–	–	–	(*17*)
6	mND	+	+	+	+	–	–	–	–	(*17*)
9	mND	+	+	+	+	–	–	–	–	(*17*)
13	mND	+	+	+	+	–	–	–	–	(*17*)
16	mND	+	+	+	+	–	–	–	–	(*2*)
17	mND	+	+	+	+	–	–	–	–	(*2*)
20	mND	+	+	+	+	–	–	–	–	(*2*)
23	mND	+	+	+	+	–	–	–	–	(*2*)
14	lND	+	+	+	+	+	–	–	–	(*17*)
15	lND	+	+	+	+	+	–	–	–	(*17*)
3	CK	++	++	++	++	++	++	–	–	(*19*)
10	CK	++	++	++	++	++	++	–	–	(*19*)
11	CK	++	++	++	++	++	++	–	–	(*19*)
	CS	++	++	++	++	++	++	++	–	

* Although mutations in 32 *cdc* genes have been discovered, only 19 of these genes are included here for consideration in developing a model of the cell cycle. Most of those not included were left out because they progress through several cycles at the restrictive temperature before development is arrested and this prevents an analysis of DNA synthesis during their terminal cycle. The mutant *cdc* 1 (*19*) was excluded because macromolecule synthesis, as well as bud emergence, is rapidly arrested in this mutant at the restrictive temperature, and we suspect that this inhibition of growth prevents the occurrence of some events which are not normally dependent upon bud emergence, but which are dependent on growth.

We shall term this event "start" (Fig. 3). In principle, completion of the "start" event can be monitored by the acquisition of insensitivity to α factor in haploids of mating type *a* or by the acquisition of insensitivity to temperature in a *cdc* 28 mutant, although this is not possible in all experimental situations.

Several observations suggest that "start" is in fact the beginning of the yeast cell cycle. First, stationary phase populations obtained by limiting any one of several nutrients (glucose, ammonia, sulfate, phosphate) consist almost exclusively of cells which are arrested at a point in the cell cycle after cell separation, but prior to bud emergence and the initiation of DNA synthesis (*12*). Stationary phase cells of mating type *a* do not undergo bud emergence after inoculation into fresh medium in the presence of α factor. It appears, therefore, that as yeast cells exhaust their nutrients they finish cell cycles and become arrested prior to "start" in the cell cycle.

Similarly, when cultures are grown with limited glucose in a chemostat there is a striking correlation between the generation time and the proportion of unbudded cells in the population (*13*). The increase in the proportion of unbudded cells as the generation time increases suggests that the unbudded cells delay the "start" of new cycles until some requirement for growth or for the accumulation of energy reserves (*14*) has been met, and that, as expected, the time necessary to meet this requirement is a function of the rate of supply of glucose.

Finally, passing "start" in the cell cycle appears to represent a point of commitment to division, as opposed to mating, for haploid cells. If a cell of mating type *a* is beyond "start" in the cell cycle at the time of exposure to α factor, it proceeds through the cell cycle to cell separation at a normal rate, and then both daughter cells become arrested at "start" (*10*). Furthermore, *cdc* mutants arrested at various positions in the cycle are unable to mate with cells of opposite mating type, with two exceptions: mutants that are arrested at "start" (*cdc* 28), and mutants that repeatedly pass through "start" at the restrictive temperature (as evidenced by the attainment of a multinucleate state, Table 1) appear to mate relatively well (*15*).

Events Necessary for "Start"

Let us consider what events of one cell cycle must be completed in order to permit the "start" event of the next cell cycle. Since in these experiments we could not monitor "start" directly, our conclusions regarding this event must be considered tentative. However, it appears from the following observations that an initial defect in cell separation, cytokinesis, or bud emergence does not prevent a cell from undergo-

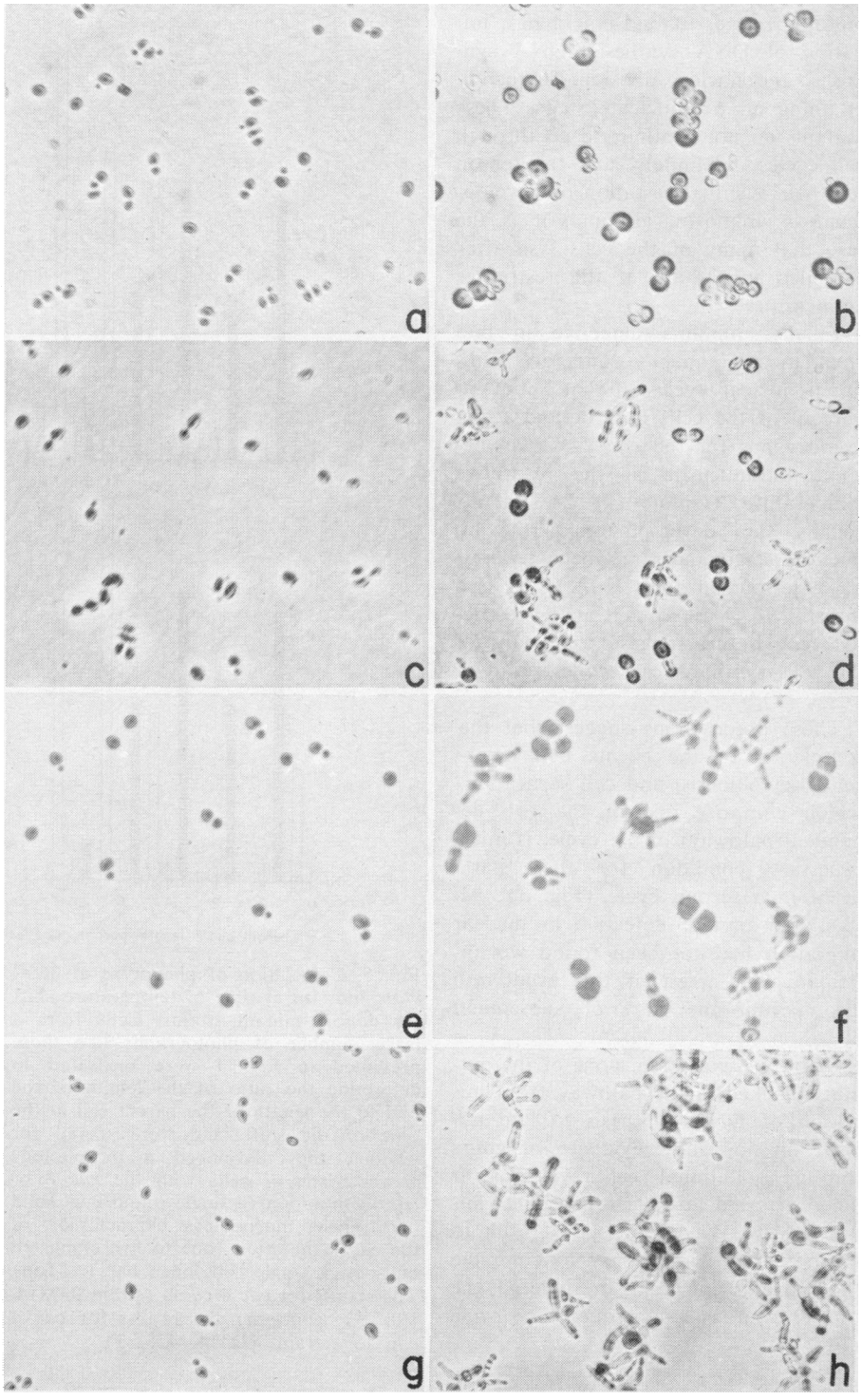

Fig. 4. Time-lapse photographs of diploid strains homozygous for two *cdc* mutations. Cells were grown at the permissive temperature (23°C) and shifted onto agar plates at the restrictive temperature (36°C). The cells were photographed at the time of the temperature shift, and at successive intervals while the plate was maintained at 36°C. (a) *cdc* 4 *cdc* 24 (strain RD314, 182-1-1, 2) at time 0; (b) *cdc* 4 *cdc* 24 after 6 hours at 36°C; (c) *cdc* 4 *cdc* 8 (strain RD314, 198, 3) at time 0; (d) *cdc* 4 *cdc* 8 after 6 hours at 36°C; (e) *cdc* 4 *cdc* 13 (strain RD314, 428, 3) at time 0; (f) *cdc* 4 *cdc* 13 after 7 hours at 36°C; (g) *cdc* 4 (strain 314D5) at time 0; (h) *cdc* 4 after 11 hours at 36°C.

ing the "start" event of subsequent cell cycles.

First, although mutants defective in cell separation have not been extensively studied, they have been isolated from mutagenized cultures after selecting for large cell aggregates by filtration through nylon mesh (*8*). A defect in cell separation does not appear to be lethal, and cells can go through an indefinite number of cell cycles despite a failure to complete this event.

Second, mutants defective in cytokinesis (*cdc* 3, *cdc* 10, *cdc* 11) undergo multiple rounds of bud emergence, initiation of DNA synthesis, DNA synthesis, and nuclear division, frequently attaining an octanucleate stage. These mutants do not continue to go through cell cycles indefinitely, and the reason for their eventual cessation of development is unknown, although it is the case that many of the cells lyse after extended incubation at the restrictive temperature.

Finally, mutants defective in bud emergence frequently undergo additional nuclear cycles in that about 50 percent of the cells in a diploid strain homozygous for the *cdc* 24 lesion become tetranucleate at the restrictive temperature. Haploid *cdc* 24 mutants usually stop development at the binucleate stage, and rarely become tetranucleate, but an analysis of DNA synthesis in the haploid cells at the restrictive temperature suggests that many of the cells synthesize a second round of DNA (*8*).

These observations suggest that the completion of the events bud emergence, cytokinesis, and cell separation, which comprise one of the two dependent pathways in the cycle, is not a necessary condition for the "start" event in a second cycle (Fig. 3). Although a mutant defective in nuclear migration has not been found we anticipate that arrest at this event will also permit the start of subsequent cycles.

With one exception, none of the mutants blocked in the pathway from initiation of DNA synthesis to late nuclear division show evidence of going through additional cell cycles after being arrested at the sites of their initial defects. We interpret this result to mean that it is necessary to complete these events in order to undergo the "start" event in a subsequent cell cycle (Fig. 3).

A Timer Controls Bud Emergence

Mutants defective in the *cdc* 4 gene (required for initiation of DNA synthesis) are exceptional in that they continue bud emergence for multiple cycles at the restrictive temperature, attaining as many as five buds on a single mononucleate cell (*16*). These successive cycles of budding continue despite the fact that the initiation of DNA synthesis, DNA synthesis, nuclear division, cytokinesis, and cell separation are not occurring. Furthermore, the time interval between successive budding events in *cdc* 4 mutants at the restrictive temperature maintains a periodicity of about one cell cycle time. This observation suggests that some type of intracellular timer initiates the successive cycles of budding, and that this timer can run independently of many of the cell cycle events.

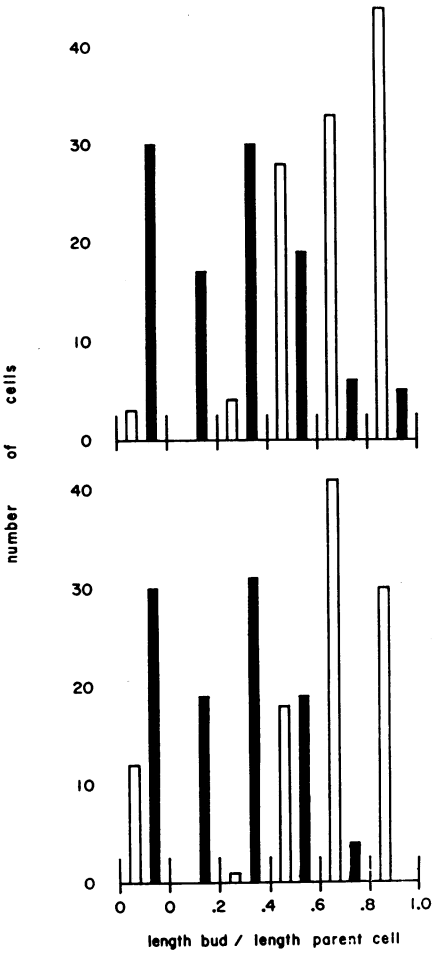

Fig. 5. Correlation of phenotype at 36°C with bud size at time of temperature shift for double mutant strains. Cells from a large number of photographs like those presented in Fig. 4 were measured to determine the ratio of the length of the bud to the length of the parent cell at the time of the shift, and were scored for whether they developed a morphology characteristic of cells with the *cdc* 8 or *cdc* 13 mutation (a single round bud, solid bars), or a morphology characteristic of the *cdc* 4 mutation (one to five elongated buds on a single cell, open bars). (Top) The results for *cdc* 4 *cdc* 8 (strain RD314, 198, 3); (bottom) the results for *cdc* 4 *cdc* 13 (strain RD314, 428, 3).

The unusual behavior of mutants defective in *cdc* 4, and the surprising conclusion that their phenotype suggests, prompted us to consider the possibility that this phenotype might not be reflecting normal control mechanisms, but might be a result of an artifact. For example, the putative buds on *cdc* 4 mutants might not be the result of normal bud emergence events, but might be caused by some unrelated morphologic alteration. Alternatively, they might be the result of normal bud emergence events, but these events might be activated in an anomolous way by the abnormal *cdc* 4 gene product. Although we cannot completely rule out the hypothesis of artifact in the behavior of *cdc* 4 mutants, the properties of a few double *cdc* mutants do eliminate some possible sources of error. Double mutant strains containing a defect in the initiation of DNA synthesis (*cdc* 4) as one mutation, and a defect in bud emergence (*cdc* 24), DNA synthesis (*cdc* 8), or medial nuclear division (*cdc* 13) as the second mutation, were constructed and examined by time-lapse photomicroscopy (Fig. 4). A diploid strain carrying only the homozygous *cdc* 4 mutation is shown in Fig. 4, g and h, for comparison.

The double mutant strain defective in *cdc* 4 and *cdc* 24 does not undergo multiple rounds of bud emergence at the restrictive temperature (Fig. 4, a and b). This result indicates that mutants defective in *cdc* 4 require a functional *cdc* 24 gene product in order to display the phenotype of repeated bud emergence and this phenotype is not, therefore, unrelated to the normal budding process.

The double mutant strains harboring lesions in *cdc* 4 and *cdc* 8, or in *cdc* 4 and *cdc* 13, exhibit an unusual pattern of development at the restrictive temperature (Fig. 4, c and d and e and f) (*16*). The result is striking in that the populations of cells from both double mutant strains behave heterogeneously. Some cells continue periodic bud emergence (characteristic of a defect in *cdc*

4 alone), and other cells terminate development with a single large bud on each parent cell (characteristic of a defect in *cdc* 8 or *cdc* 13 alone). Furthermore, the phenotype that a particular cell exhibits is correlated with the position of that cell in the cell division cycle at the time of the shift to the restrictive temperature. This correlation is evident in Fig. 4, c and d and e and f, and is recorded for a larger number of cells in Fig. 5. In both double mutant strains most of the cells that do not continue bud emergence were either unbudded or had small buds, while most of those that do continue bud emergence were unbudded or had large buds. These observations are interpreted to mean that the former class of cells are those that block at the *cdc* 8 or *cdc* 13 mediated processes, DNA synthesis, and medial nuclear division, respectively, while the latter class of cells are those that block at the *cdc* 4 mediated process, initiation of DNA synthesis. This interpretation is consistent with our previous determinations of the time of function of these gene products (*16, 17*). We may conclude, therefore, that continued bud emergence in a mutant strain is not due to the lesion in gene *cdc* 4 per se, but is merely a result of the cell's position in the cell division cycle at the time it is arrested.

These results seem to us to be best interpreted by the hypothesis of a timer that controls bud emergence and that can express itself at only one discrete stage in the cell cycle, the stage of arrest in the *cdc* 4 mutant. The role of this timer in the normal cell cycle, and, in particular, its relation to the "start" event, are at present unclear. The action of the timer might be a prerequisite for, be dependent upon, or be part of the "start" event.

Implications of the Model

Let us return now to the question we posed at the outset: How are the events of the cell cycle coordinated so that their sequence remains invariant? The phenotypes of the *cdc* mutants suggest that the following events are ordered in a single dependent pathway: "start," initiation of DNA synthesis, DNA synthesis, medial nuclear division, late nuclear division, cytokinesis, and cell separation. Hence, the temporal sequence of these events is easily accounted for by the fact that no event in this pathway can occur without the prior occurrence of all preceding events. A second dependent pathway is comprised of the events "start," bud emergence, nuclear migration, cytokinesis, and cell separation. Thus, the temporal sequence of these five events is also assured. Furthermore, the integration of the two pathways is accomplished by the facts that both diverge from a common event, "start," and that both converge on a common event, cytokinesis.

Although evidence was found for the existence of a timer that controls bud emergence, there is no indication that this timer plays any role in *coordinating* different events of the cell cycle. It is conceivable that the timer serves to phase bud emergence with respect to the events of the DNA synthesis and nuclear division pathway, but it seems to us that the joint dependence of bud emergence and initiation of DNA synthesis on "start" is sufficient to explain the coordination between the two pathways. Although the function of the timer in the cell cycle is unknown, we favor the idea that the timer is either phasing successive "start" events, perhaps by monitoring cell growth, or is phasing successive bud emergence events in order to limit the cell to one such event per cycle. A variation of the dependent pathway model appears to be sufficient, therefore, to account for the coordination of cell cycle events, and it does not appear to be necessary to invoke the model of independent pathways with a central timing mechanism.

Applicability to other organisms. The events that comprise the cell division cycle have their origin in a distant evolutionary past common to all eukaryotic organisms. The complexity of this process suggests that a high degree of conservation of its basic elements might be expected. In this context, it is interesting to note that the only events of the *S. cerevisiae* cell cycle that are not common to most eukaryotes, bud emergence and nuclear migration, are on a separate pathway from the other events, as if they were appendages added to the basic plan. We would not be surprised, therefore, if in most eukaryotes an event, "start," activates and acts as a point of commitment for the dependent pathway of events leading from the initiation of DNA synthesis, to DNA synthesis, to successive stages of nuclear division, and finally culminating in cytokinesis and, where applicable, cell wall separation. Furthermore, the completion of some stages of nuclear division, but not cytokinesis or cell separation, may in general be necessary in one cell cycle for the "start" of the next cell cycle.

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