

Genetic Control of the Cell-Division Cycle in Yeast, I. Detection of Mutants

Leland H. Hartwell,* Joseph Culotti, and Brian Reid†

DEPARTMENT OF GENETICS, UNIVERSITY OF WASHINGTON, SEATTLE

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Abstract. Time-lapse photomicroscopy has been utilized to detect temperature-sensitive yeast mutants that are defective in gene functions needed at specific stages of the cell-division cycle. This technique provides two types of information about a mutant: the time at which the defective gene function is normally performed, defined as the *execution point*, and the stage at which cells collect when the function is not performed, defined as the *termination point*.

Mutants carrying lesions in three genes that control the cell-division cycle are described. All three genes, *cdc-1*, *cdc-2*, and *cdc-3*, execute early in the cell cycle at about the time of bud initiation, but differ in their termination points. Cells carrying the *cdc-1* mutation terminate at the execution point, most cells ending up with a tiny bud that does not develop further. Cells carrying the *cdc-2* mutation terminate at mitosis. Cells carrying the *cdc-3* mutation are defective in cell separation but show no definite termination point since other processes of the cell cycle, such as bud initiation and nuclear division, continue despite the block in cell separation.

Introduction. Although many cytological and biochemical events have been precisely ordered within the cell cycle of eucaryotes, the genes that assure this orderly progression of events, the nature of their products, and the functions these products perform in the cell cycle are largely unknown. A mutational analysis of the cell cycle would therefore be advantageous, and should be feasible in the yeast *Saccharomyces cerevisiae*, a simple eucaryote combining a number of properties which facilitate biochemical and genetic investigations. Temperature-sensitive mutations provide a means of probing into genes which perform indispensable cellular functions, such as those which control the cell division cycle, and, indeed, a large number of temperature-sensitive mutants of *S. cerevisiae* have been isolated and characterized.¹ In this report we wish to describe the application of time-lapse photomicroscopy to the study of temperature-sensitive mutants and the detection, by this means, of yeast mutants blocked at specific stages of the cell division cycle.

The temporal sequence of events occurring during the cell division cycle in *S. cerevisiae* has been well characterized. The beginning of the cycle may be equated with the initiation of bud formation, and this event is rapidly followed by DNA replication that is completed within the first 25% of the cell cycle;² nuclear division occurs in a short period of time approximately half way through the cycle;³ and the entire process is terminated by cell separation. Further-

more, transcription of the yeast genome appears to be ordered with respect to the cell cycle, each enzyme being synthesized in a burst at a unique and reproducible point in the cycle.⁴ Of particular significance to the present report is the fact that bud enlargement continues throughout the cell cycle until the bud reaches approximately the size of the parent cell at the time of cell separation. Thus, visual observation of the relative size of bud and parent cell reveals the point to which a particular cell has progressed through the cycle.

Materials and Methods. Strains: The parent strain A364A (*a ad₁ ad₂ ur₁ ty₁ hi₁ ly₂ ga₁*), and the derivation from it of the haploid temperature-sensitive mutants (*ts⁻ 369*, *ts⁻ 370*, and *ts⁻ 104*) by mutagenesis with nitrosoguanidine, were described previously.¹ The diploids used in the present investigation were obtained by crossing each of these haploids to a nontemperature-sensitive strain, 79-20-3 (*αad₁₍₂₎ ur₁ le₂ ga₁*); the resulting diploid was allowed to sporulate and tetrads were dissected by micromanipulation. Each of the lesions segregated in a 2:2 pattern characteristic of a defect in a single nuclear gene. Temperature-sensitive haploids of opposite mating type segregating from this cross were in turn mated to obtain diploids homozygous for the temperature-sensitive lesion; the temperature-sensitive diploids are designated 369D-1, 370D-1, and 104D-1, and the mutations they harbor are designated *cdc-1*, *cdc-2*, and *cdc-3* respectively. The nontemperature-sensitive diploid, A364A D-4, is the product of the mating A364A × 79-20-3.

Media: The composition of YM-1 medium and YEPD-TAU plates was described previously.¹

Time-lapse photomicroscopy: The cells were grown for several generations at the permissive temperature in YM-1 medium, sonicated lightly to disperse cell clumps, and collected by centrifugation while still in the exponential growth phase. They were then resuspended at a density of about 2×10^7 cells/ml and spotted with a loop onto a YEPD-TAU plate prewarmed to 36°C. A small piece of 40-mesh stainless steel wire cut to contain a pattern of 3- by 5-squares was placed over the spot for rapid location of the same field at successive intervals; photographs were taken of one square within 10 min after spotting, and at various times thereafter, with a photomicroscope installed in an enclosure and maintained at 36°C.

Nuclear staining: Nuclei were stained with Giemsa after fixation in Helly's fixative containing formalin as described by Robinow and Marak.⁵ In some cases hydrolysis with 100 μg/ml pancreatic ribonuclease for 60 min at 60°C was substituted for the HCl hydrolysis used by these authors.

Results. Time-lapse photomicroscopy: Since the size of the bud on a yeast cell is strongly correlated with the cell's progress through the division cycle, we reasoned that a temperature-sensitive mutant defective in a specific step of the cycle could be detected by observing the morphological development of a large number of individual mutant cells after a shift to the restrictive temperature. To test this possibility an asynchronous culture of mutant cells growing at the permissive temperature (23°C) was spotted onto agar plates prewarmed to the restrictive temperature (36°C), and photographs of the same field were taken at successive time intervals, care being taken to maintain the plates at 36°C. We have examined about 100 temperature-sensitive mutants by time-lapse photomicroscopy and have found 15 in which the entire population behaves uniformly in a pattern consistent with a defect at one point in the cell cycle. The results for three mutants with distinctly different properties are presented in detail in the present report.

Figures 1 and 2 illustrate the sequence of cell development for a nonmutant

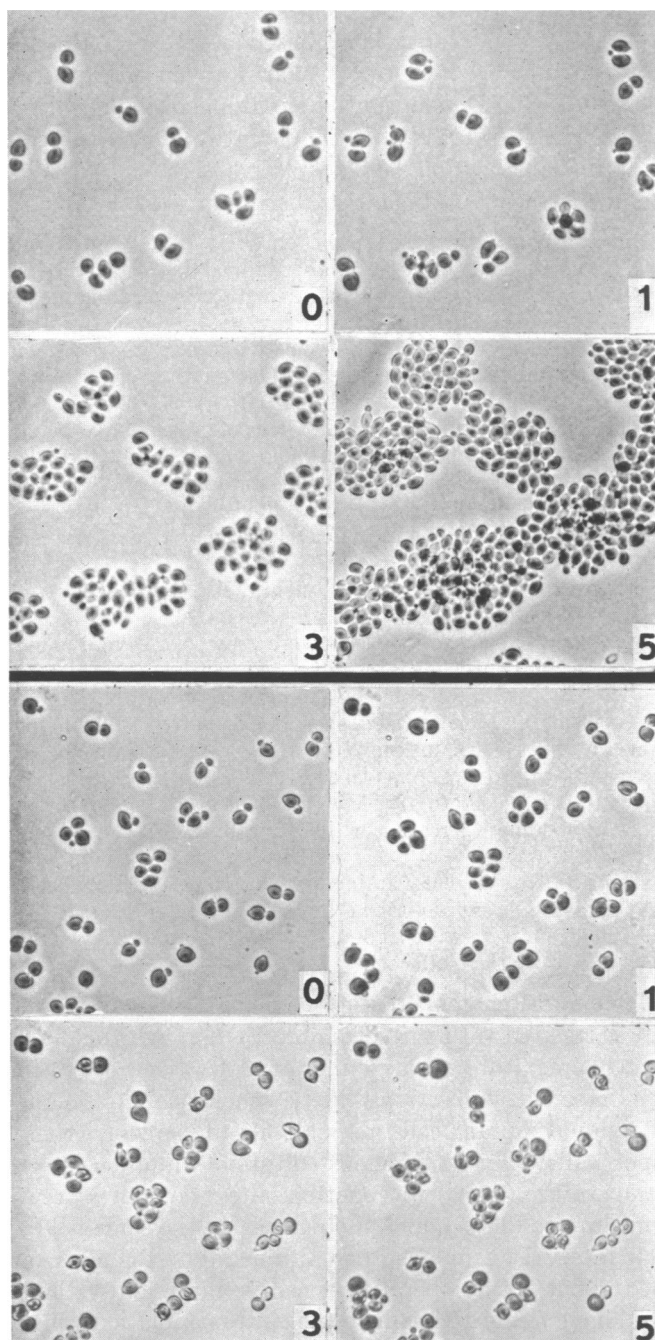


FIG. 1.—Time-lapse photomicroscopy of strains A364A D-4 (*top*) and 369 D-1 (*bottom*) at 36°C. The time in hours after the shift to 36°C is indicated in the lower right-hand corner of each picture.

strain, A364A D-4, and for three mutant strains, 369 D-1, 370 D-1, and 104 D-1; although it is only feasible to present photographs of a few cells of each mutant we have observed the development of more than 100 cells of each mutant and

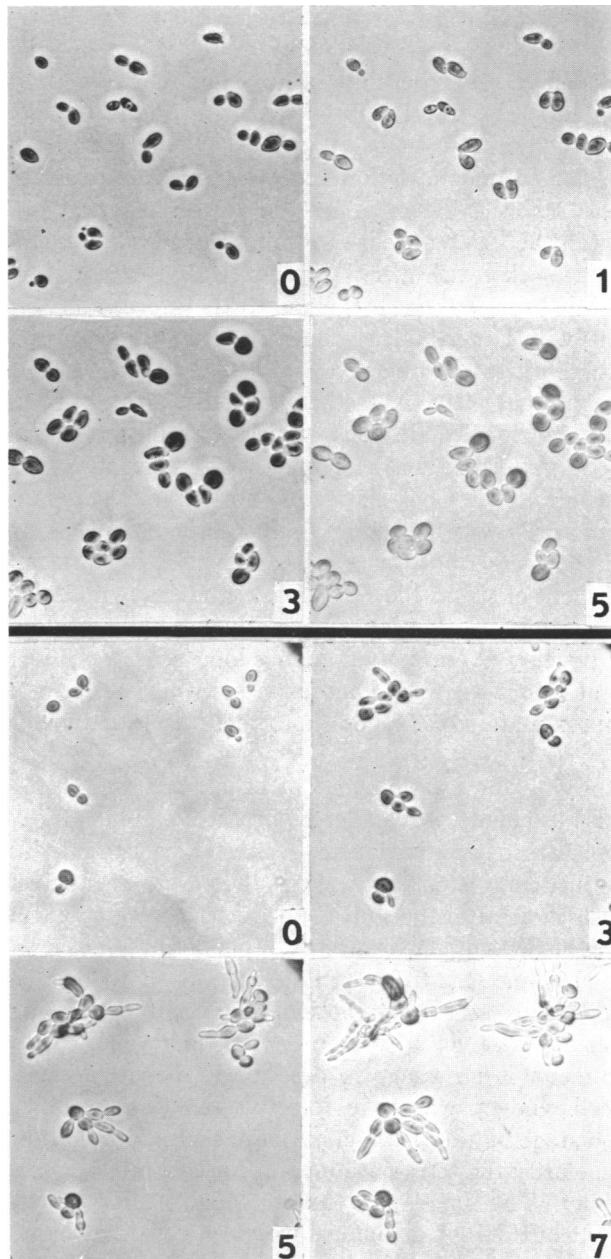


FIG. 2.—Time-lapse photomicroscopy of strains 370 D-1 (*top*) and 104 D-1 (*bottom*) at 36°C. The time in hours after the shift to 36°C is indicated in the lower right-hand corner of each picture.

have found very few exceptions to the pattern described here. Strain A364A D-4 is not temperature sensitive and divides with a generation time of approximately 2.5 hr at 36°C (Fig. 1). The three mutant strains all grow at approximately the same rate as strain A364A D-4 at 23°C; they behave quite differently, however, at the restrictive temperature of 36°C. Cells of strain 369 D-1 (Fig. 1) that have a bud at the time of the temperature shift continue the enlargement of that bud. Furthermore, a few buds issued soon after the shift to 36°C (less than 1 hr) also develop into large cells, but all buds issued after this time fail to enlarge and remain as tiny buds. Thus, at the permissive temperature just prior to bud initiation some event occurs that is necessary for bud enlargement and this event cannot be performed at the restrictive temperature in mutant 369 D-1. Cells of strain 370 D-1 (Fig. 2) that do not have a bud and a few cells with very tiny buds at the time of the temperature shift proceed through the cell cycle until they reach the stage of two large cells; other cells with larger buds at the time of the temperature shift complete the present cycle, initiate another cycle of budding, and cease development at the stage of four large cells. Note that one dead cell is evident in this figure which does not develop at all. Apparently in this strain the temperature-sensitive event is performed at the permissive temperature at about the time when buds are initiated. Cells that have not completed this event form cell doublets whereas cells with larger buds that have completed this event form quadruplets; in both cases only large cells are at the end state and no small buds are apparent. Cells of strain 104 D-1 (Fig. 2) that have buds at the time of the shift to 36°C continue to develop apparently normally while all buds issued after the shift develop abnormally, forming long processes; furthermore, each cell is capable of forming several long processes which continue to be issued at intervals of approximately one generation time. Eventually cells of this strain lyse.

Thus, by studying the development of cells after a shift to the restrictive temperature has been made, we can detect a critical period of the cell cycle during which the temperature-sensitive event occurs at the permissive temperature. Cells that have just completed this event at the permissive temperature progress through one cycle more after the shift to the restrictive temperature than do cells that at the time of the shift have reached a point in the cycle just prior to this event. We shall define this time as the *execution point* since it is the time at which the temperature-sensitive function is normally performed. Since each of these mutant strains ends up with a distinct morphology, it is clear that an additional parameter is necessary to describe its behavior—namely, the stage at which the cells end up when the defective function is not performed. We shall define this stage as the *termination point* and additional data will be presented below to clarify the termination point for each mutant strain.

Cell separation: The time-lapse studies reveal the morphology of cells at the termination point but do not distinguish between cells that have separated and those that have not. To examine this question the mutant strains were grown in liquid medium at the restrictive temperature until all cells had reached the termination point, and, after light sonication to separate any clumps of cells, the

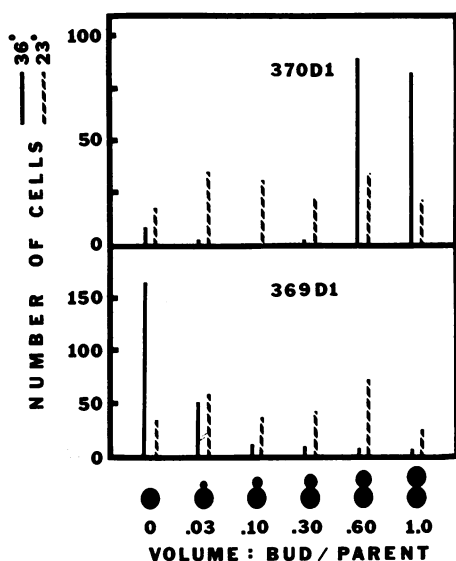


FIG. 3.—Size distribution of cells of strains 369 D-1 and 370 D-1 at the termination point. Strain 369 D-1 was incubated for 4 hr at 36°C and strain 370 D-1 was incubated for 8 hr in YM-1 medium at 36°C. After light sonication wet mounts were prepared and random fields of cells were photographed; cells in the photographs were then divided into six size-distribution classes by the measurement of individual cells.

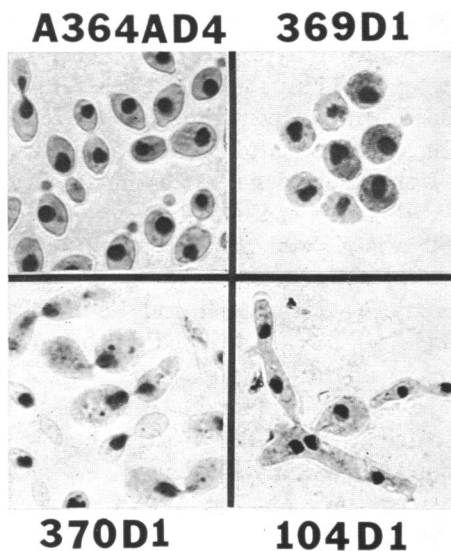


FIG. 4.—Giemsa-stained preparations of strains A364A D-4, 369 D-1, 370 D-1, and 104 D-1 after incubation at the restrictive temperature. Strain A364A D-4 was incubated for 6.5 hr at 36°C, strain 369 D-1 for 3 hr, strain 370 D-1 for 6.5 hr, and strain 104 D-1 for 6.5 hr at 36°C before fixation and staining.

size distribution of the cells was determined (Fig. 3). The size distribution of a population growing at the permissive temperature is included for comparison. It can be seen that cells of strain 369 D-1 accumulate almost exclusively as single cells which may or may not display a tiny bud; since in many cases the tiny bud is above or below the parent cell and therefore out of focus in our photographs, it is likely that most cells have a tiny bud. In fact, preparations in which the cells have been squashed between the slide and cover slip reveal that at least 75% of the cells contain tiny buds. Cells of strain 370 D-1 accumulate almost exclusively as doublets of approximately equal size; thus, these cells terminate at some stage of the cell cycle before cell separation. Cells of strain 104 D-1 accumulate as single cells with 1–5 long processes attached; the majority end up with three processes. It appears from examining these cells in liquid culture and those on solid medium that most cells of this strain also undergo one cell separation at the restrictive temperature in that there appears to be one morphologically normal cell at the center of each cluster of processes. However, the morphology of the cells is so altered that it is difficult to decide this point. Hence, 44 cells of strain 104 D-1 containing a small bud were separated by micromanipulation and allowed to develop at the restrictive temperature for 6 hr; the resulting structures were then dissected by micromanipulation to determine

the number of separate entities. In 40 cases two separate cells were present in the microcolony, in 3 cases one cell was present, and in 1 case three cells were present. Thus, most cells of strain 104 D-1 that contain a small bud at the time of the temperature shift develop that bud normally and complete the next cell separation, after which no further cell separation occurs.

Nuclear division: After incubation at 36°C cells of the wild-type and mutant strains were fixed and stained to reveal nuclei (Fig. 4). Examination of about 500 cells of an asynchronous culture of strain A364A D-4 revealed that 7% were in nuclear division as evidenced by the presence of the nucleus in the isthmus separating parent cell and bud. Measurements of the cells at the time of nuclear division indicates that the bud is about 50% of the volume of the parent cell at this time. Cells of strain 369 D-1 terminate with one nucleus per cell (Fig. 4). Cells of strain 370 D-1 terminate at mitosis with the nucleus uniformly located at the isthmus between bud and parent cell; in many cases the nucleus is found to be dumbbell shaped and to extend into both cells. Cells of strain 104 D-1 continue nuclear division at the restrictive temperature, since cells with several nuclei are found.

Discussion. The results of these studies are summarized diagrammatically in Figure 5. Cells of strain 369 D-1, homozygous for a lesion in the *cdc-1* gene,

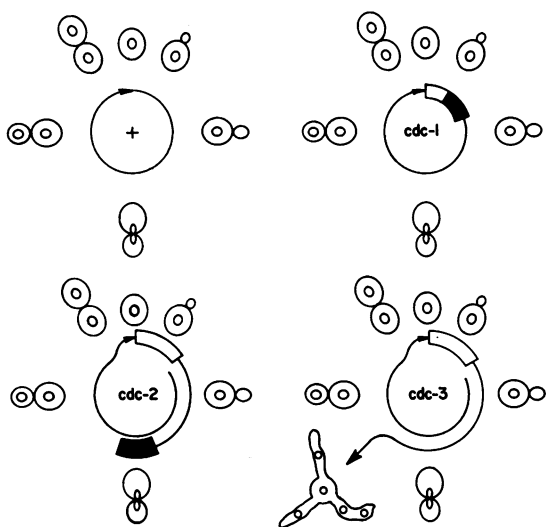


FIG. 5.—Diagrammatic representation of the behavior of cells of strains A364A D-4, 369 D-1, 370 D-1, and 104 D-1 after a shift to the restrictive temperature. Cells begin on the innermost line at the point corresponding to their stage of development at the time of the temperature shift; the mutant strains progress clockwise passing through the execution point (*open box*) and accumulate at the termination point (*closed box*).

execute the temperature-sensitive function at the time of bud initiation; those cells that have progressed past this point in the cell cycle at the time of the temperature shift progress around the cycle completing nuclear division and cell separation. These cells terminate at the execution point with most cells containing a tiny bud and a single nucleus. Cells of strain 370 D-1, homozygous for a lesion in the *cdc-2* gene, also execute a temperature-sensitive function at the time of bud initiation; cells that have progressed beyond this point at the time of the shift progress around the cycle completing nuclear division and cell separation and then terminate at mitosis. Cells of strain 104 D-1, homozygous for a

lesion in the *cdc-3* gene, execute at about the same time as the other two mutants. Cells that have progressed beyond this point at the time of the shift continue through one cell cycle completing cell separation; after this time no further cell separation takes place, but bud initiation and nuclear division continue to occur. This mutant does not have a definite termination point although the cells eventually cease development—apparently because they lyse.

The technique we have outlined for detecting yeast mutants defective in specific steps of the cell division cycle should pick up mutants defective in any gene that functions at only one stage of the cycle. The technique is dependent only upon observing that cells that have progressed beyond a specific developmental stage at the time of the shift to the restrictive temperature behave differently than those that have not progressed that far; in general, the more advanced cells complete one more cycle than the less advanced.

Furthermore, this critical stage of the cycle defines the time at which the mutant gene function is normally performed at the permissive temperature, the execution point. The observation that all three mutants described here execute at about the same time may be of general significance. It is conceivable that during the period of the cycle between cell division and bud initiation many functions that program the rest of the cycle might be performed. An unexpected finding was that some mutants did not terminate at the execution point. Thus, two of the mutants described here provide evidence for genes (*cdc-2* and *cdc-3*) that function early in the cell cycle to insure the completion of an event later in the cycle. This phenomenon might be of significance to the orderly progression of events during the cell-division cycle. Finally, mutants of this type should be useful in unraveling the interdependence of events in the cell cycle; for example, from the properties of mutant strain 104 D-1 we may conclude that cell separation is not a necessary prerequisite for the performance of other events, such as bud initiation and nuclear division, in the cycle.

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* Requests for reprints may be addressed to Dr. L. H. Hartwell, Department of Genetics, University of Washington, Seattle, Wash. 98105.

† Present address: Department of Biology, Massachusetts Institute of Technology Cambridge, Mass. 02139.

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