

REGULATION OF MATING IN THE CELL CYCLE OF *SACCHAROMYCES CEREVISIAE*

BRIAN J. REID and LELAND H. HARTWELL

From the Department of Genetics, University of Washington, Seattle, Washington 98195

ABSTRACT

The capacity of haploid a yeast cells to mate (fuse with a haploid strain of α mating type followed by nuclear fusion to produce a diploid cell) was assessed for a variety of temperature-sensitive cell division cycle (*cdc*) mutants at the permissive and restrictive temperatures. Asynchronous populations of some mutants do not mate at the restrictive temperature, and these mutants define genes (*cdc* 1, 4, 24, and 33) that are essential both for the cell cycle and for mating. For most *cdc* mutants, asynchronous populations mate well at the restrictive temperature while populations synchronized at the *cdc* block do not. Populations of a mutant carrying the *cdc* 28 mutation mate well at the restrictive temperature after synchronization at the *cdc* 28 step. These results suggest that mating can occur from the *cdc* 28 step, the same step at which mating factors arrest cell cycle progress. The cell cycle interval in which mating can occur may or may not extend to the immediately succeeding and diverging steps (*cdc* 4 and *cdc* 24). High frequency mating does not occur in the interval of the cell cycle extending from the step before the initiation of DNA synthesis (*cdc* 7) through DNA synthesis (*cdc* 2, 8, and 21), medial nuclear division (*cdc* 13), and late nuclear division (*cdc* 14 and 15).

KEY WORDS yeast · *Saccharomyces cerevisiae* · mating · cell cycle · *cdc* mutants · G1

A haploid *Saccharomyces cerevisiae* cell may embark upon any one of three avenues of the yeast life cycle depending upon the environment in which it finds itself. In the presence of an adequate nutrient supply and the absence of mating factor of the opposite mating type, the cell undergoes the mitotic cell cycle producing two cells. In the presence of a cell of opposite mating type, the two cells fuse to form a diploid zygote. When nutrients are insufficient, the cell enters a dormant stationary phase in which the cells are more resistant to killing by high temperature (16, 18), more refractory to enzymatic degradation of the cell wall (4,

5), and denser (L. Hartwell, personal communication). There is considerable evidence that the transitions between these three usually occur at a specific step in the G1 interval of the mitotic cell cycle.

The G1 interval of the cell cycle has been divided into a sequence of three steps mediated by the products of genes *cdc* 28, *cdc* 4, and *cdc* 7 (reference 12; see Fig. 4). Completion of the first step, that mediated by *cdc* 28 product, is necessary for the duplication of the spindle pole body (2), the structure from which the microtubules arise. The second step is mediated by *cdc* 4 product. Completion of this step is necessary for the separation of the spindle pole bodies (2) and the synthesis of proteins needed for DNA replication (12). The third step in G1 is mediated by the product of

gene *cdc 7* and completion of this step is necessary for the initiation of nuclear DNA replication (12).

The transition between the mitotic cell cycle and the dormant stationary phase usually occurs at the first step in G1 since populations of yeast cells experiencing certain nutritional deficiencies accumulate as unbudded cells that have neither replicated their DNA nor completed any of the G1 steps identified by the mutations in genes *cdc 28*, *cdc 4*, or *cdc 7* (reference 19; J. Pringle and R. Maddox, personal communication).

Similarly, transition from the mitotic cell cycle to the events of mating and zygote formation occurs at the first step in G1. When haploid cells of opposite mating type are mixed together, the proportion of budded cells decreases dramatically, and that of unbudded cells increases before the formation of zygotes (9). The first zygotes result from the fusion of two unbudded haploid cells. DNA replication for the first zygotic cell cycle occurs after cell fusion in the zygote (17). Furthermore, in zygotes observed by electron microscopy during the early stages of nuclear fusion, the two haploid nuclei each contained a single spindle pole body which is indicative of a position in the mitotic cycle at or before the step mediated by the product of gene *cdc 28* (3).

The synchronization of the two haploid mitotic cycles before zygote formation appears to be the consequence of mating factors produced by the two haploids. α -Cells constitutively produced a small polypeptide (α -factor) that specifically arrests cells of *a* mating type at the same step in G1 as that mediated by the product of gene *cdc 28* (1, 12). Conversely, *a* cells constitutively produce *a*-factor which transiently arrest α cells at the *cdc 28* step in the cell cycle (20). These mating factors appear to be essential for mating since single site mutations eliminate the ability to mate as well as the production of and/or sensitivity to mating factor (14) and since mutants selected solely for their resistance to mating factor invariably lose ability to mate (15).

Although it is clear that the transition from the mitotic cycle to mating can occur from a specific step in the G1 interval of the cell cycle, there is no evidence on the question of whether mating occurs at other steps as well. It is possible, for example, that mating can occur at any stage of the mitotic cell cycle but that it is more likely to occur at the *cdc 28* mediated step as a consequence of the synchronization of cells at this step by the mating factors. In order to investigate this possibility we

have studied the mating potential of cells synchronized at a variety of specific gene-mediated steps in the cell cycle with the use of temperature-sensitive mutants.

MATERIALS AND METHODS

Strains

Temperature-sensitive mutants with numerical designations were derived from the parent strain A364A (*a ade1,2 ura1 tyr1 his7 lys2 gal1*), by Dr. L. Hartwell (6). Mutants with numerical designations of greater than 1,000 were supplied by Dr. Calvin McLaughlin (University of California, Irvine, Calif.). Mutant E17 (*cdc 33-1*) was derived from A364A by mutagenesis with ethylmethane sulfonate and was supplied by Dr. Joseph Culotti. Mutant strains H135.1.1 (*a cdc 4-3 ade1,2 ura1 tyr1 his7 lys2 gal1*), H146.2.3 (*a cdc 21-1 ade1,2 ura1 lys2*), and the double mutant strain, 17C1A28C1a95 (*a cdc17-1 cdc28-1 tyr1 lys2*) were derived from mutant strains 458 (*cdc 4-3*), 17026 (*cdc 21-1*), 4028 (*cdc 17-1*), and 23019 (*cdc 28-1*), respectively, in standard crosses.

In mating experiments, H137RS64 α (*a ade1 ura1 trp1 arg4*) was used as the nontemperature-sensitive α mating type strain in the mating mixtures. In some experiments, a small proportion of the nontemperature-sensitive *a* mating type strain, H138RS56a (*a ile1 trp1 arg4*) was also included in the mating mixtures.

Media

The compositions of YM-1, YEPD, and synthetic media have been described previously (6, 7).

Mating Assay

Mating experiments were performed according to the procedure described previously (9). Aliquots of 2×10^6 cells of each mating type were removed from log phase cultures which had been grown under the desired conditions, (i.e. at the permissive temperature, with or without α -factor, or at the restrictive temperature), mixed, and filtered onto nitrocellulose filters (Millipore Corp., Bedford, Mass., type HA). In some experiments, 2×10^4 cells of strain H138RS56a were included in the mating mixtures. The filters were then incubated on YEPD plates under the desired conditions, i.e., at the permissive or the restrictive temperatures, with or without α -factor. Temperature control was achieved by wrapping the YEPD plates in plastic bags and submerging them in constant temperature water baths. Water baths were maintained at 34°C ($34.0 \pm 0.1^\circ\text{C}$), or 35°C ($34.9 \pm 0.1^\circ\text{C}$) by a Bronwill waterbath temperature regulator (Bronwill Scientific Div., Will Corp., Rochester, N.Y.). A waterbath at room temperature (between 20° and 22°C) was used for the permissive temperature. During manipulations of the YEPD plate, the plates at the restrictive temperature were placed on a slide warmer (Precision Scientific Company, Chicago, Ill.) to

minimize temperature variation. At designated times, the filters were removed from the YEPD plates, the cells were resuspended, subjected to light sonication, serially diluted, and plated for diploid colony forming units on the appropriate selective media. All resuspensions and serial dilutions involving mating mixtures containing temperature-sensitive, cell-division cycle mutants were made through sterile, 1.0 M sorbitol except in the case of mutant 4028 in which sterile, distilled water was used.

Monitoring Cell Number, Cell Morphology, Cytokinesis, and Viability

Techniques for monitoring cell number, cell morphology, and viability have been described previously (7, 8). Viability measurements were made by serial dilution in YM-1 medium in order to minimize any effects of the dilutant upon the cells.

α -Factor

Concentrated preparations of α -factor were prepared according to a modification of the procedure of Bücking-Throm et al. (1) and were generously supplied by Michael Unger.

RESULTS

Experimental Rationale

Two experimental conditions were designed to determine whether mating is limited to a specific interval of the *S. cerevisiae* cell cycle and if so to locate that interval. In the first condition (designated asynchronous 34°C mating), an asynchronous culture of a temperature-sensitive *cdc* mutant was pregrown at the permissive temperature (22°C) and mixed with the nontemperature-sensitive α tester strain H137RS64 α . The mixture was immediately placed at the restrictive temperature (34° or 35°C). Since the mutant culture was asynchronous at the time of the shift, some cells should either be in the interval of the cell cycle from which mating can occur or enter it during the incubation at the restrictive temperature even if this interval is but a small part of the cell cycle. Therefore, some diploids should be formed unless the mutant gene product plays an essential role in the mating reaction itself. The precise proportion of mutant cells that are able to mate will depend upon the length of the interval from which mating can occur and the position of this interval in the cell cycle relative to the execution point¹ of the *cdc* mutation.

¹ The execution point is defined as the point (expressed as a fraction of the total time) in the cell cycle at which the mutant cell acquires the capacity to complete the cycle after a shift to the restrictive temperature.

In some cases a variant of this experiment was also performed. Mutant *a* cells were incubated in α mating factor for approximately one cell cycle at the permissive temperature in order to synchronize them at a position in the cell cycle from which mating is known to occur. They were then mixed with α tester cells (ts^+), immediately shifted to the restrictive temperature and incubated at the restrictive temperature. This variation eliminates the complications of interpretation due to the position of the execution point. All cells should be capable of mating unless the mutant gene product is essential for the mating reaction itself.

In the second experimental condition (designated synchronous 34°C mating), the mutant cells were preincubated for approximately one cell cycle at the restrictive temperature, mixed with α tester (ts^+) cells of opposite mating type, and the mixture was incubated at the restrictive temperature. Viability was monitored at the end of the preincubation period at the restrictive temperature to be sure that a negative result was not the consequence of cell death. Therefore, under this experimental condition the mutant cells were arrested synchronously at a position in the cell cycle dictated by the particular *cdc* mutation used, and the formation of diploids would occur only if the position of arrest coincided with the interval of the cycle from which mating could occur and if the *cdc* gene product were not itself essential for the mating reaction.

Thus, with respect to the mating reaction, three possible roles may be distinguished for each *cdc* mutant by utilizing these two experimental conditions (Table I). First, a given *cdc* mutation may cause cells to arrest at a position in the cell cycle from which mating can occur. Second, the mutation may cause arrest at a position in the cell cycle from which mating cannot occur. Third, the *cdc*

TABLE I
Possible Roles and Expected Results for *cdc* Mutants
in Mating Experiments

Can mating occur within interval of arrest?	Role of <i>cdc</i> gene product in mating	Mating response expected for mutant	
		Asynchronous 34°C mating	Synchronous 34°C mating
No	Essential	—	—
	Nonessential	+	—
Yes	Essential	—	—
	Nonessential	+	+

gene product might be required for the events of the mating reaction itself. For any mutant whose gene product is essential for the mating reaction itself, it of course cannot be determined by these experiments whether the mutation causes arrest at a position in the cell cycle from which mating can occur or at a position from which it cannot.

Two controls were performed. In every case, the mutant *a* cells were pregrown at the permissive temperature and incubated with α tester (ts^+) cells at the permissive temperature (designated asynchronous 22°C mating) to assure that the strain did not carry other mutations that impaired mating. Values obtained in the first two experiments were normalized to that obtained in this control.

A second control (designated synchronous 22°C mating) was performed in many but not all experiments. The mutant *a* cells were preincubated at the restrictive temperature for approximately one cell cycle time, the *a* cells were mixed with α tester cells (ts^+) at the permissive temperature, and the mixture was incubated at the permissive temperature. This control tested for nonspecific depression of mating due to "sickness" induced by arrest at the restrictive temperature. A good yield of diploids indicates that a mutant was able to recover from the site of arrest, traverse to the interval of the cycle from which mating can occur (if it was not already there) and mate.

In some but not all of these experiments a minority (2×10^4) of a $ts^+ a$ strain was included in the mating mixtures. Diploids formed by fusion of the $ts^+ \alpha$ strain with the $ts^+ a$ strain could be distinguished from those formed with the $ts^- a$ strain by nutritional requirements on appropriate plates. Mating of the $ts^+ a$ strain under conditions where the $ts^- a$ strain failed to mate demonstrated that the mating defect was intrinsic to the $ts^- a$ strain.

The expected results are indicated in Table I and the observed results are reported in Table II.

Depression of Mating in Some Intervals of the Cell Cycle

The kinetics of diploid formation in experiments with the parent strain is shown in Fig. 1. At both 22°C and 34°C the parent strain exhibits a lag period of 60–90 min followed by a burst of diploid cells. Previously published data indicated that most of the diploids formed by 4 h were the direct result of fusions between haploid cells to produce zygotes (9). After 4 h many of the diploid cells result from the completion of cell cycles by zy-

gotes. Consequently, although the kinetics of diploid production were followed in most of these experiments only the number of diploids formed by 4 h is reported in Table II.

The parent (ts^+) strain A364A *a* was examined in numerous experiments at both 34°C and 35°C. In 12 asynchronous 22°C matings values of $1.33 \pm 0.16 \times 10^6$ diploids were produced in 4 h. The asynchronous 34°C (or 35°C) mating was performed four times at 34°C and seven times at 35°C; $146.3 \pm 15.7\%$ as many diploids formed at 34°C and $86.3 \pm 21.9\%$ at 35°C as in the corresponding control. The synchronous 34°C (or 35°C) mating was performed twice at 34°C where 147 and 169% as many diploids were formed as in the asynchronous 22°C mating and four times at 35°C where $95.5 \pm 22.7\%$ as many diploids were produced as in the asynchronous 22°C mating. Thus the parent strain mates reproducibly well at 22°C, 34°C, and 35°C in the protocols of the asynchronous and synchronous matings. Of course the parent strain is not synchronized by preincubation at 34°C or 35°C so that the designation "synchronous 34°C (or 35°C) mating" is a misnomer in this particular case. Since mating was found to be much poorer and less reproducible at 36°C, temperatures were maintained accurately at 34° or 35°C in the following experiments.

The most common response was for a mutant strain to mate well in the asynchronous 34°C mating (between 40 and 138% of the asynchronous 22°C mating) and poorly in the synchronous 34°C mating (between 0.2 and 3.7% of the asynchronous 22°C mating). This response was obtained for mutations in *cdc* 2, 3, 6, 7, 8, 10, 11, 13, 14, 15, 17, and 21 (Fig. 2 and Table II). The ratio of the number of diploids formed in the asynchronous 34°C mating to those in the synchronous 34°C mating was between 17 and 260 for all of these mutants. We consider these to be a negative response. The fact that a small proportion of diploids were formed at 34°C for these mutants may be attributed to two factors. First, some of these strains may be somewhat leaky at 34°C. Second, since the length of the preincubation at 34°C in the asynchronous 34°C mating was kept as short as possible in order to minimize the complications of lethality at the restrictive temperature, a small fraction of the population may have had insufficient time to arrest at the thermolabile step in the cell cycle (see Discussion).

The great depression of mating observed for these strains after preincubation at 34°C cannot be

TABLE II
Diploid Formation by *cdc* Mutants at Permissive and Restrictive Temperatures

Cdc gene	Allele	Strain	Temperature*	Mating response			h†	Viability‡
				Asynchronous 22°C mating	Asynchronous 34°C (or 35°C) mating			
					Synchronous 34°C (or 35°C) mating			
		A364A	34	1.33 ± .16	146 ± 16	147, 169	3	
		A364A	35		86 ± 22	96 ± 23	3	
2	1	370	35	0.80	81	1.8	2.5	56
3	3	10004	35	0.60	117	2.8	3	95
5	1	473	35	0.77	14.8	0.78	2.5	49
6	1	327	35	1.36	53	1.6	3	10
7	4	4008	34	0.99	138	3.5	2.5	67
8	1	198	35	0.72	41	0.23	2.5	75
10	1	17012	35	0.91	61	3.5	3	110
11	1	332	35	0.58	104	3.7	3	72
13	1	428	34	1.08	130	0.53	2.75	90
14	1	7041	34	0.75	77	1.8	3	
15	1	17017	34	0.72	57	2.6	3	
			35	0.83	19	1.2	3	110
16	1	281	35	0.77	8.6	0.06	2.5	27
17	1	4028	35	1.07	71	1.4	2.5	6
21	1	H146-2-3	34	0.67	51	2.1	3	86
28-17¶	1,1	17C1A28C1a95	35	1.54	77	53	2.5	
				1.65	83	94		
1	1	369	35	0.72	0.05			
	2	342	35	1.04	0.03			
4	1	314	34	0.27	3.8			
	3	H135-1-1	34	1.11	4.3			
				1.45	13			
24	1	5011	35	0.42	<0.01			
	2	E185	35	1.29	0.7			
	3	E187	35	0.99	<0.01			
33	1	E17	34	0.51	1.0			

* Temperature of mating in the asynchronous 34°C (or 35°C) mating and of preincubation and mating in the synchronous 34°C (or 35°C) mating.

† Time of preincubation at 34°C or 35° for the synchronous 34°C (or 35°C) mating.

‡ Percent of cells that are viable as monitored by colony formation at the end of the preincubation at 34°C or 35°C in the synchronous 34°C (or 35°C) mating.

|| Data for *cdc* 7 represent diploid formation after 3.5 h of mating under the three conditions.

¶ 28-17 designates a double mutant with lesions in *cdc* 28 and *cdc* 17.

attributed to lethality since in all but two cases viability was found to be greater than 50%. The strains with lesions in genes *cdc* 6 and 17 had viabilities of 10 and 6% after the preincubation at 34°C and hence interpretation of the results for these two strains is only tentative.

The synchronous 22°C mating experiment was performed with four of these mutants (genes *cdc* 7, 13, 14, and 21). Mating ability was rapidly regained at the permissive temperature, and they formed 32, 74, 58, and 52% as many diploids respectively in this regime by 4 h (or 4.5 h for *cdc*

7) as they did in the asynchronous 22°C mating experiment.

A minority of a *ts⁺ a* strain was included in the mating mixtures for the experiments with mutants in genes *cdc* 7, 13, 14, and 21. In all experiments the *ts⁺ a* strain mated well. This result indicates that the failure of these mutants to mate in the synchronous 34°C mating is intrinsic to the mutants themselves.

We interpret the high mating response in the asynchronous 34°C (or 35°C) mating to indicate that these *cdc* gene products are not directly re-

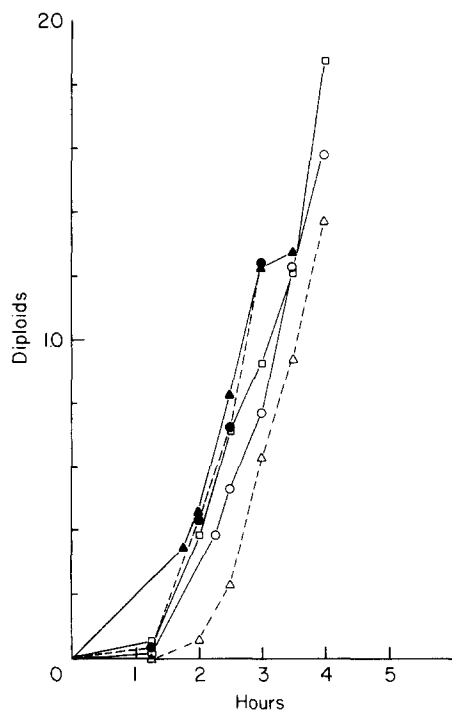


FIGURE 1 Kinetics of diploid formation with the parent strain, A364A. Aliquots of 2×10^6 cells of strain A364A and 2×10^6 cells of strain H137RS64 α were removed from liquid cultures pretreated as described in Materials and Methods, mixed, and filtered onto Millipore filters. The filters were incubated on YEPD plates at 22°C and 34°C. At designated times, the filters were removed from the YEPD plates, and the cells were resuspended in 1.0 M sorbitol, subjected to light sonication, and plated for diploid colony forming units on minimal medium containing adenine and uracil. The ordinate is in units of 10^6 diploid cells. Asynchronous 22°C mating, A364A and H137RS64 α grown and mated at 22°C (O—O); asynchronous 34°C mating, A364A and H137RS64 α grown at 22°C and mated at 34°C (●—●); synchronous 34°C mating, A364A and H137RS64 α shifted to 34°C for 3 h before being mated at 34°C (▲—▲); synchronous 22°C mating, A364A and H137RS64 α shifted to 34°C for 3 h before being mated at 22°C (Δ—Δ); A364A, preincubated for 2 h at 22°C in a 1:400 dilution of α -factor and mated with H137RS64 α at 34°C (□—□).

quired for the mating reaction itself and the low response in the synchronous 34°C mating to indicate that mating does not occur from the position of the cycle at which these mutant strains arrest. The ability of *S. cerevisiae* cells to mate is therefore limited to a portion of the cell cycle.

Strains 473 (*cdc 5*) and 281 (*cdc 16*) mated poorly in the synchronous 34°C mating but gave

only intermediate values in the asynchronous 34°C mating. We consider these two cases to be ambiguous (see Discussion). However, strain 473 does have a very late execution point (11), and it might therefore be expected to give a lower value in the asynchronous 34°C mating.

Occurrence of Mating at the *cdc 28* Step in the Cell Cycle

The following results suggest that the interval within the cell cycle where mating can occur is located between the step that is sensitive to mating factor and that mediated by the product of *cdc 7*. In parallel with the experiment reported above, one portion of cultures of strains *cdc 7*, 13, 14, and 21 were preincubated for approximately one generation time in α mating factor at the permissive temperature in order to partially synchronize the cells at the mating factor block. The cells were then mixed with α tester cells and incubated at the restrictive temperature. By 4 h these strains had

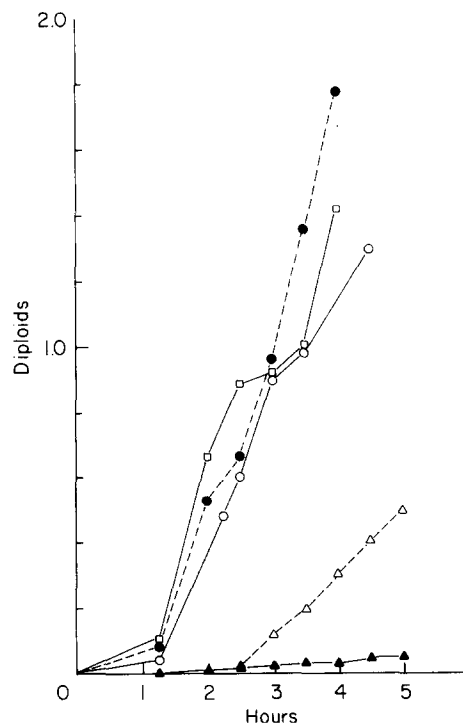


FIGURE 2 Kinetics of diploid formation with the *cdc 7* mutant strain. The experimental procedure and symbols are identical to those of Fig. 1 except strains 4008 (*cdc 7-4*) and H137RS64 α were mated, and the preincubation period at 34°C in the synchronous 34°C mating was 2.5 h.

formed 103, 92, 73, and 64% as many diploids respectively as the asynchronous 22°C mating mixtures. The result with *cdc 7* (Fig. 2) is of special significance since this block is, of the four, the step most closely succeeding the mating factor block. The fact that cells with the *cdc 7* lesion can mate efficiently at the restrictive temperature when presynchronized at the mating factor block but not when presynchronized at the *cdc 7* block (Table II) suggests that mating can occur within the interval of the cell cycle that lies at or subsequent to the α factor sensitive step but before the *cdc 7* mediated step.

Of the mutants tested, only those with a lesion in the *cdc 28* gene exhibited a high level of mating in both the asynchronous and synchronous 35°C matings (data not shown). However this result was suspect because the *cdc 28* lesion is leaky at 34–35°C; that is, at least 90% of the cells arrest transiently (for an hour or two) at the *cdc 28* step and then resume division. The lesion is tighter at 38°C, but at this temperature mating does not occur even in the parent strain. In order to be able to interpret unambiguously results obtained with this mutant, it was necessary to prevent the mutant strain from undergoing multiple cell cycles at a temperature (35°C) where mating could be tested. This was accomplished by constructing a double mutant strain containing the *cdc 28-1* lesion and the *cdc 17-1* lesion. *Cdc 17-1* was selected as the second temperature-sensitive mutation from which to construct the double mutant strain for several reasons. Asynchronous cultures of 4028 (*cdc 17-1*) mated well at both 22°C and 35°C relative to the nontemperature-sensitive control strain, A364A, indicating that there were no significant effects of secondary mutations upon the mating reaction in this strain. However, when 4028 was preincubated at the restrictive temperature to allow the cells to accumulate at the *cdc 17* block, the formation of diploids was dramatically reduced. Furthermore, the *cdc 17* mutant has a very early execution point (11). Because of this early execution point, in the population of the double mutant most of the cells would initially arrest at the *cdc 28* block. Any cells that leaked through the *cdc 28* block would arrest at the *cdc 17* block where, as discussed above, it has already been established that mating potential is dramatically reduced. Thus, if the positive mating response of *cdc 28* in the synchronous 35°C mating is due to mating at the *cdc 28* step, then the double mutant will respond like the single *cdc 28* mutant

and will mate well in the synchronous 35°C mating. But, if the positive mating response of *cdc 28* results from its leakiness, then the double mutant will respond as does a single *cdc 17* mutant and will not mate in the synchronous 35°C mating. The double mutant strain was not leaky since no significant increase in cell number occurred subsequent to one generation time at 35°C. The morphology of cells arrested at the *cdc 28* and *cdc 17* blocks differs, and microscope examination of these samples indicated that at least 70–80% of the double mutant cells arrested initially at the *cdc 28* block at the restrictive temperature.

An asynchronous population of the double mutant strain 17C1A28C1a95 which had been growing exponentially at 22°C was mated at 22°C (asynchronous 22°C mating) and at 35°C (asynchronous 35°C mating), and a population which had been preincubated at 36°C for 2.5 h to allow the cell to arrest at the temperature-sensitive cell cycle blocks was mated at 35°C (synchronous 35°C mating) with an asynchronous population of the α -tester strain. In the asynchronous 22°C mating the kinetics of diploid formation were comparable to those of A364A under similar conditions, and within 4 h, 1.65×10^6 diploids had been formed (Fig. 3). In the asynchronous 35°C mating, 77% and 83% as many diploids were formed as in the asynchronous 22°C mating on two different trials. In the synchronous 35°C mating, 53% and 94% as many diploids were formed as in the asynchronous 22°C mating in two different trials.

We interpret these results to indicate that cells can mate while arrested at the *cdc 28* mediated step. However, the alternative hypothesis that the double mutant cells mate at some point between the *cdc 28* and *cdc 17* steps cannot be rigorously excluded at the present time.

Cdc Genes with a Direct Role in Mating

A number of *cdc* mutants mated well in the asynchronous 22°C mating experiment but poorly in the asynchronous 34°C (or 35°C) mating experiment (*cdc 1, 4, 24, and 33*). It should be noted that the depression of mating in the asynchronous 34°C (or 35°C) mating is not as great for strains bearing the *cdc 4* lesion as for the other three mutants, and it may represent a special case. The failure of mating in the asynchronous 34°C (or 35°C) mating experiment cannot be attributed to a late execution point. For *cdc 33*, the execution point occurs near the time of the mating factor-sensitive step and for *cdc 1, 4, and 24* it occurs

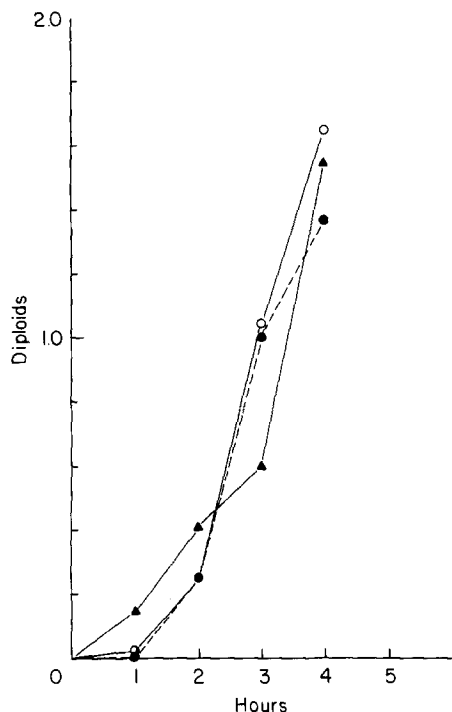


FIGURE 3 Kinetics of diploid formation with the *cdc 28-cdc 17* double mutant strain. The experimental procedure and symbols are identical to those of Fig. 1 except strains 17C1A28C1a95 (*cdc 28, cdc 17*) and H137RS64 α were mated and the preincubation period in the synchronous 35°C mating was 2.5 h at 36°C.

soon afterwards so that most of the cells in an asynchronous population would have passed through the mating factor sensitive step in the experiment. Preincubation of cells carrying either the *cdc 4* or *cdc 33* lesions with α factor for approximately one generation before incubation with the α tester strain at the restrictive temperature did not substantially increase the mating frequency, further suggesting that these genes played an essential role in the mating reaction. In experiments with α factor pretreated cells, the mating frequencies of *cdc 4* and *cdc 33* were 21% and 2.3% of the asynchronous 22°C matings, respectively (compared to values of 13% and 1.0% in the parallel asynchronous 34°C experiments). The failure of these strains to mate in the asynchronous 34°C (or 35°C) mating cannot be attributed to lethality. In separate experiments the viability of all of the strains carrying lesions in genes *cdc 1, 4, 24, and 33* have been examined with the exception of strain E187; all are greater than 50% viable after 3 h at the restrictive temperature.

In the experiments with *cdc 4* and *33* a minority of a *ts⁺ a* strain was included in the mating mixtures. The *ts⁺ a* strain mated well in all experiments. Hence the failure of strains defective in *cdc 4* and *33* to mate in the asynchronous 34°C (or 35°C) mating is an intrinsic property of the mutant cells.

DISCUSSION

These experiments were designed to test the ability of *S. cerevisiae* cells to mate at specific sites in the cell cycle by using synchronous populations of temperature-sensitive *cdc* mutants arrested at specific points within the cell cycle. Mutant strains that mated well in the asynchronous 34°C (or 35°C) mating experiment but poorly in the synchronous 34°C (or 35°C) mating experiment are interpreted as defining steps (*cdc 7, 2, 8, 21, 13, 14, and 15*) in the normal cell cycle at which mating does not occur and mutant strains that mated well in the synchronous 34°C (or 35°C) mating define steps (*cdc 28*) at which mating does occur. Some strains did not mate well in either experiment at 34°C or 35°C, and these mutants define gene products that are essential for the mating reaction (*cdc 1, 4, 24, and 33*). Two considerations influence the interpretation of the results. First, in view of the fact that the quantitative mating response varies over two orders of magnitude, what response is to be considered positive and what negative? Second, since cells arrested at a mutant block are not identical to normal cells passing this point in the cell cycle, to what extent do the results obtained with the mutants reflect the characteristics of normal cells?

Mating experiments performed with the parent strain (A364A) gave quantitative results in the asynchronous 34°C (or 35°C) mating which differed by as much as twofold on different days when normalized to the asynchronous 22°C mating. Furthermore, if mating is limited to certain intervals of the cell cycle, then the execution point of the temperature-sensitive mutation would be expected to influence the maximum response in the asynchronous 34°C (or 35°C) mating. Therefore, a certain amount of variation was to be expected in the results. However, as discussed above, values for the asynchronous 34°C (or 35°C) matings were commonly 40% or more of the asynchronous 22°C matings. These results may be considered to represent a positive response. Values of 5% or less were considered to represent a negative response. In fact only three experi-

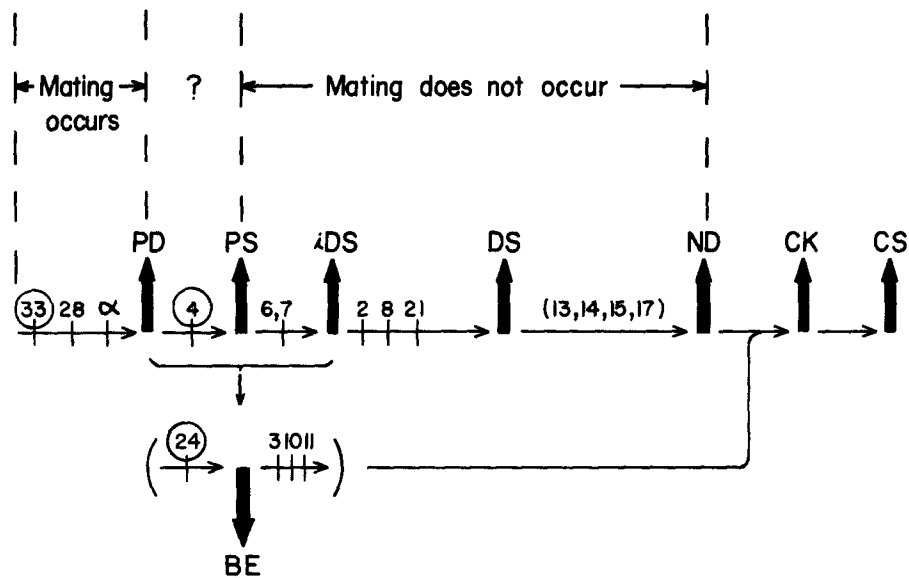


FIGURE 4 Mating capacity within the dependent steps of the *S. cerevisiae* cell cycle. The dependent steps are taken from Hartwell (10). Each distinguishable step is designated by an arrow. Designations over the same arrow represent interdependent events. Mating occurs at the *cdc 28* step but not at all others that could be tested. Numbers enclosed within circles designate steps mediated by genes whose mating ability was low in the asynchronous 34°C (or 35°C) mating. The ability of cells to mate at these steps could not therefore be tested in the synchronous 34°C (or 35°C) mating experiment and hence remains unknown. Designations: numbers, *cdc* genes; α , mating factor; *PD*, spindle pole body duplication; *PS*, spindle pole body separation; *iDS*, initiation of DNA synthesis; *DS*, DNA replication; *ND*, nuclear division; *CK*, cytokinesis; *CS*, cell wall separation; and *BE*, bud emergence.

ments gave values between 5 and 40%, and these may be considered ambiguous cases.

In the synchronous 34°C (or 35°C) matings, only one case of a response greater than 3.7% of the value for the asynchronous 22°C mating was found. In this instance (strain 17C1A28C1a95, the *cdc 28* and 17 double mutant), the response was obviously positive with values of 53% and 94% being recorded in two experiments. The remaining mutants which gave values of 3.7% or less were considered to have negative responses. As discussed above, we consider the residual diploid formation observed in the synchronous 34°C (or 35°C) matings of these mutants to be the result of two factors. First, some of the mutants may be somewhat leaky at 34°C or 35°C. Second, the mutants were preincubated at the restrictive temperature only for approximately one cell cycle time in order to avoid as much as possible complications due to abnormal development of the cells at the mutant block. Since it has been observed that cells in a mass population exhibit a broad distribution of generation time (M. Unger and L. H. Hartwell, personal communication), a small

proportion of the original population may have failed to reach the mutant block during the preincubation.

The most serious criticism of our conclusions is that the arrested mutant cell may not be comparable to a normal cell. Some but not all of the potential artifacts inherent in the use of mutants can be eliminated. In most experiments viability measurements indicate that the negative response was not due to lethality. The results of the synchronous 22°C mating experiment with mutants in genes *cdc 7*, 13, 14, and 21 demonstrated that the negative response was rapidly reversed when the arrested cells were returned to the permissive temperature. In some experiments a minority of *ts⁺ a* cells were incubated in the mating mixture to show that the negative response was limited to intracellular effects. Finally, since one mutant mated well at the restrictive temperature in the synchronous 35°C mating experiment, any "sickness" induced by the preincubation at the restrictive temperature cannot be a general property of all mutants.

Some mutants (those defective in *cdc 1*, 4, 24, or 33) did not mate well in the asynchronous 34°C

(or 35°C) mating experiment. Our interpretation of these results is that these mutants carry a lesion in a gene required for both cell division and the mating reaction. Mutants defective in *cdc 4* may represent a special case since the depression of mating in the asynchronous 34°C mating was not as great as for the other three mutants. Although the precise nature of the defects in these mutants is unknown, plausible hypotheses for their pleiotropy can be suggested. Mutants defective in *cdc 1* and *cdc 33*, unlike most cell cycle mutants, arrest growth as well as division at the restrictive temperature (13), and the failure of these two mutants to mate at the restrictive temperature may be due to their inability to grow. Unlike *cdc 1* and *cdc 33* mutants, *cdc 24* and *cdc 4* mutants do continue to grow after cell cycle arrest at the restrictive temperature (13). *Cdc 24* fails to bud at the restrictive temperature (8); perhaps this mutant is defective in some aspect of cell wall metabolism that is needed both for bud emergence and for the fusion of cell walls during mating. *Cdc 4* mutants arrest with duplicated but unseparated nuclear plaques at the restrictive temperature (2). Perhaps changes in the nuclear plaque that occur during or preparatory to mating (3) require this gene product. Because these four mutants are indispensable to mating they cannot be used to determine whether mating occurs or not at the *cdc 1*, 4, 24, or 33 steps of the cell cycle.

If we assume that the arrested mutants are behaving like normal cells at the same point in the cycle, then the results indicate that mating can occur at the *cdc 28* step and that mating cannot occur or is greatly depressed for the interval of the cycle extending from initiation of DNA synthesis (*cdc 7*) through late nuclear division (*cdc 14* and 15). Interestingly, the *cdc 28* step is identical to the step at which the mating factors cause cell cycle arrest. The limitation of mating to one (or a few) step(s) in the cell cycle is not surprising. Since mating involves the fusion of two haploid cells and nuclei to form a diploid zygote which then reproduces by mitotic cell division, it seems reasonable to suppose that synchronization of the two haploid cell cycles must occur at some point in this process. In *S. cerevisiae* this synchronization is apparently achieved by mating factors which arrest both *a* and α cells at the *cdc 28* mediated step in the cell cycle and by controls that limit mating to this particular step.

Brian J. Reid was supported by a National Institutes of

Health predoctoral fellowship. We wish to thank Dennis Livingston and Susan Dutcher for their critical reading of the manuscript. This research was supported by a grant from the National Institute of General Medical Sciences, United States Public Health Service.

Received for publication 3 March 1977, and in revised form 5 July 1977.

REFERENCES

1. BÜCKING-THROM, E., W. DUNTZE, L. H. HARTWELL, and T. R. MANNEY. 1973. Reversible arrest of haploid yeast cells at the initiation of DNA synthesis by a diffusible sex factor. *Exp. Cell Res.* **76**:99-110.
2. BYERS, B., and L. GOETSCH. 1974. Duplication of spindle plaques and integration of the yeast cell cycle. *Cold Spring Harbor Symp. Quant. Biol.* **38**:123-131.
3. BYERS, B., and L. GOETSCH. 1975. Behavior of spindles and spindle plaques in the cell cycle and conjugation of *Saccharomyces cerevisiae*. *J. Bacteriol.* **124**:511-523.
4. DEUTCH, C. E., and J. M. PARRY. 1974. Sphaeroplast formation in yeast during the transition from exponential phase to stationary phase. *J. Gen. Microbiol.* **80**:259-268.
5. EDDY, A. A., and D. H. WILLIAMSON. 1959. Formation of aberrant cell walls and of spores by the growing yeast protoplast. *Nature (Lond.)*. **183**:1101-1104.
6. HARTWELL, L. H. 1967. Macromolecule synthesis in temperature-sensitive mutants of yeast. *J. Bacteriol.* **93**:1662-1670.
7. HARTWELL, L. H. 1970. Periodic density fluctuation during the yeast cell cycle and the selection of synchronous cultures. *J. Bacteriol.* **104**:1280-1285.
8. HARTWELL, L. H. 1971. Genetic control of the cell division cycle in yeast. IV. Genes controlling bud emergence and cytokinesis. *Exp. Cell Res.* **69**:265-276.
9. HARTWELL, L. H. 1973. Synchronization of haploid yeast cell cycles, a prelude to conjugation. *Exp. Cell Res.* **76**:111-117.
10. HARTWELL, L. H. 1976. Sequential function of gene products relative to DNA synthesis in the yeast cell cycle. *J. Mol. Biol.* **104**:803-817.
11. HARTWELL, L. H., R. K. MORTIMER, J. CULOTTI, and M. CULOTTI. 1973. Genetic control of the cell division cycle in yeast. V. Genetic analysis of *cdc* mutants. *Genetics*. **74**:267-286.
12. HEREFORD, L. M., and L. H. HARTWELL. 1974. Sequential gene function in the initiation of *S. cerevisiae* DNA synthesis. *J. Mol. Biol.* **84**:445-461.
13. JOHNSTON, G. C., J. R. PRINGLE, and L. H. HARTWELL. 1976. Coordination of growth with cell division in the yeast *Saccharomyces cerevisiae*. *Exp. Cell*

- Res.* **105**:79–98.
14. MACKAY, V., and T. R. MANNEY. 1974. Mutations affecting sexual conjugation and related processes in *Saccharomyces cerevisiae*. I. Isolation and phenotypic characterization of nonmating mutants. *Genetics*. **76**:255–271.
 15. MANNEY, T. R., and V. WOODS. 1976. Mutants of *Saccharomyces cerevisiae* resistant to the α mating factor. *Genetics*. **82**:639–644.
 16. SCHENBERG-FRASCINO, A., and E. MOUSTACCHI. 1972. Lethal and mutagenic effects of elevated temperature on haploid yeast. I. Variations in sensitivity during the cell cycle. *Mol. & Gen. Genet.* **115**:243–257.
 17. SENA, E. P., D. N. RADIN, and S. FOGEL. 1973. Synchronous mating in yeast. *Proc. Natl. Acad. Sci. U. S. A.* **70**:1373–1377.
 18. SHERMAN, F. 1957. The heat inactivation and production of cytochrome deficiency in yeast. *Exp. Cell Res.* **11**:659–660.
 19. UNGER, M. W., and L. H. HARTWELL. 1976. Control of cell division in *Saccharomyces cerevisiae* by methionyl-tRNA. *Proc. Natl. Acad. Sci. U. S. A.* **73**:1664–1668.
 20. WILKINSON, L. E., and J. R. PRINGLE. 1974. Transient G1 arrest of *S. cerevisiae* cells of mating type α by a factor produced by cells of mating type a . *Exp. Cell Res.* **89**:175–187.