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Mating Ability During Chemically Induced G1 Arrest of Cells of the Yeast *Saccharomyces cerevisiae*

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Diploid formation by haploid cells of *Saccharomyces cerevisiae* was tested during and after treatment with chemical agents which bring about arrest at the cell cycle regulatory step "start." All compounds, except sinefungin, allowed efficient mating. During sinefungin treatment, zygote formation, but not karyogamy, was affected.

For the budding yeast *Saccharomyces cerevisiae*, regulation of the cell division cycle occurs in the G1 interval of the cell cycle, at the step referred to as "start" (7, 8). Cells can be arrested in the cell cycle at start by the actions of certain temperature-sensitive cell division cycle (*cdc*) mutations (1, 8, 14). By comparing the phenotypes of cells arrested at start through the effects of different *cdc* mutations with those of cells arrested at start by environmental conditions, Reed (14) described two classes of arrest at start. Class I start arrest is characterized by cells which, when arrested at the block, continue to grow and increase in size and retain the ability to mate with cells of the opposite mating type. Such cells resemble cells treated with mating pheromones, which arrest cells at start in preparation for mating (15) while growth activities continue (12, 19). In contrast, class II start arrest is characterized by cells which fail to grow or mate at the block. Such cells resemble cells arrested at start because of starvation.

The start event can also be affected by certain chemical agents. Reciprocal-shift experiments show that each of these agents reversibly arrests cells at a cell cycle stage which is functionally interdependent with start (2, 11, 12a, 17, 18). Some of these chemical agents produce significant effects on macromolecular metabolism which accompany start arrest (reviewed in reference 17). To determine if these physiological changes affect the mating ability of the start-arrested cells and to compare the start arrest provoked by each of these agents with those brought about by class I and class II mutations, we assessed the mating abilities of cells arrested by treatment with start-arresting compounds. Results indicate that start-arresting compounds can generally be grouped with respect to the class I and class II start arrest categories.

Three types of quantitative mating experiments were performed by procedures adapted from the work of Reid and Hartwell (15). In the first type of experiment, exponentially growing cells of the **a** mating type were mixed with cells of the α mating type and incubated on solid medium under start-arresting conditions to permit cell fusion. These cells were then transferred to solid medium on which only diploid cells could grow. This type of experiment was designed to test the ability of cells to mate under start-arresting conditions (referred to in Table 1 as mating by asynchronous cells).

The second type of experiment was designed to find out if cells already blocked at the start event retained the ability to mate. The procedure was essentially the same as that described above, except that *MAT***a** cells were first exposed to start-arresting conditions to block cells at start before they were mixed with cells of the opposite mating type.

In the third type of experiment, cells were arrested at start but, in this protocol, were then tested for mating ability under permissive conditions. This procedure was included to determine if effects on mating seen in the first two experiments were readily reversible upon removal of the start-arresting conditions.

The mating ability of cells at start was originally quantitated for cells arrested by various *cdc* mutations (14, 15). For comparison, we have included the results of matings involving cells bearing a *cdc28* start mutation. Mutations in this start gene produce class I cell cycle arrest at start because they allow mating at the block (14). Also included are results for cells which bear a *cdc64-1* start mutation (1) and which fail to mate at the block; thus, the *cdc64-1* mutation produces class II start arrest. The quantitative aspects of mating shown by these two *cdc* mutant strains (Table 1) were similar to those found earlier for the same strains (1, 14). Because in the absence of arresting conditions all strains used in this study mated equally well (Table 1, footnote *e*), the mating abilities of the *cdc* mutant strains are useful in interpreting the mating abilities of cells arrested by other means.

The mating abilities of cells exposed to start-arresting conditions are shown in Table 1. Cells undergoing cell cycle arrest during treatment with the compounds 2,2′-dipyridyl (17), *o*-phenanthroline (11), or 8-hydroxyquinoline (11) all displayed high levels of mating during the asynchronous mating procedure (Table 1). More importantly, when first arrested at start by exposure to any of these compounds, cells were still able to mate (Table 1). Control experiments in which *MAT***a** cells alone were put through the asynchronous mating protocol showed that cells became arrested as unbudded cells (data not shown) and thus ensured that these (and the other) start-arresting agents were in fact active during the 3-h mating period. Exposure of cells to other start-arresting compounds, such as thienylalanine (2) and ethionine (18), allowed only moderate levels of mating. Nevertheless, these levels were similar to those of the strain bearing the class I *cdc28-4* start mutation (Table 1) and were greater than or equal to those of class I *cdc28*, *cdc36*, and *cdc37* mutants in similar experiments incorporating prior start arrest (14). Thus, ethionine-treated and thienylalanine-treated cells also show a class I arrest response.

Cells treated with the start-arresting agent sinefungin (SF)

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TABLE 1. Quantitative mating after different start-arresting treatments[a]

Strain	Arresting condition	% Mating efficiency of:		
		Asynchronous cells[b]	Synchronous cells	
			G1-arresting conditions[c]	Permissive conditions[d]
GR2	8-Hydroxyquinoline (20 μg/ml)	104[e]	63	67
	o-Phenanthroline (20 μg/ml)	50	80	60
	2,2'-Dipyridyl (31 μg/ml)	96	32.5	37
	β-2-DL-Thienylalanine (10 μg/ml)	8.0	10.0	80
	L-Ethionine (10 μg/ml)	5.0	4.0	6.0
	SF (1.5 μg/ml)	0.5	0.06	0.4
S104	*cdc64-1*; 37°C	0.15[e]	0.03	13
EP-1	*cdc28-4*; 37°C	28[e]	12	22

[a] Approximately 3×10^6 cells of *MAT***a** strain GR2 (*his6 ura1* [11]), EP-1 (*cdc28-4 his6 ura1*), or S104 (*cdc64-1 leu1* [1]) were mixed with equal numbers of exponentially growing cells of strain A4840A (*MAT*α *ade2 his5* [Cold Spring Harbor Laboratory]) and suspended in 10 ml of 1 M sorbitol. The mixed-cell population was collected on a 0.45-μm-pore-size nitrocellulose filter (Millipore Corp.) previously sterilized by autoclaving. The filter was then rinsed once with 10 ml of 1 M sorbitol and placed "cell side up" on solid minimal medium (yeast nitrogen base [Difco Laboratories] [10]) supplemented with adenine, histidine, uracil, or leucine and, in some cases, the start-arresting compound at the arresting concentration. After incubation for 3 h to permit cell fusion, cells from the filter were suspended in 5 ml of ice-cold 1 M sorbitol, sonicated briefly to separate individual cells, and spread on solid minimal medium without nutritional supplements or start-arresting agents to select for diploid cells. For each mating mix, parental strains were tested separately for reversion of auxotrophs, which was found in each case to be negligible. The viability of strain GR2 cells before and after all mating incubations (>90% in every case) was assessed by spreading cell mixtures on solid minimal medium supplemented with only histidine and uracil; under these conditions strain A4840A does not grow.

[b] Exponentially growing *MAT***a** cells were mixed with *MAT*α cells. Mating mixtures were incubated in the presence of start-arresting compounds at the indicated concentrations or at 34°C for matings involving strains EP-1 or S104 before plating for diploid colonies.

[c] *MAT***a** cells were arrested by exposure either to start-arresting agents at the indicated concentrations or to the nonpermissive temperature for the start mutation. For each compound, cells were treated for that minimum period (3.5 h) found necessary by dose-response experiments to bring about start arrest (>75% nonbudding cells). Cell cycle arrest was monitored in each experiment. Mating mixtures were incubated as described in footnote *b*.

[d] Matings were performed as described in footnote *c*, except that mating mixtures were incubated in the absence of start-arresting agents at 23°C.

[e] For each strain, 100% mating efficiency was the value for exponentially growing cells in mating mixtures incubated in the absence of start-arresting conditions. Diploids formed per input *MAT***a** cell for the data shown were 0.17, 0.53, and 0.20 to 0.57 for strains EP-1, S104, and GR2, respectively.

(12a) showed very low levels of mating. Concentrations of SF sufficient to produce start arrest inhibited the mating of cells at the start block by at least two orders of magnitude, as compared with the mating abilities of cells arrested at start by other treatments. The low level of mating shown by cells arrested by SF (Table 1) was similar to that for cells arrested at start through the effect of the class II *cdc64-1* start mutation. The changes imposed by the *cdc64-1* mutation and by SF must each in some way affect the mating process itself, as cells arrested by other means at start can mate efficiently. However, these two types of class II arrest differ in the time needed for arrested cells to recover mating ability when returned to permissive conditions. The *cdc64* mutant cells rapidly regained mating ability (Table 1), but the SF-treated cells did not. It has been shown (12a) that after SF is removed there is a 4-h lag before cells begin to bud (an activity indicative of the performance of start and of the initiation of a new cell cycle [8]). Thus, cells tested for mating under permissive conditions after SF-mediated start arrest will still be at start during the 3-h period available for zygote formation. The low frequency of mating of these cells even under permissive conditions after SF-mediated start arrest (Table 1) suggests that the mating defect is caused by slowly reversible physiological changes in SF-treated cells.

Mating is at least a two-step process. Cells of opposite mating type must fuse cytoplasmically to form a zygote, and nuclei must subsequently undergo karyogamy. The *kar1-1* mutation allows zygote formation but interferes with karyogamy (4); cytoductants, from which haploid nuclei are segregated in hybrid cytoplasm, are thus formed. To test the effects of SF on zygote formation and on karyogamy, we mixed SF-treated cells of strain GR2 (see Table 1) with cells of the cycloheximide (CYH)-resistant and mitochondrially deficient strain R10 (*cyh2 MAT*α *his6 met* [*rho*$^-$]) and used the general mating procedure described in Table 1, footnote *a*, with incubation at 23°C to permit cell fusion. Diploids formed from these two haploid strains are CYH sensitive, as the *cyh2* mutation confers a CYH-resistant phenotype which is recessive (14). Cytoplasmic fusion in the absence of karyogamy would generate some cytoductants with the CYH-resistant haploid nucleus from strain R10 and the [rho$^+$] oxidative capacity of strain GR2. Therefore, cytoductants were selected by plating cell mixtures on CYH-containing solid medium with glycerol as the sole carbon source. The ratio of cytoductants selected by these criteria to diploids selected by nutritional criteria was <0.7%, similar to normal frequencies for cytoductant formation (0.1 to 0.6% [5]). Because most cell fusion events went on to yield diploid cells, SF must not markedly affect karyogamy. Other experiments showed that SF affected zygote formation. No zygotes (of 400 unbudded cells scored) were observed during microscopic examination of mating mixtures containing *MAT***a** cells first arrested at start by treatment with SF and then incubated for cell fusion in the presence of SF. This should be compared with cells arrested and incubated for cell fusion in the presence of *o*-phenanthroline which produced 9.4% zygotes. At least one mating step necessary for zygote formation, the induction of cell surface agglutinins by mating pheromones, has been shown to depend on protein synthesis (3, 6, 16). SF is the only compound listed in Table 1 which, under start-arresting conditions, has even moderate effects on protein synthesis (12a), which may account for its effect on zygote formation. In addition, SF has been shown (9, 13; Li et al., manuscript in preparation) to inhibit various methyltransferase reactions, and so in this way may affect a methyltransferase activity involved in initial mating processes.

For the compounds listed in Table 1 which permit a class I mating response, effects on macromolecular metabolism have been characterized (reviewed in reference 17). All of

the chemically induced start arrests are accompanied by minor effects on protein synthesis and major effects on RNA metabolism. The production of precursor rRNA is decreased when cells are treated with a compound which brings about a mating-competent start arrest. Because this metabolic response did not prevent cells from mating, it appears that continued synthesis of rRNA is not necessary for mating.

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