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"Start" Mutants of *Saccharomyces cerevisiae* Are Suppressed in Carbon Catabolite-Derepressing Medium

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Temperature-sensitive cell division "start" mutants *cdc28*, *cdc36*, *cdc37*, and *cdc39* of the yeast *Saccharomyces cerevisiae* arrested cell division in the G1 phase of the cell cycle in glucose medium. I report here that *cdc28*, *cdc36*, and *cdc39* mutants were suppressed when grown in carbon catabolite-derepressing medium.

The biochemical basis for the control of conjugation by oligopeptide mating factors in the yeast *Saccharomyces cerevisiae* is unknown. Recent work suggests that one of the genes involved in the regulation of conjugation, *STE5*, affects adenosine 3',5'-phosphate (cAMP) metabolism (3). The *cdc* start mutants *cdc28*, *cdc36*, *cdc37*, and *cdc39* (7) arrested cell division in G1 at the same point in the cell cycle as a mating factor arrests mating-competent cells. The *cdc36* and *cdc39* mutations can suppress a mutation in the *STE5* locus (J. Shuster, Mol. Cell. Biol., in press) and are therefore also involved in the regulation of conjugation. I report that the defect in some of the *cdc* start mutants was suppressed by carbon catabolite derepression.

The strains used are listed in Table 1. Complex YM-1 and minimal yeast nitrogen base (YNB) media were as described previously (1, 2). Cell number was determined with a particle counter (Coulter counter model Zb; Coulter Electronics, Inc., Hialeah, Fla.). Growth rates for cells after a temperature shift were often biphasic; the rates reported are those determined about 5 h after the shift.

The CDC^+ control strain, 260-8-2, divided well in glucose- or pyruvate-based medium; the growth rate in pyruvate was approximately ½ of that observed in glucose for all temperatures tested (Fig. 1A and Table 2). The rates of division of *cdc28*, *cdc36*, *cdc37*, and *cdc39* at the permissive temperature in pyruvate were also about ½ the rates observed in glucose; however, the *cdc28*, *cdc36*, and *cdc39* strains divided well at the high temperature (34 to 36°C) in pyruvate medium but not in glucose medium (Fig. 1B through D). The division rates for *cdc39*, *cdc36*, and *cdc28* were approximately eightfold, fivefold, and threefold greater, respectively, than the rates observed in glucose at the restrictive temperature (Table 2). The *cdc37* mutant was not suppressed by growth in pyruvate (Table 2). Since *cdc36* and *cdc39* demonstrated the strongest effects, these mutants were investigated in detail.

The ability of *cdc36* and *cdc39* to divide at the restrictive temperature in pyruvate may be explained as a result of (i) the presence of pyruvate or (ii) the absence of glucose. These two possibilities were tested by assaying the ability of the *cdc* mutants to divide in medium containing both glucose and pyruvate. The *cdc39* mutant was pregrown overnight in pyruvate at the restrictive temperature (36°C). The culture was split, and ½ received glucose to a concentration of 2%. The *cdc39* cells could not sustain division after the addition of glucose. In a similar experiment, *cdc36* was pregrown at the permissive temperature in medium containing both glucose and acetate (*cdc36* is incapable of prolonged growth at the restrictive temperature in any medium) and then shifted to the restrictive temperature. The cells did not grow in glucose plus acetate at the high temperature (doubling rate, >24 h). I conclude that the presence of glucose inhibits the cell division of *cdc36* and *cdc39* mutants when shifted to the restrictive temperature.

Both *cdc36* and *cdc39* were able to divide in acetate but not fructose at restrictive temperatures. Strain 4050-2-4 (*cdc36 MAL GAL*) could not divide in maltose (doubling rate, >24 h) and divided only weakly (doubling rate, 17.7 h) in galactose. Thus, cell division at the restrictive temperature in *cdc36* and *cdc39* mutants is not specifically inhibited by glucose, but rather by the presence of a readily fermentable carbon source.

One possible explanation for the suppression of *cdc36* and *cdc39* mutants in nonfermentable carbon sources states that the mutants are suppressed solely by a reduction in growth rate. To eliminate this possibility, *cdc36* strain 4050-2-4 was grown at the permissive temperature in the presence of glucose and low levels of the protein synthesis inhibitor trichodermin (8) to

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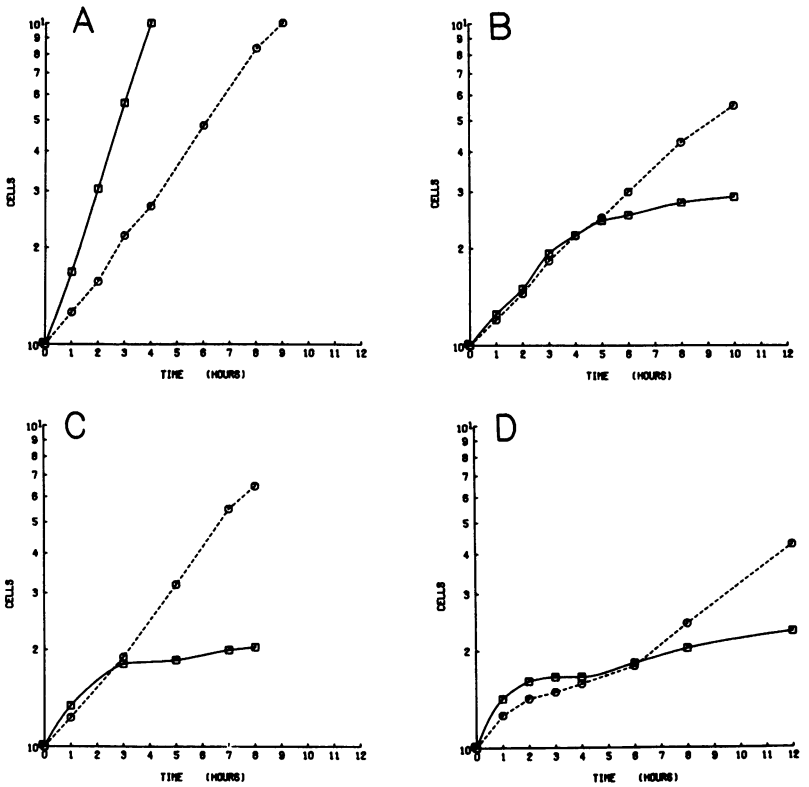

FIG. 1. Kinetics of division of *cdc* mutants in pyruvate. Strains were pregrown in YM-1 at 23°C to mid-log phase, diluted to 0.5 × 10^6 to 1.0 × 10^6 cells per ml, and shifted to restrictive temperatures at 0 h. Ordinate, normalized cell number increase = (cells per milliliter at $t = x$)/(cells per milliliter at $t = 0$). Solid line, 2% glucose as carbon source; dashed line, 3% sodium pyruvate as carbon source. Restrictive temperatures: 34°C for *cdc36*, *cdc28*, and *cdc37*; 36°C for *cdc39*. (A) 260-8-2 (CDC$^+$); (B) 661-2 (*cdc36*); (C) 665-1 (*cdc39*); (D) 653-1 (*cdc28*).

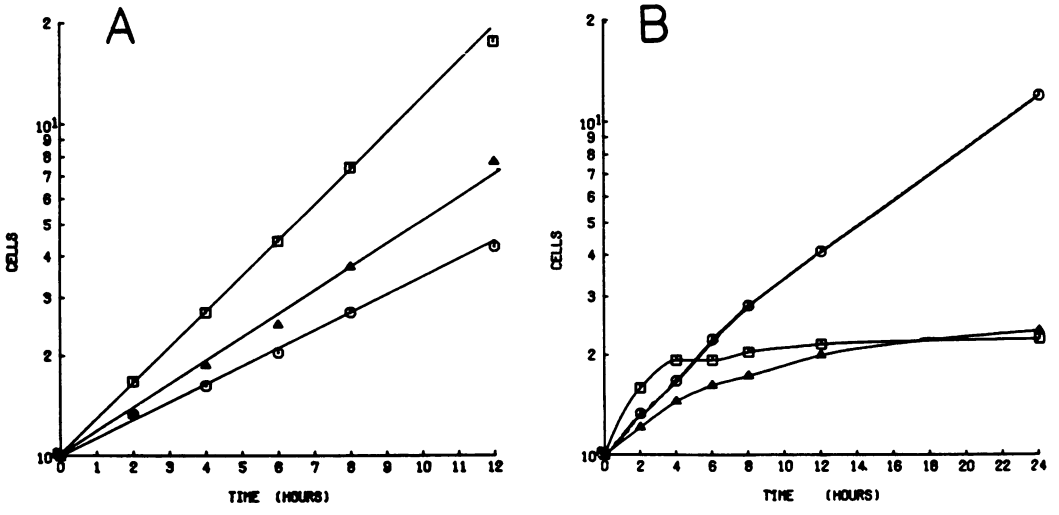

FIG. 2. Effect of slow growth on cell division arrest of *cdc36* in glucose. (A) Strain 4050-2-4 was grown in YNB defined medium at 23°C containing: 4% glucose (□); 4% glucose plus 0.75 μg of trichodermin per ml (△); or 3% sodium pyruvate (○). Ordinate, normalized cell number increase = (cells per milliliter at $t = x$)/(cells per milliliter at $t = 0$). (B) Cultures were shifted to 34°C at 0 h. Symbols are as described above.

TABLE 1. List of strains

Strain	Markers	Source
260-8-2	*MATα met2*	Reed (7)
661-2	*MATα cdc36-16 trp1 ura1 cyh2*	Reed (7)
665-1	*MATα cdc39-1 met2 tyr1 cyh2*	Reed (7)
653-1	*MATα cdc28-4 met8 trp1 tyr1 ile1 cyh2*	Reed (7)
674-3	*MATα cdc37-2 hom2 aro1 his2*	Reed (7)
4050-2-4	*MATα cdc36-16 met2 ura1 cyh2 MAL GAL*	This study

TABLE 2. Suppression of start mutants in pyruvate

Strain	Temp (°C)	Doubling time (h)[a] in: Glucose	Pyruvate	Glucose/ pyruvate
260-8-2	23	1.92	3.33	0.58
(CDC)	34	1.25	2.75	0.45
	36	1.12	2.67	0.42
661-2	23	3.00	4.00	0.75
(*cdc36*)	34	>24	4.75	>5.1
4050-2-4	23	2.93	5.89	0.50
(*cdc36*)	34	>24	5.82	>4.1
665-1	23	2.62	3.93	0.67
(*cdc39*)	36	23.9	3.00	8.0
653-1	23	2.83	3.78	0.75
(*cdc28*)	34	16.8	5.58	3.0
674-3	23	2.83	13.3	0.21
(*cdc37*)	34	7.50	>24	<0.31

[a] Strains were pregrown at 23°C to mid-log phase and then shifted to restrictive temperatures. The doubling times were calculated from plots of time versus log (increase in cell number). The values represent the rates of cell division about 5 h after the temperature shift. All of the strains were grown in YM-1 containing either 2% glucose or 3% sodium pyruvate, except strain 4050-2-4, which was grown in YNB defined medium (with amino acids) and either 4% glucose or 3% sodium pyruvate.

reduce the division rate, then shifted to the restrictive temperature. The results (Fig. 2) indicate that cells grown in glucose plus trichodermin show a reduced capacity to divide as compared with glucose-grown cells. Similar results were obtained with *cdc39* (data not shown). Therefore, suppression of the start mutants in pyruvate is not due simply to a reduction in growth rate.

The morphologies of the start mutants were examined. The temperature-sensitive cell division cycle mutants *cdc36* and *cdc39* arrested cell division in the G1 phase of the cell cycle at "start" and formed an aberrant shmoo morphology when shifted to the restrictive temperature in glucose medium (7). I report here that strains bearing either of these mutations also arrested cell division in the G1 phase of the cell cycle and formed shmoos when shifted to the restrictive temperature in the presence of the catabolite-repressing carbon source fructose or maltose. Galactose, which causes only partial catabolite repression (6), allowed only weak growth at the restrictive temperature for *cdc36*; these cells formed the shmoo morphology. In contrast, *cdc36* and *cdc39* strains continued to divide at the restrictive temperature when grown in the catabolite-derepressing carbon source pyruvate or acetate; these cells displayed a morphology similar to that of wild-type cells.

The CDC36 and CDC39 gene products have been implicated in the regulation of cell division mediated by oligopeptide mating pheromones (Shuster, in press). Thus, this work describes a significant link between the regulation of cell division as mediated by mating factors and the catabolite repression system; the data suggest that an element is common to both systems. Analogy with the second messenger hypothesis of the mammalian peptide hormone response (9) and the fact that cAMP has been implicated in catabolite repression (5) suggest that this link may be cAMP metabolism. In support of this idea, Liao and Thorner (4) have recently observed that inhibitors of phosphodiesterase activity are capable of affecting the response of yeast *MAT***a** cells to the α-mating factor.

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