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Mating-Defective *ste* Mutations Are Suppressed by Cell Division Cycle Start Mutations in *Saccharomyces cerevisiae*

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Temperature-sensitive mutants which arrest in the G1 phase of the cell cycle have been described for the yeast *Saccharomyces cerevisiae*. One class of these mutants (carrying *cdc28*, *cdc36*, *cdc37*, or *cdc39*) forms a shmoo morphology at restrictive temperature, characteristic of mating pheromone-arrested wild-type cells. Therefore, one hypothesis to explain the control of cell division by mating factors states that mating pheromones arrest wild-type cells by inactivating one or more of these *CDC* gene products. A class of mutants (carrying *ste4*, *ste5*, *ste7*, *ste11*, or *ste12*) which is insensitive to mating pheromone and sterile has also been described. One possible function of the *STE* gene products is the inactivation of the *CDC* gene products in the presence of a mating pheromone. A model incorporating these two hypotheses predicts that such *STE* gene products will not be required for mating in strains carrying an appropriate *cdc* lesion. This prediction was tested by assaying the mating abilities of double mutants for all of the pairwise combinations of *cdc* and *ste* mutations. Lesions in either *cdc36* or *cdc39* suppressed the mating defect due to *ste4* and *ste5*. Allele specificity was observed in the suppression of both *ste4* and *ste5*. The results indicate that the *CDC36*, *CDC39*, *STE4*, and *STE5* gene products interact functionally or physically or both in the regulation of cell division mediated by the presence or absence of mating pheromones. The *cdc36* and *cdc39* mutations did not suppress *ste7*, *ste11*, or *ste12*. Lesions in *cdc28* or *cdc37* did not suppress any of the *ste* mutations. Other models of *CDC* and *STE* gene action which predicted that some of the *cdc* and *ste* mutations would be alleles of the same locus were tested. None of the *cdc* mutations was allelic to the *ste* mutations and, therefore, these models were eliminated.

The yeast *Saccharomyces cerevisiae* reproduces as either a haploid or a diploid (29). The bridge between the haploid and diploid phases is the process of conjugation which occurs between cells of opposite mating types. These mating types, **a** and α, are controlled by a pair of complex mating type loci, *MAT***a** and *MAT*α, respectively (12, 17; see references 8 and 26 for reviews). The initial event in conjugation which occurs after cells of opposite mating types are mixed together is the synchronization of the cells at a stage in the cell cycle known as "start" (7). This synchronization is a result of the interaction of oligopeptide mating pheromones with their appropriate target cells. *MAT*α cells produce constitutively α-mating factor, which arrests cell division of *MAT***a** cells (3, 4), and similarly *MAT***a** cells produce **a**-factor, which arrests *MAT*α cells (1, 2, 27). Cells arrested by mating factor for extended periods of time without conjugating continue to increase in mass and form an aberrant morphology, a pear-shaped shmoo. Cell division arrest in G1 followed by shmoo formation is thus characteristic of cells undergoing a conjugatory response. In an attempt to identify the genes involved in the conjugatory response, Reed (21) isolated four complementation groups of temperature-sensitive mutants which undergo G1 arrest, exhibit the shmoo morphology, and have the ability to mate at the restrictive temperature. These mutants, Cdc28, Cdc36, Cdc37, and Cdc39, may represent constitutive expression of the mating system at the restrictive temperature in that the cells appear to prepare for conjugation in the absence of the appropriate mating factor signals. If this is true, the mutations identify gene products which must be inactivated for conjugation to occur. These mutations are not mating type specific; that is, they affect both *MAT***a** and *MAT*α cells. Collectively, these mutants have been referred to as "start" mutants since they have been shown to arrest cell division at the same stage in the cell cycle as do the mating factors.

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A different approach to the identification of the genes involved in the conjugatory process was the selection of mutants defective in conjugation (15). MacKay and Manney isolated mutants incapable of conjugation (sterile mutants). Many of the mutants were mating type nonspecific; that is, they conferred a sterile phenotype when present in either a *MAT***a** or a *MAT*α background (13, 14). This suggested that many of the genes required for conjugation may have similar functions in both **a** and α cells. Hartwell isolated a series of temperature-sensitive mutants in *MAT***a** cells which were insensitive to the cell division arrest mediated by α-factor (6). These mutants, as expected, were sterile. Eight complementation groups were obtained of which only one (*ste2*) was mating type specific. Two of the groups, *ste8* and *ste9*, proved to express silent copies of mating type information (J. D. Rine, Ph.D. thesis, University of Oregon, Eugene, 1979). Thus, the mutants become insensitive to mating factor by mimicking an *MAT***a**/*MAT*α diploid cell. The roles of the remaining class of mutations, *ste4*, *ste5*, *ste7*, *ste11*, and *ste12*, remain unexplained. The mutants identify the gene products essential for conjugation to occur.

Although a large number of models of gene action can be constructed based upon the preliminary characterization of the Cdc and Ste mutants, two models are attractive since they make strong predictions. The test of these predictions is the subject of this report.

MATERIALS AND METHODS

Strains and media. The strains used in this study are listed in Table 1. YM-1 liquid growth medium, YNB minimal medium, and YEPD solid medium were described previously (5, 9). The YM-1 medium contained 2% glucose. The YEPD medium was made without adding adenine or uracil.

Plate assay for mating ability. Segregants from tetrads with four viable spores were inoculated onto a YEPD plate and grown for 2 to 3 days at 23°C. The plates were replica plated to a prewarmed (34°C) YEPD plate and immediately inoculated with PT1 (*MAT***a**) or PT2 (*MAT*α) mating testers. The plates were incubated at 34°C overnight and then replica plated to minimal plates to select for diploids. Plates were scored after 1 and 2 days at 34°C. Mating ability was scored as follows: + = confluent growth in cross-stamp area after 1 day; +/− = zero to six colonies in the cross-stamp area after 1 day and three to six colonies after 2 days; − = less than three colonies after 2 days. Mating type was determined by performing the assay at 23°C, at which temperature all strains mated well.

Allelism and linkage. Genetic crosses and dissections were performed as described by Mortimer and Hawthorne (18).

Complementation tests. The presence or absence of *ste4* in *MAT*α segregants from the crosses *cdc36* × *ste4* and *cdc39* × *ste4* were determined as follows.

TABLE 1. List of strains

Strain	Genotype	Source
4000-3	*MAT***a** *cdc28-4 lys2 tyr1 cyh2*	This study
4000-4	*MAT*α *cdc28-4 lys2 tyr1 cyh2*	This study
4005-5-1	*MAT*α *cdc28-15 lys2 tyr1 thr4*	This study
4005-8-1	*MAT***a** *cdc28-15 lys2 tyr1 trp1*	This study
4036-1	*MAT***a*** *ste4-3 trp1 met2 ura3 can1*	This study
PT1	*MAT***a** *hom3 iso1 can1*	This study
PT2	*MAT*α *hom3 iso1 can1*	This study
381G	*MAT***a** *ade2 lys2 tyr1 his4 trp1 cry1 SUP4-3*	L. Hartwell (6)
381 Derivatives		
−63b	*ste4-3*	L. Hartwell (6)
−82b	*ste4-5*	L. Hartwell (6)
−42c	*ste5-3*	L. Hartwell (6)
−82a	*ste5-6*	L. Hartwell (6)
−73a	*ste7-1*	L. Hartwell (6)
915-6-3	*MAT*α *ste5-6 his4 met2 ura1*	L. Hartwell (6)
922-6-4	*MAT*α *ste4-3 his4 met2 ura1*	L. Hartwell (6)
942-7-4	*MAT*α *ste12-1 his4 met2 lys2*	L. Hartwell (6)
2037-3-3	*MAT*α *ste11-2 leu2 trp1 ade2*	L. Hartwell (6)
2038-2-2	*MAT*α *ste12-1 leu2 trp1 ade2*	L. Hartwell (6)
2040-1-1	*MAT*α *ste4-3 leu2 trp1 ade2*	L. Hartwell (6)
2041-3-4	*MAT*α *ste4-5 leu2 trp1 ade2*	L. Hartwell (6)
2044-5-2	*MAT*α *ste5-3 leu2 trp1 ade2*	L. Hartwell (6)
623-2	*MAT*α *cdc36-3 trp1 cyh2*	S. Reed (21)
626-1	*MAT***a** *cdc36-5 ade1 ura1 cyh2*	S. Reed (21)
653-1	*MAT*α *cdc28-4 met8 trp1 tyr1 ile1 cyh2*	S. Reed (21)
661-2	*MAT***a** *cdc36-16 trp1 ura1 cyh2*	S. Reed (21)
661-3	*MAT*α *cdc36-16 trp1 tyr1 lys2 cyh2*	S. Reed (21)
665-1	*MAT*α *cdc39-1 met2 tyr1 cyh2*	S. Reed (21)
665-2	*MAT***a** *cdc39-1 met2 ura1 trp1 cyh2*	S. Reed (21)
674-1	*MAT***a** *cdc37-2 hom2 arg4*	S. Reed (21)
674-3	*MAT*α *cdc37-2 hom2 aro1 his2*	S. Reed (21)

*MAT*α segregants which were phenotypically temperature sensitive for growth and fertile were crossed with strain 4036-1 (*MAT***a*** *ste4-3*). The resultant *MAT***a***/*MAT*α diploids (*MAT***a***/*MAT*α cells mate as *MAT*α cells [10]) were tested for their ability to mate. The presence of *ste4* in the original segregant was indicated by a failure of the corresponding diploid to mate at 34°C.

The presence or absence of *ste5* in *MAT*α segregants in the cross *cdc36* × *ste5* was tested in a similar manner. *MAT*α segregants were crossed to strain 381-42e (*MAT***a** *cry1 ste5-3*), mating type was rendered homozygous by selecting cryptopleurine-resistant (23) mitotic recombinants, and the cells were tested for their ability to mate.

Quantitative mating. Quantitative mating assays were performed by a modification of the method of Reid and Hartwell (22). Strains to be tested were grown up overnight in YM-1 at 23°C to mid-log phase (1×10^6 to 5×10^6 cells per ml). The *cdc ste* double mutants to be tested were chosen in order of appearance from the segregants recovered in the crosses. A total of 5×10^6 cells were inoculated into 5 ml of YM-1 preincubated at 34°C in a water bath. A total of 5×10^6 cells of the appropriate *MAT***a** or *MAT*α tester strain (PT1 or PT2, respectively) were added, and the mixture was filtered onto a 0.45-μm nitrocellulose filter (Millipore Corp.) and then rinsed with 5 ml of 34°C YM-1. The filter was placed onto a prewarmed YEPD plate containing 8% dextrose and incubated at 34°C for 8 h. The filters were then placed into 5 ml of YNB without glucose, and the cells were resuspended in a Vortex mixer at maximum speed for 10 s. The cells were diluted and plated onto minimal plates to select for diploids. The plates were incubated for 2 days at 34°C, and the number of diploid colonies was determined.

Growth kinetics of isogenic *MAT*a/*MAT*a, *MAT*α/*MAT*α, and *MAT*a/*MAT*α strains. Isogenic diploid strains homozygous for the *cdc* start mutations were constructed by crossing *MAT***a** *cdc* and *MAT*α *cdc* haploid strains and then exposing the *MAT***a**/*MAT*α diploids to 6,000-rad X-rays to induce mitotic recombination. *MAT***a**/*MAT***a** and *MAT*α/*MAT*α recombinants were recovered by screening the irradiated cells for the ability to conjugate with appropriate mating type tester strains. The isogenic sets of strains were pregrown in YM-1 at 23°C overnight and then shifted to the restrictive temperatures. At intervals, a 0.90-ml sample was withdrawn and fixed with 0.10 ml of Formalin. The fixed samples were diluted, sonicated for 10 s at 80 W, and counted in a particle counter (Coulter Counter model Zb; Coulter Electronics, Hialeah, Fla.). The percentage of a population which was present as unbudded cells was determined directly with a phase-contrast microscope (×400).

RESULTS

Allelism and linkage. One model of gene action during conjugation states that the *ste* mutations and the *cdc* mutations are alleles of the same locus. Under this hypothesis, one allele results in the production of an inactive gene product whereas the other allele codes for an aberrantly active product. One form of this model assumes that the Ste mutant produces an inactive gene product and the Cdc mutant produces an active form. In this case, the function of mating factor is to activate the *STE*/*CDC* gene product which, when activated, can mediate cell division arrest. The *ste* mutations are incapable of conjugation since they cannot form an active product. The temperature-sensitive *cdc* gene product assumes the activated conformation at restrictive temperature and results in cell division arrest in the absence of mating factor.

An alternative form of the allelism model states that the Cdc mutant produces the inactive gene product and the Ste mutant produces the active form. Under this hypothesis, activated wild-type *CDC*/*STE* gene product is required for cell division. The function of mating factor is the inactivation of this gene product. The temperature-sensitive Cdc mutant arrests cell division at the restrictive temperature in the absence of mating factor because the *STE*/*CDC* gene product is inactivated. The *ste* mutation codes for a gene product which is insensitive to the inactivation by mating factor and is therefore always active. Thus, cell division cannot be arrested in the Ste mutant and the cells are incapable of conjugation.

Both of the models outlined above predict that some of the *cdc* and *ste* mutations will be alleles of a single locus. The models were tested directly by crossing the Cdc mutants to the Ste mutants to determine the linkage relationships between the *CDC* and *STE* genes. Table 2 shows the results of linkage tests between the *cdc* lesions and the *ste* mutations.

cdc37 is linked to *ste5* with a map distance between them of approximately 30 centimorgans. Since the location of *cdc37* has been reported to be close to the *aro1* locus on chromosome IV (21), the positions of *ste5* and *cdc37* relative to the *aro1* locus were determined (Table 3). The results indicated that the *aro1* gene is

TABLE 2. Genetic linkage between *cdc* and *ste* mutations[a]

cdc mutation	PD:NPD:T				
	ste4	*ste5*	*ste7*	*ste11*	*ste12*
cdc28	8:7:22	4:4:24	3:3:12	5:7:13	3:2:13
cdc36	NL[b]	NL	82:0:2	9:3:9	8:5:33
cdc37	7:6:33	8:0:11	1:8:21	3:1:6	2:1:8
cdc39	NL	NL	5:3:15	3:3:14	4:4:17

[a] Standard genetic crosses were performed, and the resultant tetrads were analyzed. The data presented in some cases represent results obtained from more than one cross involving different alleles. PD, Parental ditype; NPD, nonparental ditype; T, tetratype.

[b] NL, No linkage; determined by locating the map positions of *cdc36*, *cdc39*, *ste4*, and *ste5*.

TABLE 3. Mapping of *cdc* and *ste* mutations[a]

Locus 1	Locus 2	PD	NPD	T	Map distance[b]
cdc36	*ste7*	82	0	2	1.2
cdc36	*cdc2*	11	0	5	15.6
cdc36	*cdc9*	40	0	1	1.2
ste7	*cdc2*	17	0	8	16.0
ste7	*cdc9*	53	0	5	4.3
cdc39	*thr4*	31	2	34	34.3
cdc39	*MAT*	70	18	200	53.5
cdc37	*aro1*	44	0	17	13.9
cdc37	*hom2*	100	0	4	1.9
cdc37	*ste5*	8	0	11	28.9
ste5	*aro1*	6	0	3	16.7
ste5	*hom2*	4	0	9	34.6
hom2	*aro1*	40	0	8	8.3
ste4	*met7*	18	0	10	17.9
ste4	*his3*	10	0	3	11.5
met7	*his3*	6	0	7	26.9

[a] PD, Parental ditype; NPD, nonparental ditype; T, tetratype.

[b] Map distance was calculated as [(1/2)T + 3NPD]/total, as described by Perkins (20).

located between *cdc37* and *ste5*. Therefore, *cdc37* and *ste5* cannot be allelic.

cdc36 and *ste7* are tightly linked, with the distance between them, ca. 1 centimorgan, not much greater than some intragenic distances previously reported. Therefore, a "bifunctional" complementation test was performed. If *cdc36* and *ste7* are allelic, one of the phenotypes (temperature sensitivity or sterility) should be the result of an inactivated protein, whereas the alternative phenotype should represent the aberrantly activated gene product. When the two alleles are present in a diploid, the mutant-active gene is functionally hemizygous; therefore, the diploid should display the phenotype characteristic of one or the other haploid parent strains. Strain 661-3 (*MAT*α *cdc36*) was crossed to strain 381-43a (*MAT***a** *ste7*), *MAT***a** was rendered homozygous (by the technique described in Materials and Methods), and the resultant *MAT***a**/*MAT***a** diploid was tested for its ability to grow at 38°C and its ability to mate at 34°C. This strain displayed wild-type phenotypes with respect to division and fertility at the restrictive temperature. I conclude that *cdc36* and *ste7* are tightly linked but not allelic.

The sterile phenotype did not segregate 2 sterile:2 fertile in the crosses of *cdc36* (or *cdc39*) by *ste4* (or *ste5*) due to suppression of the sterile phenotype. Therefore, a different test of allelism was performed. If two mutations are allelic, they will be located at the same position on the genetic map. I report here the map locations of *cdc36*, *cdc39*, *ste4*, and *ste5* (Table 3; Fig. 1). All four of these mutations are unlinked and are therefore not alleles of each other. The map location of *ste7* is also reported. From these data, I conclude that none of the *cdc* start mutations, *cdc28*, *cdc36*, *cdc37*, or *cdc39*, are alleles of the *ste* mutations, *ste4*, *ste5*, *ste7*, *ste11*, or *ste12*.

Suppression of sterility by *cdc* start mutations. A different model concerning the mechanism of gene action of the *ste* and *cdc* genes assumes that the gene products act in the following sequential pathway. *CDC* gene product is necessary for cell division. Mating factor activates the *STE* gene product, and this activated form inhibits the *CDC* gene product. The absence of *CDC* gene product causes the cells to arrest in G1 and prepare for conjugation. Under this hypothesis, Ste mutants are sterile because they cannot inactivate the *CDC* gene product. If the *CDC* gene product was inactivated by independent means, such as a temperature-sensitive mutation which results in a thermolabile gene product, the Ste$^-$ cells would be rendered fertile. According to the model, this type of pathway suppression would not be allele specific since any defect in the appropriate *CDC* gene product should obvi-

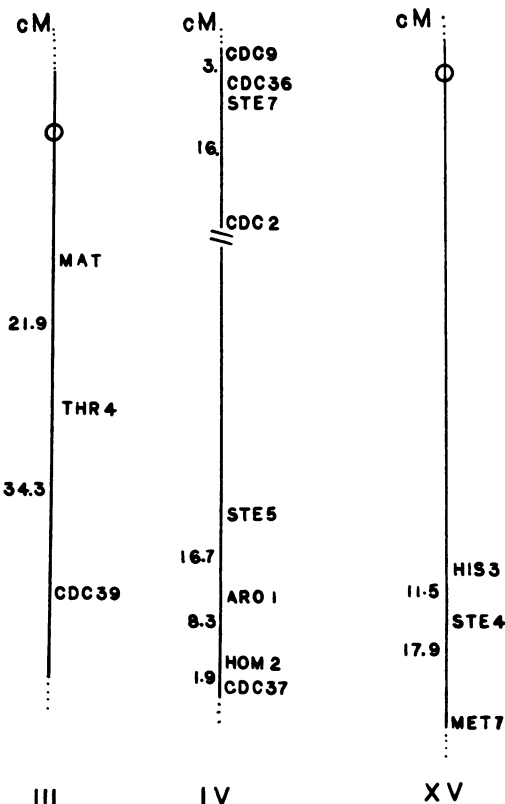

FIG. 1. Genetic map of *cdc* and *ste* mutations. cM, Centimorgan.

ate the requirement for the particular *STE* gene product.

Suppression of *ste* mutations by *cdc* mutations was analyzed by assaying the mating abilities of the *cdc ste* double mutants. If the *cdc* and *ste* mutations are unlinked, one expects tetrads of the type PD (parental ditype):T (tetratype): NPD (nonparental ditype) in the ratio 1:4:1 (18). If the *cdc* mutation does not suppress the *ste* lesion, all of the tetrads will segregate 2 fertile:2 sterile segregants. If the defect in the *CDC* gene does obviate the requirement for functional *STE* gene product, only the PD class will yield asci which segregate 2 fertile:2 sterile.

***cdc36* and *cdc39* mutations suppress *ste4*.** Significant deviations from the expected 2:2 segregation for sterility were observed for the crosses *cdc36* × *ste4* and *cdc39* × *ste4* (Table 4). The data for *cdc36* and *cdc39* indicate an excess of Ste^+ segregants consistent with the hypothesis that these *cdc* lesions can suppress the *ste4* mutation. The allele specificity of the interaction was investigated by performing crosses with alternative alleles of both the *cdc* and *ste* mutations. The results (Table 4) show that the suppression of *ste4* by *cdc36* is not allele dependent. The *cdc39* mutation suppressed *ste4-3* but not *ste4-5*, indicating an allele-specific interaction.

If the *cdc36* and *cdc39* mutations suppress the *ste4* lesion in all double-mutant segregants, no phenotypically temperature-sensitive and sterile segregants should be recovered in the *cdc* × *ste4* crosses. However, some segregants of this class were observed, suggesting heterogeneity in the *cdc ste* strains. This heterogeneity was determined to be due to the *MAT* locus. Phenotypically temperature-sensitive and sterile segregants were all ***MAT*a**. Therefore, *ste4* was suppressed strongly by *cdc36* and *cdc39* only in *MAT*α segregants. To determine the degree of suppression, quantitative mating experiments were performed. The *MAT*α *cdc36* (or *cdc39*) *ste4* segregants were identified by complementation (see Materials and Methods). In cases where fertility segregated 3 fertile:1 sterile in a tetrad, the complementation test proved that the *ste4* gene had actually segregated in a Mendelian fashion (2:2). The complementation tests also demonstrated that the suppression of *ste4* by *cdc36* or *cdc39* is recessive in that the diploids (constructed in the complementation test) heterozygous for the *cdc* defect and homozygous for *ste4* are sterile (data not shown). The results of quantitative mating experiments (Table 5) indicate that the *cdc36* and *cdc39* lesions strongly suppress the *ste4* defect in *MAT*α cells. The mating efficiency of the *MAT*α *cdc ste4* strains is about 10% of wild type compared with about 5×10^{-6} for the *MAT*α *CDC ste4* control. In contrast, ***MAT*a** double mutants mate at frequencies only about 0.01% of the wild-type level.

***cdc36* and *cdc39* mutations suppress *ste5*.** Sterility failed to segregate in the expected ratio of 2 sterile:2 fertile in the crosses *cdc36* × *ste5* and *cdc39* × *ste5*. The excess of Ste^+ segregants was more pronounced with the *ste5-3* allele than the *ste5-6* allele for all of the *cdc* lesions tested (Table 6). Some phenotypically temperature-sensitive and sterile segregants were recovered, indicating heterogeneity in the *cdc ste* strains. This heterogeneity was not due to the *MAT* locus.

The segregation of *ste5* in these crosses could be determined in the plate assay. Two of the segregants in each tetrad displayed wild-type mating ability, and the other two segregants mated weakly or were nonmaters. The weak mating phenotype was observed only in Cdc^- segregants and never in Cdc^+ segregants. This suggested that the *cdc* lesion suppressed the *ste5* mutation and that the weakly mating cdc segregants contained the *ste5* mutation. This was tested directly in a subset of the segregants by complementation. This analysis demonstrated that (i) the weakly mating segregants carry the *ste5* mutation and (ii) the suppression of *ste5* by *cdc* mutations is recessive. Quantitative mating experiments (Table 5) demonstrated that *cdc36 ste5-3* and *cdc39 ste5-3* strains mated at frequencies of 0.1% of wild type. The *ste5-6* allele was not suppressed in the quantitative mating test.

TABLE 4. Suppression of *ste4* by *cdc* mutations

cdc locus	*ste* locus	STE:ste[a]				
		4:0	3:1	2:2	1:3	0:4
28-4	*4-3*	1	4	19	0	0
28-4	*4-5*	0	3	8	0	0
28-15	*4-3*	0	3	9	0	0
36-3	*4-3*	3	23	9	0	0
36-3	*4-5*	0	7	6	0	0
36-5	*4-3*	4	15	7	0	0
36-5	*4-5*	0	6	6	0	0
36-16	*4-3*	3	20	18	0	0
36-16	*4-5*	0	6	8	0	0
37-2	*4-3*	0	4	46	0	0
39-1	*4-3*	1	30	30	0	0
39-1	*4-5*	0	6	36	0	0

[a] Standard genetic crosses were performed, and the segregants were analyzed for mating ability and temperature sensitivity. STE = more than three colonies present in the plate mating test after 2 days at 34°C; ste = less than four colonies present in the mating test. The ratios represent the segregation of the appropriate phenotype in the four-spored tetrads.

TABLE 5. Mating frequencies of *cdc ste4* and *cdc ste5* double mutants[a]

cdc mutation	Mating frequency with given *ste* mutation				
	+	4-3	4-5	5-3	5-6
CDC	1.00	**a** $<10^{-6}$ α 3.9×10^{-6}	**a** $<10^{-6}$ α 2.1×10^{-6}	$<10^{-6}$	$<10^{-6}$
cdc28-4		**a** $<10^{-6}$ α 2.4×10^{-5}		3.7×10^{-6}	
cdc36-3		**a** 2.7×10^{-3} α 4.0×10^{-2}	**a** 3.0×10^{-6} α 1.0×10^{-1}	2.6×10^{-2}	$<10^{-6}$
cdc36-16		**a** 6.6×10^{-5} α 1.8×10^{-1}	**a** 6.7×10^{-5} α 1.1×10^{-1}	7.7×10^{-4}	$<10^{-6}$
cdc37-2		**a** $<10^{-6}$ α 1.0×10^{-6}		8.1×10^{-6}	
cdc39-1		**a** 3.6×10^{-5} α 1.0×10^{-1}	**a** 2.1×10^{-6} α 3.1×10^{-3}	9.0×10^{-4}	$<10^{-6}$

[a] Frequencies represent the numbers of diploids produced after 8 h of mating at 34°C normalized to the number of diploids produced by wild type in that same interval: 1.8×10^7/ml for wild-type *MAT***a**; 5.1×10^7/ml for wild-type *MAT*α. Frequencies reported are the mean log raised to the power of 10 of three different segregants of each mating type for *ste4* and four different segregants (2 *MAT***a** and 2 *MAT*α) for *ste5*. Frequency = $10^{(\Sigma \log(\text{diploids}_{(\text{segregant})}/\text{diploids}_{(\text{wild type})})/n)}$.

There was no effect of mating type for either allele.

***CDC36* and *CDC39* revertants do not suppress *ste4* or *ste5* mutations.** Some heterogeneity was observed in the suppression of *ste4* and *ste5* by *cdc36* and *cdc39*. This is likely due to the genetic background of the various segregants tested. To test whether the suppression of *ste4* and *ste5* was due to the *cdc* lesions or some other gene, the following test was performed. Non-temperature-sensitive revertants of 661-2 (*cdc36-16 STE*) and 665-2 (*cdc39-1 STE*) were selected. The reversion events were deemed to be intragenic suppressors or back mutations since they were inseparable from the original *cdc36* and *cdc39* lesions in 48 and 46 tetrads, respectively. The revertants were crossed to strains 922-6-4 (*CDC ste4-3*) and 2044-5-2 (*CDC ste5-3*). Sterility segregated 2 sterile:2 fertile as expected if there was no suppression of sterility. Quantitative mating experiments demonstrated that the *CDC ste* segregants were as sterile as the *CDC ste* parent strains (data not shown). These data indicate that the suppression of *ste4* and *ste5* is due primarily to the *cdc36* and *cdc39* lesions and not to other suppressors closely linked to the *CDC36* and *CDC39* loci. As an additional test of the significance of the *cdc36* or *cdc39* lesions in the suppression of *ste4* and *ste5* mutations, non-temperature-sensitive revertants of fertile *cdc ste* double mutants were selected and assayed for mating ability. These *CDC* revertants were sterile (Table 7).

***cdc28* and *cdc37* mutations do not strongly suppress *ste4* or *ste5*.** Some of the tetrads resulting from the crosses *cdc28* × *ste4*, *cdc28* × *ste5*, *cdc37* × *ste4*, and *cdc37* × *ste5* did not display 2:2 segregation for fertility (see Tables 4 and 6). In these cases, the mating abilities of only two of

TABLE 6. Suppression of *ste5* by *cdc* mutations

cdc locus	*ste* locus	STE:ste[a]				
		4:0	3:1	2:2	1:3	0:4
28-4	*5-3*	0	3	9	0	0
28-4	*5-6*	0	0	10	0	0
28-15	5-3	0	1	13	0	0
36-3	*5-3*	0	5	6	0	0
36-3	*5-6*	0	16	36	0	0
36-5	*5-3*	1	4	2	0	0
36-5	*5-6*	0	9	28	0	0
36-16	*5-3*	0	7	20	0	0
36-16	*5-6*	0	2	56	0	0
37-2	*5-3*	1	2	19	0	0
39-1	*5-3*	6	14	16	0	0
39-1	*5-6*	1	8	25	0	0

[a] Standard genetic crosses were performed, and the segregants were analyzed for mating ability and temperature sensitivity. STE = more than three colonies present in the plate mating test after 2 days at 34°C; ste = less than four colonies present in the mating test. The ratios represent the segregation of the appropriate phenotypes in the four-spored tetrads.

TABLE 7. Mating frequencies of *CDC* revertants derived from fertile *cdc ste* segregants[a]

Segregant	*MAT*	Mating frequency		
		cdc36-16 ste4-3	*CDC36R ste4-3*	*cdc/CDC*
4020-72-3	α	1.9×10^{-1}	6.2×10^{-5}	3,100
4020-5-1	α	2.6×10^{-1}	3.1×10^{-6}	84,000
4020-72-4	**a**	2.0×10^{-5}	$<10^{-6}$	
4020-82-1	**a**	7.9×10^{-5}	$<10^{-6}$	
		cdc36-16 ste5-3	*CDC36 ste5-3*	
4056-1-4	α	8.4×10^{-6}	$<10^{-6}$	
4056-8-3	α	8.3×10^{-3}	9.8×10^{-5}	85
4056-1-1	**a**	6.5×10^{-3}	1.1×10^{-5}	586
4056-2-1	**a**	7.8×10^{-4}	7.5×10^{-6}	104
		cdc39-1 ste4-3	*CDC39 ste4-3*	
4034-1-2	α	1.95×10^{-2}	4.10×10^{-6}	4,800
4034-1-4	**a**	9.92×10^{-5}	$<10^{-6}$	
		cdc39-1 ste5-3	*CDC39 ste5-3*	
4055-2-2	α	8.30×10^{-2}	1.66×10^{-4}	500

[a] Frequencies represent the number of diploids produced after 8 h of mating at 34°C normalized to the number of diploids produced by wild type in that same interval.

the segregants in a tetrad appeared wild type, whereas the other two segregants were weak maters or nonmaters (scored as +/− or −, respectively, as described in Materials and Methods). Results of quantitative mating assays performed on the *cdc ste* double mutants indicated that *cdc28* and *cdc37* do not strongly suppress either *ste4* or *ste5* (see Table 5): The discrepancy between the plate assay data and the results from the quantitative mating tests reflects the fact that a few of the *cdc ste* double mutants are capable of low-frequency mating (0.01 to 0.1% of wild type) and therefore score as weak maters in the plate assay, resulting in non-2:2 segregation of fertility. Thus, although the mating ability of strains containing *ste4* or *ste5* is not strongly affected by a *cdc28* or *cdc37* mutation, it is possible that *cdc28* or *cdc37* is capable of weakly suppressing *ste4* (or *ste5*) and that this weak suppression is dependent upon other genetic factors in the backgrounds of the strains utilized in the construction of the double mutants.

ste7*, *ste11*, and *ste12* mutations are not suppressed by *cdc* start mutations.** Sterility segregated 2 sterile:2 fertile in all crosses involving *ste7*, *ste11*, and *ste12* (Table 8), indicating that the *cdc28*, *cdc36*, *cdc37*, or *cdc39* mutation was unable to strongly suppress lesions in these *ste* genes. Since the results obtained in the plate assay for fertility are variable at low mating frequencies (<0.1% of wild type), quantitative mating tests were performed on a subset of the *cdc ste* double mutants to determine whether any of the *ste* mutations were weakly suppressed. Four *cdc ste* double mutant segregants (two *MATa** and two *MAT*α) were assayed from each cross. The results (Table 9) indicate that there is no significant difference in the mating abilities between the *cdc ste* double mutants and the *CDC ste* controls for *ste7*, *ste11*, and *ste12*.

Suppression of *cdc36* and *cdc39* by mating type heterozygosity. Strains homozygous for *cdc36* or *cdc39* have been observed to be leaky in *MAT***a**/*MAT*α diploids (21). I report here a quantitative

TABLE 8. Sterile segregation in *cdc* × *ste* tetrads[a]

cdc locus	*ste* locus	STE:ste				
		4:0	3:1	2:2	1:3	0:4
28	*7*	0	0	10	0	0
	11	0	1	7	0	0
	12	0	0	8	0	0
36	*7*	0	0	12	0	0
	11	0	0	14	0	0
	12	0	0	14	0	0
37	*7*	0	0	8	0	0
	11	0	0	10	0	0
	12	0	0	11	0	0
39	*7*	0	0	23	0	0
	11	0	0	18	0	0
	12	0	0	25	0	0

[a] Standard genetic crosses were performed, and the segregants were analyzed for mating ability and temperature sensitivity. STE = more than three colonies present in the plate mating test after 2 days at 34°C; ste = less than four colonies present in the mating test. The ratios represent the segregation of the appropriate phenotypes in the four-spored tetrads.

TABLE 9. Mating frequencies of *cdc ste7*, *cdc ste11*, and *cdc ste12* double mutants[a]

cdc mutation	Mating frequency with given *ste* mutation			
	+	*7-1*	*11-2*	*12-1*
CDC	1.0	$<10^{-6}$	$<10^{-6}$	2.26×10^{-6}
cdc28-4	1.4	$<10^{-6}$	2.3×10^{-6}	$<10^{-6}$
cdc36-16	1.0	$<10^{-6}$	3.1×10^{-6}	$<10^{-6}$
cdc37-2	0.54	$<10^{-6}$	$<10^{-6}$	$<10^{-6}$
cdc39-1	1.5	$<10^{-6}$	9.6×10^{-6}	$<10^{-6}$

[a] Frequencies represent the number of diploids produced after 8 h of mating at 34°C normalized to the number of diploids produced by wild type in that same interval, as described in Table 5.

analysis of the effects of mating type heterozygosity upon the phenotypes of *cdc36* and *cdc39* mutants.

The ability of *MAT***a**/*MAT*α *cdc* diploids to proliferate at restrictive temperatures was determined by assaying the growth kinetics of diploid strains homozygous for *cdc36* or *cdc39* but differing at the *MAT* locus. The results (Fig. 2A and B) demonstrate that both *cdc36* and *cdc39* *MAT***a**/*MAT*α strains are capable of increased cell division at restrictive temperature compared with the isogenic *MAT***a**/*MAT***a** and *MAT*α/*MAT*α strains. In addition, the *MAT***a**/*MAT*α strains did not arrest cell division as unbudded cells (Fig. 2C and D). The effect of mating type heterozygosity upon the increase in mass of *cdc36* strains was determined. The results (Fig. 3) indicate that the *MAT***a**/*MAT***a** and *MAT*α/*MAT*α strains cease both the accumulation of mass and cell number increase after about 6 h. However, whereas *MAT***a**/*MAT*α and *MAT***a**/*MAT***a** cells displayed similar increases in mass (4.07- and 3.39-fold, respectively), the *MAT***a**/*MAT*α population increased 3.93-fold in cell

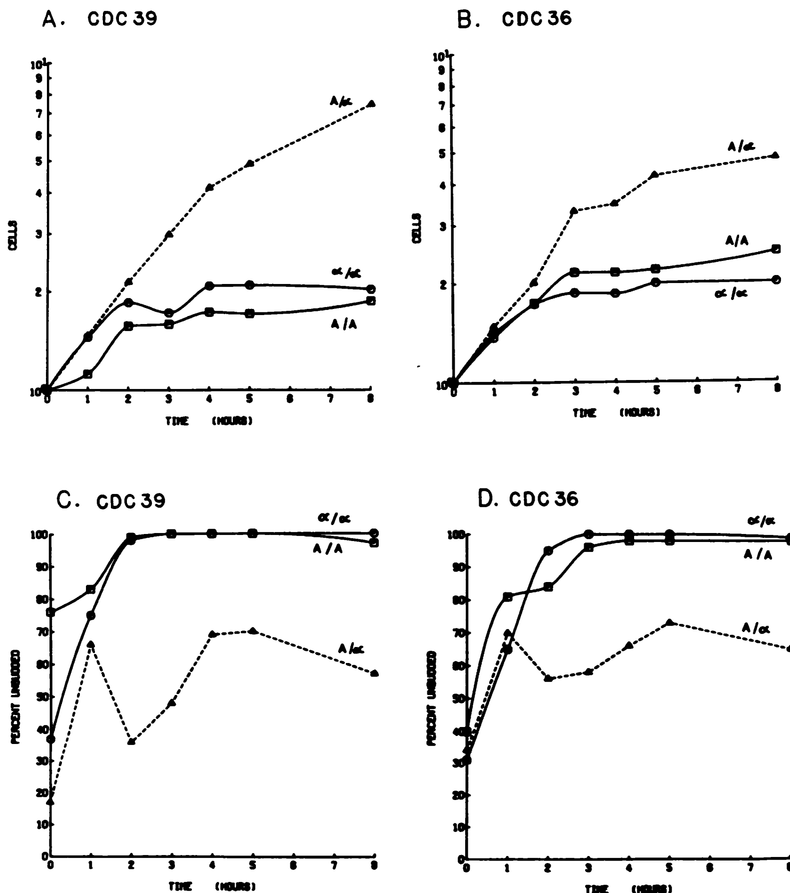

FIG. 2. Effect of *MAT* locus on cell division arrest of *cdc36* and *cdc39*. Strains were grown in YM-1 at 23°C to mid-log phase, diluted to 0.5×10^6 to 1.0×10^6 cells per ml, and shifted to restrictive temperatures at time = 0 h: 34°C for *cdc36* and 36°C for *cdc39*. (A and B) Ordinate = normalized cell number increase [(cells per milliliter at $t = x$)/(cells per milliliter at $t = 0$)]: A, *cdc39*; B, *cdc36*. (C and D) Ordinate = percent unbudded cells in the population: C, *cdc39*; D, *cdc36*.

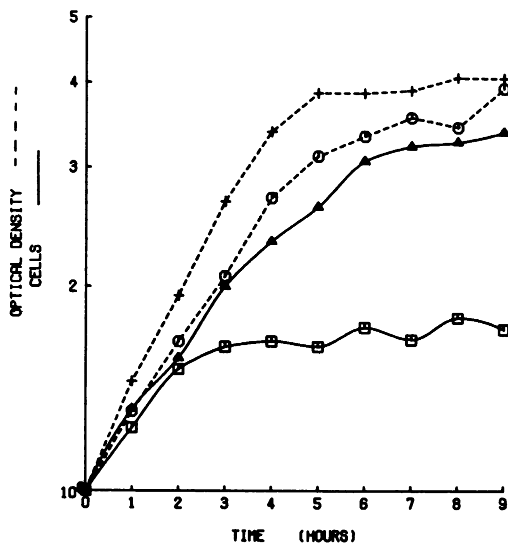

FIG. 3. Effect of *MAT* locus on growth arrest of *cdc36*. Strains were grown in YM-1 at 23°C to mid-log phase, diluted to 0.5×10^6 to 1.0×10^6 cells per ml, and shifted to 36°C at time = 0 h. (Solid line) Normalized cell number increase = (cells per milliliter at $t = x$)/(cells per milliliter at $t = 0$); (dashed line) normalized mass increase based upon optical density (OD) at 660 nm and 1 cm = (OD at time = x)/(OD at time = 0). Symbols: □—□, *MATa/MATa*; △--△, *MATa/MATα*; O-----O, *MATa/MATa*; +-----+, *MATa/MATα*.

number compared with a 1.73-fold increase for the *MATa/MATa* population. The *MATa/MATα* strain arrested cell division asynchronously, with 61% of the cells unbudded compared with 95% unbudded for the *MATa/MATa* strain. I conclude from these data that mating type heterozygosity suppresses the cell division G1 arrest phenotype, but is incapable of suppressing a growth defect resulting from a mutation in the *CDC36* locus. Mating type heterozygosity did not suppress the cell division defect of *cdc28* (Fig. 4).

DISCUSSION

The process of conjugation in the yeast *S. cerevisiae* was investigated utilizing the Cdc start mutants and the Ste sterile mutants. Two models of gene action were tested. The first model predicted that some of the *cdc* mutations and some of the *ste* mutations were alleles of the same locus. This model assumes that either the *cdc* or the *ste* allele results in the production of an aberrantly active gene product. This model is based upon the observation that some regulatory genes have been shown to mutate to either inactive or aberrantly active gene products. For example, *lacI* mutations in *Escherichia coli* cause constitutive expression of the *lac* operon

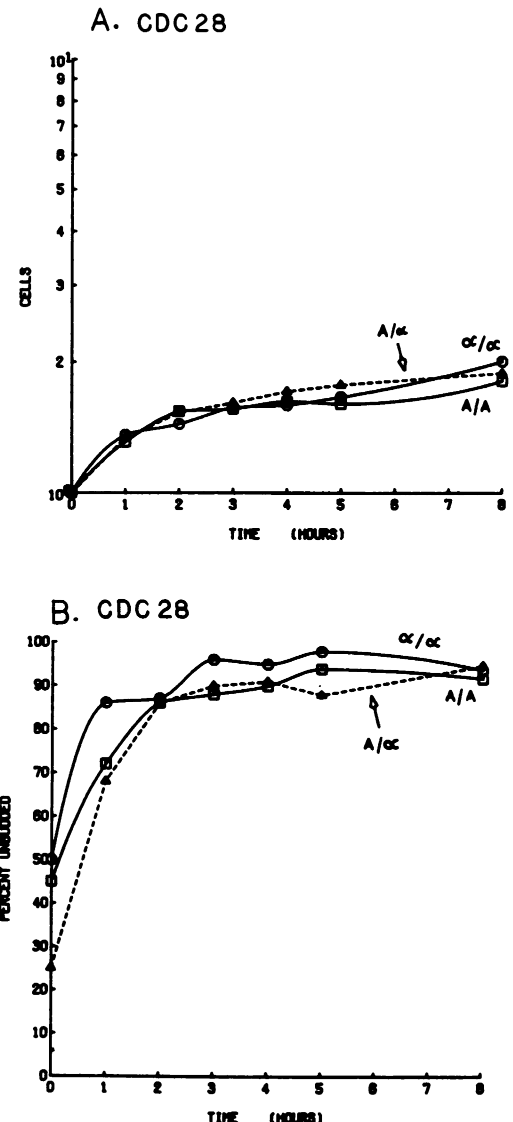

FIG. 4. Effect of *MAT* locus on cell division arrest of *cdc28*. (A) Strains were grown in YM-1 at 23°C to mid-log phase, diluted to 0.5×10^6 to 1.0×10^6 cells per ml, and shifted to restrictive temperature, 34°C, at time = 0 h. (Ordinate) Normalized cell number increase = (cells per milliliter at $t = x$)/(cells per milliliter at $t = 0$). (B) Cultures treated as described above. (Ordinate) Percent unbudded cells in the population.

(19), whereas *lacI*^{s} mutations result in cells which are noninducible (28). A similar result has been observed in *Saccharomyces*, where one allele of a *GAL* locus, *GAL81*, causes constitutive expression of enzymes required for galactose utilization whereas an alternative allele, *gal4*, results in an inability to utilize galactose (16). The results of this study indicate that the *cdc* start mutations are not allelic to any of the

ste mutations and therefore eliminate the allelism model. Although *cdc36* was observed to be tightly linked to *ste7* (ca. 1 centimorgan), they do not appear to be allelic. The map positions of *cdc36*, *cdc37*, *cdc39*, *ste4*, *ste5*, and *ste7* are reported.

The second model of gene action which was tested stated that the *CDC* and *STE* gene products act in the following sequential manner. *CDC* gene product is required for cell division. Mating factor causes the activation of *STE* gene product whose function is the inhibition of the *CDC* gene product. This inhibition results in a cessation of cell division in preparation for conjugation. This model predicted that the appropriate *cdc* mutation would obviate the requirement for the functional *STE* gene product and, therefore, *cdc ste* double mutants would be capable of conjugation. This expectation was realized in the double mutants *cdc36 ste4*, *cdc36 ste5*, *cdc39 ste4*, and *cdc39 ste5*. However, the simple model is not sufficient to explain all of the data.

The suppression of *ste4* was not allele specific for three independently isolated alleles of *cdc36* and two alleles of *ste4*, as expected if the *cdc* mutation obviates the requirement for the *STE4* gene product. The suppression of *ste4* by *cdc39* was allele dependent for *ste4* (only one allele of *cdc39* has been isolated). These allele-specific interactions may indicate that there is a physical interaction between the *cdc39* and *ste4* gene products (25). The *cdc36* and *cdc39* mutations suppressed *ste4* strongly only in *MAT*α cells. This was surprising since both *ste4* and the *cdc* lesions have the same phenotype in *MAT***a** and *MAT*α cells. One possibility is that the *ste4* gene is under the control of the mating type locus. The *MAT*α locus of *S. cerevisiae* consists of two cistrons; *MAT*α*1* codes for a positive regulator of α functions and *MAT*α*2* codes for a repressor of **a** functions (8, 24). Only one gene, *MAT***a***1*, has been identified genetically at the *MAT***a** locus. The product of this gene is not essential for conjugation (10, 11). Therefore, *MAT*α cells contain a positive effector of conjugatory functions whereas *MAT***a** cells do not. If the *STE4* gene is regulated by the *MAT* locus, more *STE4* gene product may be synthesized in response to the *MAT*α*1*-positive regulator than in its absence. The levels of *STE4* gene product would thus be higher in *MAT*α cells than in *MAT***a** cells. This idea is consistent with the observation that *MAT*α *CDC ste4* cells mate at slightly higher frequencies than *MAT***a** *CDC ste4* cells (see Table 5). If the temperature-sensitive Ste4 mutants tested had residual Ste4 activity at restrictive temperature, the residual activity may be higher in α cells; thus, *cdc36* and *cdc39* mutations can suppress the *ste4* defect in *MAT*α cells specifically. In this context, it should be noted that, although *ste4* mutations in *MAT***a** cells were not suppressed well by *cdc36* or *cdc39*, a decrease in the mating ability of *MAT***a** *cdc36 ste4* and *MAT***a** *cdc39 ste4* strains was observed when the temperature sensitivity phenotype was reverted (see Table 7). This result indicates that the *cdc36* and *cdc39* mutations have a small but detectable effect upon the mating ability of *MAT***a** *ste4* cells and suggests that the interaction among the *CDC36*, *CDC39*, and *STE4* gene products differs quantitatively in *MAT***a** and *MAT*α cells.

The suppression of *ste5* by *cdc36* and *cdc39* was weak. The *cdc36-16 ste5-3* and *cdc39-1 ste5-3* double mutants mated at about 0.1% of wild type (100- to 1,000-fold above the *CDC ste5* controls). The *cdc36-3 ste5-3* double mutants mated at about 2% of the wild-type level. Of the *ste5* alleles tested, only *ste5-3* was suppressed at all. Three possible explanations for the observed results are the following. First, since the restrictive temperatures for the Cdc start mutants are 36 to 38°C, it may be that the *cdc* gene products are not sufficiently inactivated at the temperature of the mating assays (34°C) to suppress the *ste5* defect. However, mating experiments cannot be performed at temperatures much greater than 34°C since wild-type strains are largely infertile at these higher temperatures (22). That the leaky allele *cdc36-3* displays the highest degree of suppression makes this possibility less likely. Second, it is possible that the interaction between *cdc36* or *cdc39* and *ste5* is highly allele specific due to a physical interaction between the gene products (25). A third possibility is that the *ste5* gene product performs more than one function during the response to the mating pheromone and that *cdc36* or *cdc39* can suppress only one of those functions. Under this model, the mutation, *ste5-3*, can be suppressed by mutations in the *cdc* genes because only one of its functions (the one which interacts with the *cdc* gene products) is defective.

None of the four *cdc* mutations (*cdc28*, *cdc36*, *cdc37*, or *cdc39*) suppressed the sterility resulting from *ste7*, *ste11*, or *ste12* lesions. In addition, *cdc28* or *cdc37* did not significantly suppress *ste4* or *ste5*. Hartwell (6) reported that *MAT***a** *cdc28-4 ste* strains were as sterile as *MAT***a** *CDC ste* controls. The work presented in this paper confirms those results and, in addition, demonstrates that *cdc28* mutations are incapable of significantly suppressing *ste* mutations in *MAT*α cells. The simplest interpretation of these data is that *CDC28*, *CDC37*, *STE7*, *STE11*, and *STE12* gene products act independently from the *CDC36*/*CDC39*-*STE4*/*STE5* pathway.

Mating type heterozygous *MAT***a**/*MAT*α diploids homozygous for either *cdc36* or *cdc39* do not arrest cell division in G1 when shifted to

restrictive temperatures; however, the *MATa*/*MAT*α diploids remain temperature sensitive for growth. *MATa*/*MATa* and *MATa*/*MAT*α *cdc36*/*cdc36* diploids display similar increases in mass when shifted to restrictive temperature. However, the *MATa*/*MATa* cells arrest division at a single point in the cell cycle in G1, whereas the *MATa*/*MAT*α cells divide more than once and then arrest cell division asynchronously. I infer from these data that a mating-competent strain carrying a mutation in *cdc36* is defective in two cellular processes, progression through start and cellular growth. This is also likely for strains which bear a *cdc39* mutation. One hypothesis consistent with these data states that the *CDC36* or *CDC39* genes are responsible for the production of a substance which is essential for cellular growth. Cell division in mating-competent cells may be sensitive to a threshold level of this substance, and consequently mating-competent *cdc36* or *cdc39* cells arrest division when the amount of the substance drops below a critical value. If this threshold concentration is greater than the amount required for cell growth (mass increase), growth and division will be transiently uncoupled. Mating-incompetent cells may lack the (conjugatory) system for response to a threshold value of the substance and, therefore, cease division and growth simultaneously. It would be interesting to determine whether mating factor is capable of depressing cellular growth of mating-competent Cdc^{+} strains after extended periods (more than two doubling times) after the addition of mating factor.

The suppression of *ste4* or *ste5* by *cdc36* or *cdc39* and the effects of mating type heterozygosity upon *cdc36* and *cdc39* strains suggest that the *CDC36* and *CDC39* gene products are involved directly in the regulation of cell division mediated by mating factors.

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