GENETIC CONTROL OF THE CELL DIVISION CYCLE IN YEAST: V. GENETIC ANALYSIS OF *cdc* MUTANTS

LELAND H. HARTWELL, ROBERT K. MORTIMER*, JOSEPH CULOTTI AND MARILYN CULOTTI

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

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ABSTRACT

One hundred and forty-eight temperature-sensitive cell division cycle (*cdc*) mutants of *Saccharomyces cerevisiae* have been isolated and characterized. Complementation studies ordered these recessive mutations into 32 groups and tetrad analysis revealed that each of these groups defines a single nuclear gene. Fourteen of these genes have been located on the yeast genetic map. Functionally related cistrons are not tightly clustered.

Mutations in different cistrons frequently produce different cellular and nuclear morphologies in the mutant cells following incubation at the restrictive temperature, but all the mutations in the same cistron produce essentially the same morphology. The products of these genes appear, therefore, each to function individually in a discrete step of the cell cycle and they define collectively a large number of different steps.

The mutants were examined by time-lapse photomicroscopy to determine the number of cell cycles completed at the restrictive temperature before arrest. For most mutants, cells early in the cell cycle at the time of the temperature shift (before the execution point) arrest in the first cell cycle while those later in the cycle (after the execution point) arrest in the second cell cycle. Execution points for allelic mutations that exhibit first or second cycle arrest are rather similar and appear to be cistron-specific. Other mutants traverse several cycles before arrest, and its suggested that the latter type of response may reveal gene products that are temperature-sensitive for synthesis, whereas the former may be temperature-sensitive for function.

The gene products that are defined by the *cdc* cistrons are essential for the completion of the cell cycle in haploids of *a* and *α* mating type and in *a*/*α* diploid cells. The same genes, therefore, control the cell cycle in each of these stages of the life cycle.

SACCHAROMYCES cerevisiae is an appropriate organism for the genetic dissection of the mitotic cell division cycle. First, this organism undergoes the same sequence of events during cell division in both the haplophase and diplophase. Thus, recessive mutations that impair the ability of the cell to complete cell division can be isolated in the haplophase and the ability of two such mutations to exhibit complementation can be studied in the diplophase. Second, a single cell of *S. cerevisiae* begins a mitotic cycle with the emergence of a bud on its sur-

* On leave from Division of Medical Physics, University of California, Berkeley, California 94720.

face and this bud grows in size throughout the mitotic cycle. The size of the bud on a cell reveals the position of that cell in the cell division cycle, and this fact facilitates the recognition of cell cycle mutants. Finally, the genetic system of *S. cerevisiae* has been extensively studied and lends itself with facility to mutational analysis (MORTIMER and HAWTHORNE 1969).

Consequently, we initiated a search among a collection of temperature-sensitive yeast mutants for mutations that specifically arrest mutant cells at a discrete step of the division cycle. Our expectation was that the cells from an asynchronous culture of such a mutant would assume a uniform and possibly unusual morphology at the restrictive temperature. Previous reports have described the detection of cell division cycle (*cdc*) mutants (HARTWELL, CULOTTI and REID 1970) and the physiological properties of some *cdc* mutants as studied in synchronous cultures (HARTWELL 1971a,b; CULOTTI and HARTWELL 1971). The purpose of this communication is to report on the genetic characterization of *cdc* mutants. We have determined the number of complementation groups represented by these mutants, have found that the lesions are each due to single, recessive mutations in nuclear genes, and in some cases have located the map positions of the defective genes. We have compared the phenotypes for most of the independently isolated mutations that reside in the same cistron in order to determine whether the phenotypes are cistron- or allele-specific. The effect of at least one mutation from each complementation group upon the mitotic cycle has been examined in haploids of *a* and α mating type and in diploids to determine whether or not the cell cycle is controlled by the same genes in each of these stages of the life cycle.

MATERIALS AND METHODS

Yeast strains and origin of mutants: All of the temperature-sensitive mutants with numerical designations were derived from the parent strain, A364A (*a ade1 ade2 ura1 tyr1 his7 lys2 gal1*) by mutagenesis with N-methyl-N′-nitro-N-nitrosoguanidine as described earlier (HARTWELL 1967). Mutants with numbers greater than 1000 were generously supplied by DR. CALVIN MCLAUGHLIN (University of California, Irvine). Mutants prefixed with the letter E were obtained from A364A by mutagenesis with ethylmethane sulfonate as described by HAWTHORNE 1969. For all the temperature-sensitive mutants the permissive temperature is 23°C and the restrictive temperature is 36°C.

Strains of α mating type for complementation studies were obtained as segregants of the mating: mutant $\times$ H79–20–3 (α *ade1 ura1 leu2 gal1*). A variety of strains were used for the genetic mapping experiments.

Genetic techniques: Standard techniques of genetic analysis of *S. cerevisiae* have been described by MORTIMER and HAWTHORNE (1969).

Detection of temperature-sensitive cell cycle mutants: Temperature-sensitive mutant clones were examined either by time-lapse photomicroscopy (HARTWELL, CULOTTI and REID 1970) or, in most cases, by a liquid screening procedure. In the latter, mutant clones were grown overnight in YM-1 medium at 23°C, and while in the exponential growth phase, were adjusted to a density of 2×10^6 cells per ml and shifted to 36°C. Two 1-ml samples were withdrawn from the culture at 0, 3, 6, and 9 hours after the temperature shift and added to 4 ml of saline-formaldehyde (0.15M NaCl containing 3.7% formaldehyde). The samples were left at room temperature for one to several days before being examined. The cells from one set of samples were then centrifuged down, the supernatant was discarded, the cells were resuspended in a 1:20 dilution of glusulase (Endo Laboratories, Garden City, N.Y.) in distilled water, and they were incubated

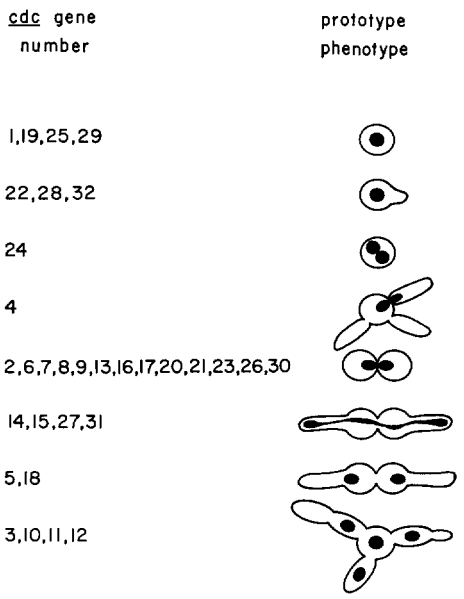

FIGURE 1.—Prototype phenotypes of *cdc* mutants after the termination of cell cycle development at the restrictive temperature.

for 1 hour at 23°C. The cells were then centrifuged down, the glusulase was decanted, the cells were resuspended in a drop of the liquid remaining in the tube and they were examined in a phase contrast microscope. The glusulase treatment serves to digest off the cell walls and, thereby, to separate any clumps of cells that are not held together by cytoplasmic membranes. That is, if a bud and its parent cell have completed cytokinesis but not cell wall separation, they will appear as two separate cells after the glusulase treatment. The spheroplasts resulting from glusulase treatment do not lyse in the low osmolarity medium that they are suspended in due to the prior fixation in formaldehyde. Essentially the same morphologies are found for each of the mutants if they are sonicated prior to examination rather than digested with glusulase, although sonication is slightly more likely to lead to artificial breakage of cells. Mutant clones in which at least 80% of the cells exhibited a uniform morphology were considered candidates for cell cycle mutants.

The nuclear morphologies of these mutants were then examined by a modification (HARTWELL 1970) of the ROBINOW and MARAK technique of Giemsa staining (ROBINOW and MARAK 1966). Most of the mutant clones in which the cell population exhibited a uniform cellular morphology also exhibited a uniform nuclear morphology, as depicted in Figure 1, and all such mutants were retained as prospective *cdc* mutants. In a few cases, although most of the cells of a mutant clone exhibited a uniform cellular morphology, the nuclear morphologies were not uniform, and these mutants were not retained as cell cycle mutants.

Determination of execution points: A culture of mutant cells in exponential growth phase at the permissive temperature was sonicated to disrupt clumps of cells, shifted to the restrictive temperature onto prewarmed solid medium, and photographed by time-lapse photomicroscopy as described previously (HARTWELL, CULOTTI and REID 1970). The cells of most mutants traverse one to several division cycles and then arrest development at a characteristic morphology. The morphology of the individual cells in each mutant strain after the termination of development at the restrictive temperature was known from the liquid screening technique described above. With this information, it was relatively easy to determine the number of cells produced by each mutant cell following the temperature shift from the photomicrographs for most mutant strains.

However, when more than four cells are present in each microcolony, there may be some error due to the stacking of cells on top of one another. Furthermore, some mutants, in particular the cytokinesis mutants (*cdc* 3, 10, 11, and 12) form such aberrant and diverse morphologies that it is not possible to determine unambiguously the number of cells per microcolony.

Execution points were calculated for those mutant strains in which the cells arrest in the first or second cell cycle following the temperature shift in the following way. Approximately 50 to 100 cells of a particular mutant strain were scored as to whether they produced one or two cells at the restritive temperature. After correction for the age distribution in an asynchronous population, the fraction of cells producing two cells should give directly the fraction of the cell cycle at which execution (acquisition of the ability to complete the present cycle and arrest in the second cell cycle at the restrictive temperature) occurs.

However, two difficulties complicate the simple interpretation of this data, only one of which can be easily corrected for. First, many of the mutants grow more slowly at the permissive temperature than the parent strain, probably because the thermolabile defect is partially expressed at the permissive temperature. If this is the case, a portion of the mutant population is always transiently arrested at the cell cycle block at the permissive temperature and the population is not, therefore, accurately represented by the age distribution equation. Since this perturbation would be extremely difficult to correct for, it is ignored, and to the extent that the distribution of cells within the cycle differs for any particular mutant from that assumed for a random exponentially growing culture, an error is introduced.

A second difficulty is introduced by the fact that two cells may appear as one, either because the sonication did not disrupt all clumps of cells or because two cells may fortuitously sit down side by side. If one or both of the two cells has a bud, they are scored as two cells and no error is introduced. If, however, the pair is composed of two unbudded cells, they will be indistinguishable from one cell with a large bud. This possible source of error can be corrected for. In a well-sonicated, exponential culture of the haploid parent strain A364A, 40% of the cells are unbudded and 60% are budded. Therefore, in time-lapse studies where the proportion of unbudded cells is less than 40%, a correction is made by reallocating some of the cells that were scored as cells with large buds into the unbudded class; one large budded cell becomes two unbudded cells.

Let b_o equal the observed number of budded cells and u_o be the observed number of unbudded cells. If $u_o/(u_o + b_o)$ is less than 0.4, then assume that a fraction of the cells with large buds, x, are really two unbudded cells. The true number of unbudded cells, u, is then equal to $u_o + 2\,x\,b_o$ and the true number of budded cells is $b = b_o - x\,b_o$. By setting $u = 0.4(u + b)$ we can determine x:

$$x = \frac{0.4\,b_o - 0.6\,u_o}{1.6\,b_o}.$$

The execution point (Ex) is then calculated from the fraction of cells, N, that produce two cells at the restrictive temperature by correcting for the age distribution in an asychronous population: $Ex = 1 - \log\,(1 + N)/.301$ (PUCK and STEFFEN 1963).

All the execution points in this paper are for haploid strains and are consistently later in the cell cycle than the execution points determined previously in homozygous diploid strains (HARTWELL, CULOTTI and REID 1970; HARTWELL 1971a,b; CULOTTI and HARTWELL 1971). This observation is probably a reflection of the fact that the unbudded portion of the cycle occupies a greater fraction of the cycle in haploid strains.

RESULTS

Detection of cell division cycle (cdc) mutants: Cell cycle mutants were detected among a collection of 1500 temperature-sensitive mutants of *S. cerevisiae* by the rather uniform and sometimes unusual cellular and nuclear morphologies that the mutant cells assume after an interval of growth at the restrictive temperature. These morphologies are illustrated in Figure 1. The *cdc* numbers in

TABLE 1

Notes on the phenotypes of mutants after termination of cell cycle development at the restrictive temperature

cdc	Descriptive notes
1	Some cells terminate with a tiny bud.
2, 16	Some cells terminate with most of the nucleus on one side of the isthmus separating parent cell and bud.
3, 10, 11, 12	The number of nuclei per cell varies at termination from about 2 to 16, with most cells having 4 to 8 nuclei.
4	The number of buds per cell at termination varies from 1 to 5, with most cells having 3 buds.
5, 14, 15, 18, 27, 31	The number of elongated processes which may represent second cycle buds varies from none to 4, with most cells displaying two as shown.
7	In haploid cultures some alleles display 20% to 40% of the cells as unbudded cells at termination. However, other alleles and the same alleles in homozygous diploids show greater than 80% of the cells with the morphology given.
22, 28, 32	The morphology displayed is typical of that after about one generation time at the restrictive temperature. The cells continue to elongate at the restrictive temperature and after two or more generation times assume very elongated and, in some cases, even branched structures.
24	The number of nuclei per cell varies from about 1 to 4 at termination, with most haploid cells displaying two while many homozygous diploid cells display four.
all	Most of the mutant cells lyse after extended periods of incubation at the restrictive temperature. This lysis is apparently the result, in most cases, of continued growth without cell division, as most mutants (except those in *cdc* 1, 19, and 25) continue to enlarge at the restrictive temperature after the termination of cell cycle development.

this figure refer to the complementation groups, and the phenotype illustrated is defined as the *prototype phenotype* for that complementation group. That is, most mutants within the complementation group display the phenotype illustrated. Some of the subtle variations exhibited among mutants of the same complementation group and among strains with the same allele, but with different genetic backgrounds are briefly noted in Table 1. In addition, a few exceptional mutants in some of the complementation groups do not exhibit the prototype phenotype and these will be discussed in a subsequent section.

Segregation of temperature sensitivity and phenotype in tetrads: The mutants were all derived from strain A364A (mating type a). Some of the mutants were mated with a non-temperature-sensitive strain of α mating type in order to study the segregation of the mutation in tetrads from the resulting heterozygous diploid and the phenotype that the mutation produces in a variety of segregants, and to derive temperature-sensitive mutants of α mating type for complementation studies. The complementation studies were performed in concert with the tetrad analysis so that, in most cases, only a single allele (allele 1) has been analyzed by tetrad analysis—except in those cases to be described below, where a particular mutant exhibited an unusual characteristic that needed further clarification.

In most cases, the mutant by nonmutant crosses exhibited a 2:2 segregation for temperature sensitivity to temperature insensitivity in the four spores of the tetrad derived from the heterozygous diploid and the clones grown from the temperature-sensitive spores exhibited the prototype phenotype after a period of growth at the restrictive temperature. These results indicate that the lesions producing temperature sensitivity are the result, in each case, of a mutation in a single nuclear gene, and that the phenotype exhibited by the mutant at the restrictive temperature is a result of this single mutation and not the result of modifiers acting in concert with it. It was possible to determine, therefore, in these and in subsequent crosses, the second division segregation frequencies for each of the *cdc* cistrons by comparing their segregation patterns with those of known centromere-linked markers (Table 2). Ten (*cdc* 4, 5, 7, 8, 10, 11, 14, 15, 16, 29) of 31 *cdc* genes display significant centromere linkage (gene *cdc* 32 could not be examined for reasons that are explained below). Further localization of the centromere-linked genes are described in a following section.

There were four exceptions to the 2:2 pattern for $ts^-:ts^+$ indicative of more than one temperature-sensitive lesion in the parent strain (mutant 104, *cdc* 3; 4028, *cdc* 17; 5011, *cdc* 24; 23019, *cdc* 28). In all four cases it was apparent from tetrad analysis that the mutant strains harbored two independently segregating temperature-sensitive mutations. Only one of the two lesions in each strain produced the prototype phenotype. In all four cases it was possible to derive a spore that contained only the temperature-sensitive *cdc* lesion from a NPD ascus (all four spores temperature-sensitive). The clone from this spore was then used in subsequent crosses to demonstrate that the lesion in genes *cdc* 3, 17, 24, and 28

TABLE 2

Centromere linkage of cdc *genes*

cdc	FDS	SDS	Percent SDS	*cdc*	FDS	SDS	Percent SDS
1	10	30	75	17	6	25	81
2	26	75	74	18	8	18	69
3	4	10	ns†	19	4	6	ns
4	59	11	16*	20	16	25	61
5	52	21	29*	21	5	8	ns
6	12	22	65	22	5	9	ns
7	15	2	12*	23	8	9	ns
8	29	29	50*	24	4	10	ns
9	9	18	67	25	3	8	ns
10	24	0	0*	26	4	7	ns
11	29	33	53*	27	5	9	ns
12	6	17	74	28	4	15	79
13	12	30	72	29	42	14	25*
14	70	71	50*	30	17	25	60
15	26	4	13*	31	3	3	ns
16	41	14	26*				

* Percent SDS significantly less than 2/3 ($P < .05$). FDS, first division segregation; SDS, second division segregation.

† ns = not statistically significant enough to warrant calculation of the percent SDS.

segregated 2:2 ts^-:ts^+ following sporulation of a heterozygous diploid. The second division segregation frequencies for genes *cdc* 3, 17, 24, and 28 in Table 2 were obtained in these latter crosses.

Finally, one mutant 212 (*cdc* 32) could not be analyzed due to the low viability of the spores deriving from the heterozygous diploid. Mutant 212 is probably an *a*/*a* diploid, since tetrads derived from a cross with an α/α strain gave fair viability and since it has a DNA content per cell twice that of the other mutant strains. The genetic analysis of mutant 212 is not yet complete.

Complementation tests: Thirty-one temperature-sensitive mutant strains of α mating type were derived by tetrad dissection or random spore preparations from diploids heterozygous for one of the temperature-sensitive cell cycle mutations. Each of these strains represents a different complementation group. All of the 140 cell cycle mutants of *a* mating type were mated with each of the thirty-one strains of α mating type and the resulting diploids were tested for temperature sensitivity. For the most part, the mutant strains fail to complement with only one of the α tester strains. In this way, the thirty-two complementation groups listed in Table 1 were derived from the original collection of 140 temperature-sensitive cell cycle mutants (mutant 212 is assigned to group 32 since it complements with all 31 α tester strains). The cellular morphologies (but not nuclear morphologies) of each of the noncomplementing, heteroallelic diploids were examined after incubation at the restrictive temperature, and all appeared like the prototype. These results suggest the presence of thirty-two cistrons, each of whose products are essential for one of the discrete steps of the cell cycle. All of the mutations that we have found in each of these cistrons are recessive, with one possible exception. Mutation *cdc* 12-1 appears to be semi-dominant in some strains and recessive in others.

There were, however, nine mutants (4028, *cdc* 1 and 17; 23019, *cdc* 1 and 28; 23012 *cdc* 4 and 15; 19021 *cdc* 4 and 14; 18045 *cdc* 9 and 27; 371, *cdc* 14 and 27; 284; *cdc* 16 and 29; 9042, *cdc* 16 and 23; 5011, *cdc* 23 and 24) that failed to complement with two of the α tester strains. They appear, therefore, in two complementation groups in Table 3. These mutant strains might harbor two temperature-sensitive lesions or they might be the result of a single lesion that is unable to complement with two intra-cistronic complementation groups. In order to decide between these two alternatives, each of these exceptional mutants was mated with a non-temperature-sensitive strain and the resulting diploid was subjected to tetrad analysis. The clones resulting from the four spores of the tetrads were tested for temperature sensitivity, examined for their morphology at the restrictive temperature, and analyzed for complementation with tester strains from each of the two complementation groups with which the original mutant strain had given a negative complementation test. Two temperature-sensitive mutations were segregating in each of these crosses, as evidenced by an excess of ts^- over ts^+ segregants, by the presence in most cases of two morphological phenotypes among the segregants and by the presence of independently segregating mutations by the complementation tests. The complementation tests permitted independent scoring of the two lesions segregating in the crosses and the classification

TABLE 3

Complementation groups and phenotypes of cdc *mutants*

cdc	Allele	Mutant	Morphology*	Number of cells†	Execution‡	*cdc*	Allele	Mutant	Morphology*	Number of cells†	Execution‡
1	1	369	P	1–2	.32		7	247	P	1–2	.62
	2	342	P	1–2	.15		8	292	P	1–2	.40
	3	4028	A				9	288	P	1–2	.33
	4	131	P	4–10			10	9008	P	1–2	.24
	5	296	A				11	446	A		
	6	456	P	1–4			12	399	A	1–2	.33
	7	E1–6	P	2–4			13	13088	P		
	8	23019	A	1–2			14	7070	P	1–2	.30
2	1	370	P	1–2	.34	10	1	17012	P		
	2	336	P	1–2	.17		2	191	P		
	3	19041	P	1–2	.24		3	310	P		
	4	346	P	1–2	.34		4	12007	P		
	5	15003	P	3–7			5	22051	P		
	6	256	P	3–8			6	21021	A		
	7	E7–7	P	15–25			7	E110	A		
3	1	104	P				8	E2–15	P		
	2	7058	P				9	E8–2	P		
	3	10004	P			11	1	332	P		
	4	161	A				2	449	P		
	5	201	A				3	12020	P		
4	1	314	P	1–2	.21		4	13010	A		
	2	374	P	1–2	.25		5	261	A		
	3	458	P	1–2	.27	12	1	471	P		
	4	23012	P	1–2	.26		2	9019	P		
	5	19036	P				3	23028	P		
	6	19021	P				4	2040	P		
	7	20066	P	1–2	.27	13	1	428	P	1–2	.63
5	1	473	P	1–2	.74	14	1	7041	P	1–2	.72
6	1	327	P	1–2	.24		2	305	A		
7	1	124	P				3	214	P	1–2	.82
	2	325	P				4	367	P	1–2	.63
	3	18032	P				5	147	P	1–2	.92
	4	4008	P	1–2	.32		6	19021	A		
	5	379	P				7	272	P	1–2	.77
	6	20055	P				8	289	P	1–2	.79
	7	E247	P				9	3026	P	1–2	.67
8	1	198	P	1–2	.61		10	371	P	3–4	
	2	172	P	1–2	.48		11	7049	P	1–2	.55
	3	13052	P	1–2	.62		12	10039	P	1–2	.86
	4	12022	P	1–2	.67		13	E8–1	P	1–2	
	5	141	A			15	1	17017	P	1–2	.53
	6	363	P				2	13053	P	1–2	.55
9	1	244	P	1–2	.56		3	23012	A		
	2	280	P	1–2	.49		4	7038	P	1–2	.79
	3	236	P	1–2	.50		5	19031	P		
	4	436	A				6	E275	P	1–2	
	5	18045	P	1–2	.46		7	E280	P	1–2	
	6	16011	A				8	E4–1	P	1–2	

TABLE 3—Continued

Complementation groups and phenotypes of cdc *mutants*

cdc	Allele	Mutant	Morphology*	Number of cells†	Execution‡	*cdc*	Allele	Mutant	Morphology*	Number of cells†	Execution‡
16	1	281	P	1–2	.48		3	E5–5			
	2	284	P	1–2	.34	21	1	17026	P	1–2	.63
	3	486	P	3–6			2	E117	P	1–5	
	4	9049	P	3–6		22	1	248	P	1–2	.19
	5	13074	P	2–4		23	1	9013	P	1–2	.76
	6	249	P	2–4			2	5011	A		
	7	16001	P	2–6			3	9042	P		
	8	7054	P	4–8			4	E73	A		
	9	10051	P				5	E344	P	1–6	
	10	9042	P	1–2	.27		6	E6–2	P	20–25	
	11	7035	P			24	1	5011	P	1–2	.35
	12	246	P	1–2	.22		2	E185	P	1–2	.19
	13	17009	P	2–6			3	E187	P	1–2	
	14	E140	P	2–3		25	1	321	P	2–5	
	15	E221	P	1–5		26	1	7027	P	2–4	
	16	E226	P	4–8		27	1	9002	P	2–5	
	17	E1–15	P	2–3			2	E115	P	8–10	
	18	E7–4	P	3–5			3	371	A		
17	1	4028	P	1–2	.18		4	18045	A		
	2	E366	P	5–7		28	1	23019	P	1–2	.16
18	1	14028	P	3–4		29	1	17048	P	1–3	
	2	3057	P	1–8			2	284	A		
	3	E331	P	2–10		30	1	23015	P	2–8	
19	1	395	P	2–6			2	15023	P	2–4	
20	1	127	P	1–2	.81	31	1	12021	P	2–4	
	2	9025	P	1–4		32	1	212	P	1–2	.15

* P = prototype phenotype; A = atypical phenotype.

† The number of cells produced by a single cell after a shift from the permissive to the restrictive temperature.

‡ The execution point for the double mutants that appear in two complementation groups is recorded only under the complementation group that appears by morphological criteria to be epistatic in the double mutant.

of tetrads as PD, NPD or TT for the two ts⁻ mutations (Table 4). Segregants from these crosses that failed to complement with only one of the two complementation groups being tested exhibited the prototype phenotype of that complementation group. Thus, in each of the nine exceptional cases, the original mutant strain had acquired two independent *cdc* mutations during the mutagenesis.

Comparison of the phenotypes of mutants within a complementation group: Two parameters of a mutant's phenotype were examined and compared among mutants of the same complementation group. First, cells from an asynchronous culture were shifted from the permissive temperature onto agar plates at the restrictive temperature and followed by time-lapse photomicroscopy. This analysis determines the number of cells that a single cell can form after the temperature shift (Table 3).

TABLE 4

Tetrad analysis of mutants that belong to two cdc *complementation groups*

Mutant	*cdc* alleles	Ascus types		
		PD	NPD	TT
4028	1–3; 17–1	5	0	6
23012	4–4; 15–3	5	5	2
19021	4–6; 14–6	6	0	6
18045	9–5; 27–4	3	0	8
371	14–10; 27–3	1	4	7
284	16–2; 29–2	4	4	2
9042	16–10; 23–3	2	1	3
5011	23–2; 24–1	1	1	2

For some mutant strains it is difficult to determine the number of cells per microcolony and, hence, no entry is made in Table 3. The cytokinesis mutants, *cdc* 3, 10, 11, and 12, produce such aberrant and diverse morphologies at the restrictive temperature that it is difficult to estimate the number of cells per microcolony. Most of the haploid mutants of the *cdc* 7 group produce a mixture of two morphological types (see Table 1), and this fact complicates the interpretation of the photographic data on these mutants.

With most mutants a single cell produces one cell or two cells at the restrictive temperature before development is arrested. In all such cases, there is a strong correlation between the ability of a cell to form one cell (arrest in the first cell cycle) or two cells (arrest in the second cell cycle) and the position of the cell in the cell cycle at the time of the temperature shift. That is, cells that are early in the cycle (with no bud or a small bud) at the time of the shift form one cell while those that are later in the cycle (with larger buds) form two. By analyzing the response of 50 to 100 cells for many of the mutant strains, it was possible to estimate the time in the cell cycle at which the mutant cell acquires the capacity to complete the first cell cycle and arrest in the second following a shift from the permissive to the restrictive temperature (the execution point). Execution points are expressed in fractions of a cell cycle in Table 3 and were derived as described in MATERIALS AND METHODS.

If we compare execution points among mutants in the same complementation group (Table 3), there is a fair degree of correlation (we exclude from this comparison mutants that form more than two cells at the restrictive temperature). This correlation is evident in Table 5 where the average execution points and the standard deviation of execution points have been calculated for six complementation groups in which four or more execution points have been determined. Considering the inherent uncertainties in the calculation of the execution point (see MATERIALS AND METHODS) and the relatively small number of cells that were scored for each mutant (50 to 100), the data suggests that the execution point is a cistron-specific and not an allele-specific parameter.

It must be emphasized that this conclusion is based upon only those mutants that produce one or two cells at the restrictive temperature. Other mutants that

TABLE 5

Standard deviations of execution points

cdc	Number of alleles determined	Average of execution points		Standard deviation
4	5	.23	±	.02
8	4	.60	±	.07
9	10	.42	±	.11
2	4	.27	±	.07
14	9	.75	±	.11
16	4	.33	±	.10

produce more than two cells (15003, 256, E7–7 in *cdc* 2; 371 in *cdc* 14 and twelve mutants in *cdc* 16) were excluded from the calculations recorded in Table 5. Many cells produce more than two cells following the shift to the restrictive temperature, and this type of response can occur among some alleles in a complementation group in which other alleles produce one or two cells. These two types of responses may be the result of fundamentally different types of mutational alteration to the gene product. Mutants that produce more than two cells might be temperature-sensitive for the synthesis (i.e., assembly) of the gene product while the mutants that produce one or two cells might be temperature-sensitive for the function of the gene product.

The second parameter of phenotype to be compared among mutants of the same complementation group is the cellular and nuclear morphology after the mutant cells have terminated development at the restrictive temperature. Mutants are classified in Table 3 according to whether they display the prototype phenotype (designated P; see Figure 1) or are atypical (designated A). Although some subtle differences were occasionally noted, in almost all cases, independently isolated alleles of the same cistron produce the prototype phenotype at the restrictive temperature. Cellular morphology following arrest at the restrictive temperature, therefore, appears to be cistron-specific rather than allele-specific.

However, some mutants of some complementation groups were atypical in the sense that they did not display the prototype phenoytpe after growth at the restrictive temperature. If the products of the *cdc* genes function exclusively in a single event of the cell division cycle, then their alteration by mutation might be expected to result in the same cellular morphology irrespective of the allele. On the other hand, if these gene products function in other cellular processes as well, then mutations in these genes might produce different phenotypes, depending upon the type of alteration sustained by the gene and its product. In other words, the observation of alleles with atypical phenotypes raises the possibility that the gene product in question may not be specifically employed in a discrete step of the cell cycle. A second, trivial explanation for the atypical behavior of some mutants might be that these mutants have incurred two mutations with one of the two mutations having an epistatic effect upon the cell's phenotype. Three distinct classes of atypical mutant will be discussed in succession, and it will be demonstrated that all atypical mutants are the result of two mutations.

One class of atypical cell cycle mutant is explained by the fact that the mutants are double cell cycle mutants which display the prototype phenotype characteristic of the epistatic cell cycle mutation (mutants 4028, 23019, 23012, 19021, 5011, 371, 18045, 284). These mutants are, therefore, atypical in one of the two complementation groups in which they occur and display the prototype phenotype for the second complementation group. These mutants were discussed in a previous section where it was pointed out that the two mutations can be separated in segregants from tetrads and that both mutations produce the prototype phenotype in mutants harboring only one of the two mutations.

A second class of atypical mutant fails to complement with only one *cdc* tester. These five mutants (16011, *cdc* 9–6; 21021, *cdc* 10–6; E110, *cdc* 10–7; 13010, *cdc* 11–4; E73, *cdc* 23–4) exhibit a cell cycle phenotype at the restrictive temperature, but the phenotype differs from the prototype. For two of these mutants, 16011 and 21021, the results of tetrad analysis were consistent with the presence of two mutations. Strain 16011 harbors one temperature-sensitive mutation and one mutation that alters the morphology of temperature-sensitive and temperature-insensitive strains alike. Strain 21021 harbors two temperature-sensitive mutations. The remaining three mutants were analyzed by random spore analysis (Table 6). The results demonstrate the presence in each mutant of two independent temperature-sensitive mutations in that ts⁻ progeny were found that did complement with the appropriate *cdc* tester strain as well as ts⁻ progeny that did not. Some of the latter exhibited the prototype phenotype.

Hence, although some subtle differences exist among mutants within a complementation group, all the cell cycle mutations within the same complementation group that have been found by screening for a uniform phenotype at the restrictive temperature produce very similar cellular and nuclear morphologies. All the apparent exceptions to this generalization were found to be the result of double mutations.

TABLE 6

Random spore analysis of atypical mutants

Mutant	Allele	ts⁺ progeny*	ts⁻ progeny*	
			Do complement with appropriate *cdc* tester strains	Do not complement with appropriate *cdc* tester strain
E110	10–7	20	23	18
13010	11–4	29	16	15
E73	23–4	15	43	36
296	1–5	40	43	14
161	3–4	29	11	46
201	3–5	42	32	18
141	8–5	43	7	46
261	11–5	7	43	43
305	14–2	56	4	33

* Only haploid clones, as determined by ability to mate, are recorded.

A more rigorous search for exceptional mutants: It is, of course, possible that our failure to find non-prototype mutants of the *cdc* cistrons is due to our selection of mutants displaying only cell cycle phenotypes (i.e., mutants that terminate development in a uniform morphological state). If these gene products function in cellular processes other than a specific step of the cell cycle, then some temperature-sensitive lesions in these genes might produce cells with a nonuniform morphology (for example, an asychronous cell population) at the restrictive temperature. If mutations that produce a non-prototype phenotype occur with a frequency as great as those that produce a cell cycle phenotype, then we would expect (from the repeat frequency of cell cycle mutants) several per cistron in a set of several hundred randomly chosen temperature-sensitive mutants.

In order to test for this possibility, α tester strains from genes *cdc* 1 through *cdc* 16 were each examined for complementation with 379 temperature-sensitive mutants of *a* mating type. Nineteen noncomplementing mutants that fell into 9 of the 16 complemetation groups used for testing were discovered by these complementation tests. Ten of the nineteen mutants exhibited the prototype phenotype of their complementation group and were not studied further; these mutants were missed in the first screening for cell cycle mutants either because they did not grow well and, hence, were not examined or because of an oversight. Nine of the nineteen mutants did not exhibit the prototype phenotype and these nine constitute the third class of atypical mutants. Three of the nine mutants (436, *cdc* 9–4; 446, *cdc* 9–11; 399, *cdc* 9–12), when arrested at the restrictive temperature, did have greater than 50% but less than 80% of the cells displaying the prototype phenotype; they were not studied further. The other six mutants (296, *cdc* 1–5; 161, *cdc* 3–4; 201, *cdc* 3–5; 141, *cdc* 8–5; 261, *cdc* 11–5; and 305, *cdc* 14–2) arrest at the restrictive temperature as an asynchronous cell population with a wide distribution of bud sizes and a variety of nuclear morphologies.

For reasons outlined above, it was important to determine whether the atypical phenotype of these mutants was due to a second epistatic temperature-sensitive mutation in these strains or an alternative phenotypic expression of the *cdc* gene. First, the cellular phenotypes of heteroallelic diploids resulting from the mating of an α *cdc* tester strain with the atypical mutant were examined after growth at the restrictive temperature. In all cases, the heteroallelic diploid exhibited the prototype cellular phenotype. The nuclear morphologies were not examined. This result suggests, but does not prove, that the atypical strains are double mutants with the non-*cdc* lesion's being epistatic in the haploid and recessive in the heteroallelic diploid.

Random spore analyses were undertaken on diploids resulting from the mating of these atypical strains with a non-temperature-sensitive strain. The progeny spores were tested for temperature sensitivity, for complementation with the appropriate *cdc* tester strains, and for cellular morphology at the restrictive temperature (Table 6). In all cases, the presence of a second, non-*cdc*, temperature-sensitive mutation was evident by the fact that a significant proportion of the progeny were temperature-sensitive, yet did complement with the appropriate *cdc* tester strains. Furthermore, the presence of a temperature-sensitive muta-

tion which produced the prototype phenotype was also evident by the fact that some of the temperature-sensitive progeny, which failed to complement with the *cdc* tester strain, exhibited the prototype phenotype. Some of the temperature-sensitive progeny which failed to complement with the *cdc* tester strain did not exhibit the prototype phenotype, and these are probably the expected double mutant progeny.

We conclude, therefore, that within the limits of this investigation all temperature-sensitive mutations in a particular *cdc* gene produce the prototype phenotype.

Mapping of the cdc *genes:* One of the approaches for establishing that the 32 complementation groups into which the cell cycle mutants were grouped represented unique genes was to determine the genetic map positions of mutants from the different groups. Fourteen of these complementation groups, *cdc 1*, *2*, and *4* to *15*, were included in another mapping study (MORTIMER and HAWTHORNE, in preparation) and some of the results which will be presented are from this study. In Table 2 are presented the numbers of first and second division segregations for each of the genes *cdc 1* to *cdc 31*. As discussed previously, the strain carrying the mutation that represents *cdc 32* had diploidized and was not analyzed further. Of the 31 mutants, 10 show significant centromere linkage—i.e. a second division segregation (SDS) frequency which is significantly less than two-thirds. Strains carrying these ten mutations, *cdc 4*, *5*, *7*, *8*, *10*, *11*, *14*, *15*, *16*, and *29* were crossed to a set of strains that collectively carried genetic markers near the centromers of each of the 16 already established chromosomes (HAWTHORNE and MORTIMER 1968). Tetrad analysis of these crosses revealed that each of these ten genes was linked to a gene near the centromere of one of these chromosomes (Table 7). The assignment of these centromere-linked cell cycle mutants to the various chromosomes, based on the above results, is as follows: CHR I—*cdc 15*, CHR III—*cdc 10*, CHR IV—*cdc 7*, CHR VI—*cdc 4* and *cdc 14*, CHR IX—*cdc 29*, CHR X—*cdc 8* and *cdc 11*, CHR XI—*cdc 16*, and CHR XIII—*cdc 5*.

To determine the specific location of these centromere-linked genes on their

TABLE 7

Tetrad data demonstrating linkage of cell-division-cycle mutants to centromere genes

Gene pair	Chromosome	Ascus type PD	NPD	T
cdc 15—ade 1	I	30	0	0
cdc 10—α	III	14	0	10
cdc 7—trp 1	IV	15	0	2
cdc 4—his 2	VI	15	2	21
cdc 14—his 2	VI	43	0	7
cdc 29—his 6	IX	29	0	7
cdc 8—ilv 3	X	21	0	20
cdc 11—ilv 3	X	26	1	32
cdc 16—met 14	XI	25	0	13
cdc 5—lys 7	XIII	20	3	32

TABLE 8

Tetrad data demonstrating linkage of cell-division-cycle mutants to miscellaneous genes

Gene pair	Chromosome	Ascus type PD	NPD	T
cdc 28—tyr 1	II	37	0	0
cdc 2—trp 1	IV	23	3	75
cdc 2—cdc 9	IV	20	1	24
cdc 4—SUP 11	VI	13	0	3
cdc 14—SUP 6	VI	46	0	4
cdc 12—pet 3	VIII	7	0	4
cdc 8—SUP 4	X	17	0	0
cdc 8—cdc 11	X	17	0	10

chromosomes, secondary crosses were made that included in heterozygous condition other genes whose map positions on these chromosomes were already known. Additionally, the remaining 21 mutants were screened for chance linkage to markers dispersed over the genome. Some of these mutants were initially screened by mitotic crossing over, trisomic, or random spore analyses, and any suggested linkages were then confirmed by tetrad analysis (MORTIMER and HAWTHORNE, in preparation). The tetrad analysis results are summarized in Table 8.

The genes *cdc 15* and *cdc 28* failed to recombine with *ade 1* and *tyr 1*, respectively, so their positions on CHR I and II are specified. Similarly, *cdc 10* failed to recombine with the centromere of CHR III and is thereby automatically located. The placement of *cdc 7*, *cdc 2*, and *cdc 9*, in that order, on the left arm of CHR IV involved a number of crosses and both meiotic and mitotic analysis (MORTIMER and HAWTHORNE, in preparation). On CHR VI, *cdc 4* has been located on the left arm and *cdc 14* on the right arm between *his 2* and *SUP 6*. The order of *cdc 12* and *pet 3* relative to the centromere of CHR VIII has not been determined. On the left arm of CHR IX, *cdc 29* has been located distal to *his 6* on the basis of asci in which there was an exchange between these genes that was associated with a first division segregation of *his 6*. Similarly, the two cell cycle mutants *cdc 8* and *cdc 11* have been placed distal to *ilv 3* on the right arm of CHR X in the order centromere—*ilv 3*—*cdc 8*—*cdc 11*. The mutant *cdc 8* fails to recombine with *SUP 4*, already mapped distal to *ilv 3*. On CHR XI, *cdc 16* has been positioned across the centromere from *met 14*, while *cdc 5* has been mapped on the left arm of CHR XIII, across the centromere from *lys 7*.

The crosses described in Table 4 were segregating for two cell cycle mutants as a result of two independent mutations in the original mutant strain. Two cases of linkage are suggested by these results: *cdc 1—cdc 17* and *cdc 4—cdc 14*. The latter linkage was expected because both genes are linked to *his 2* on CHR VI (Table 7). Random spore results (MORTIMER and HAWTHORNE, in preparation) suggest linkage of *cdc 1* to *can* 1 on the left arm of CHR V. Thus, *cdc 1* and *cdc 17* may be located on this chromosome. The map positions of the *cdc* genes are summarized in Figure 2.

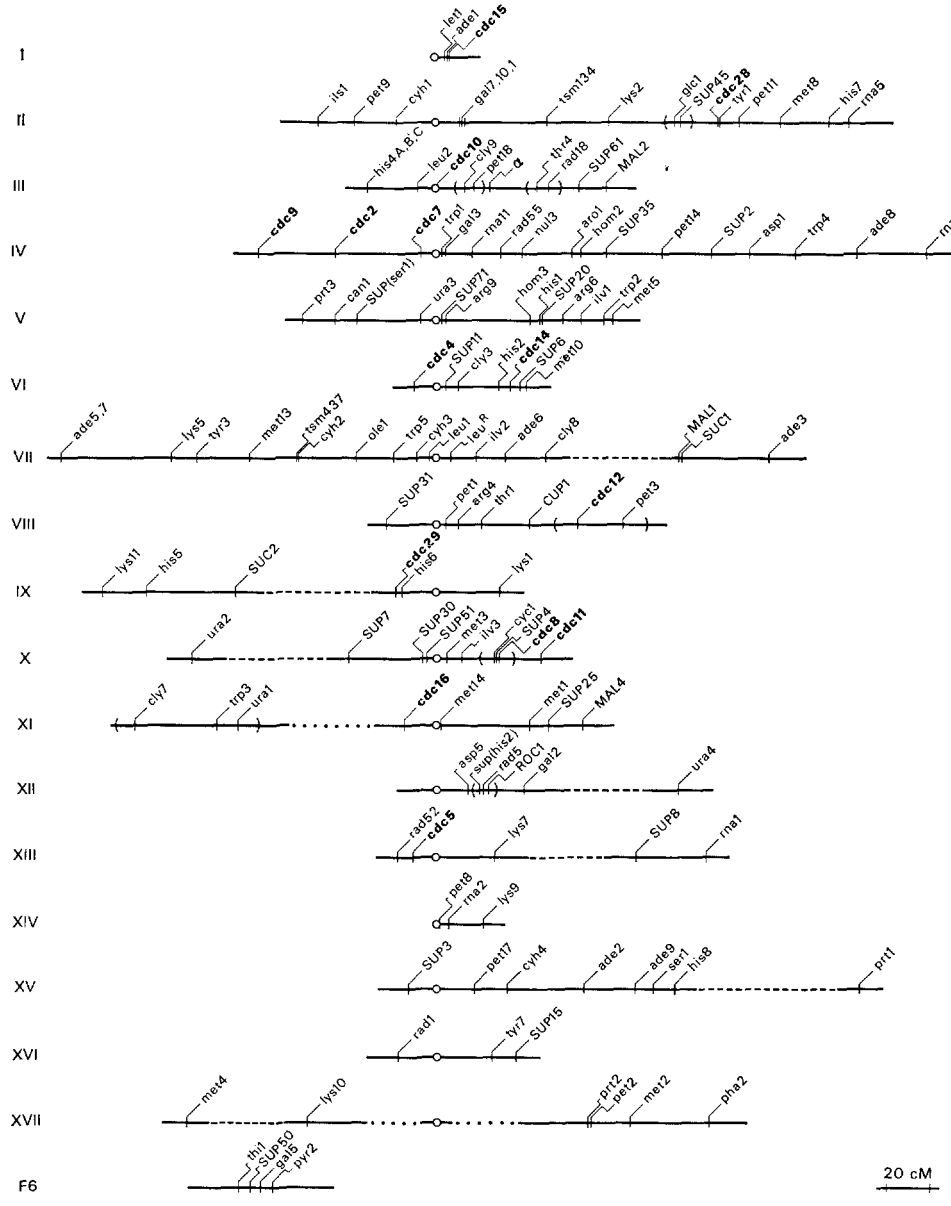

FIGURE 2.—The genetic map of *Saccharomyces cerevisiae* showing the positions of the *cdc* genes.

DISCUSSION

The budding habit of *Saccharomyces cerevisiae* facilitated the detection of temperature-sensitive mutants defective in specific steps of the cell division cycle by the simple technique of observing cellular morphologies following an interval of incubation at the restrictive temperature. Cell cycle mutants (*cdc*) are evident

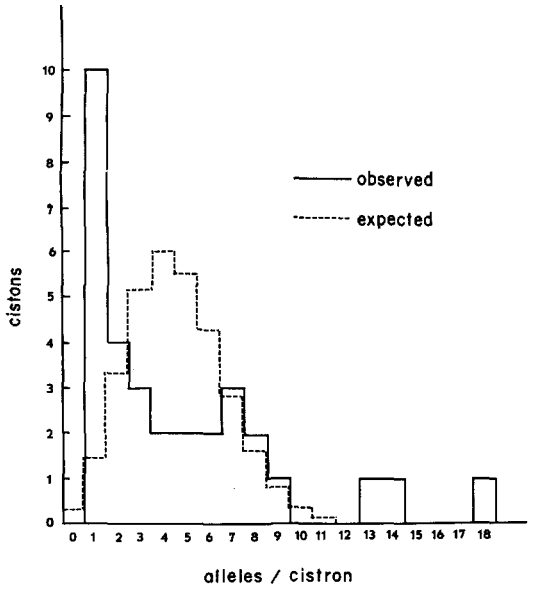

FIGURE 3.—The observed distribution of *cdc* alleles per cistron compared with the distribution of alleles per cistron expected on the basis of an average of 4.625 alleles per cistron from the Poisson distribution.

by the uniform and in some cases abnormal cellular morphologies attained by an asynchronous population of mutant cells following arrest of development at the restrictive temperature. The mutants were isolated in haploid cells of *a* mating type and genetic analysis demonstrated that these same gene products were essential for the mitotic cycle in haploids of α mating type or in a/α diploids. The fact that both haploid and diploid cells of *S. cerevisiae* undergo mitosis and call upon the same gene products to do so allowed the isolation of the recessive *cdc* mutants in haploid cells and their grouping by complementation in diploids.

An examination of approximately 1500 temperature-sensitive mutants revealed 148 *cdc* mutants that define 32 complementation groups. These results suggest that approximately 10% of the genes essential for vegetative growth function at specific steps of the cell cycle and that there are at least 32 such genes. If the distribution of mutations among genes were to fit a Poisson distribution, it would be possible to estimate the total number of *cdc* genes; but the fit is too poor to warrant such an estimate (Figure 3). It is clear, however, from the fact that there are an average of 4.6 mutations per complementation group that we have reached the stage of diminishing returns in attempting to identify new *cdc* genes by this screening procedure.

It is our expectation that many more than 32 genes are necessary for the complex sequence of events that comprises the cell division cycle of *S. cerevisiae* and indeed of all cells. Several possible explanations can be proposed for our inability to detect more than 32. It may be that although some gene products function at only one time during the cell cycle, a defect in one of these genes fails to arrest

the mutant cell with a morphology that is recognizable as a cell cycle arrest. Secondly, it is possible that some genes mutate to temperature sensitivity with a much lower frequency than other genes and that a more exhaustive search would uncover new *cdc* genes. Indeed, a comparison of the distribution of alleles per gene with that expected from a Poisson distribution suggests a wide distribution in the susceptibility of different genes to the occurrence of ts^- mutations (Figure 3). Thirdly, it may be that some genes are present in more than one copy per genome, and that a recessive mutation in any one of the redundant set of genes goes undetected.

The fact that most of the *cdc* genes contain more than one allele permitted a comparison of the phenotypes of mutant cells bearing different alleles of the same cistron. This comparison allows one to determine whether a particular phenotypic characteristic of a mutant is an allele-specific or cistron-specific character. After a thorough examination of most mutants that appeared to be atypical within a complementation group, it became clear that the cellular and nuclear morphologies of the mutant cells after arrest at the restrictive temperature were cistron-specific rather than allele-specific characteristics. The implication of this result is that each of these genes functions in one specific step of the cell division cycle and that the mutant phenotypes are the consequence of the failure of that gene product to perform its normal function. The hypothesis that a particular mutant phenotype is the result not of the inability of a gene product to perform its function, but rather, is due to an abnormal function performed by the mutant gene product, is essentially ruled out by this observation. The acquisition of an abnormal function by a mutant gene product would be expected to be highly allele-specific and not cistron-specific.

A second parameter that was compared among various alleles of the same complementation group was the number of cells that a mutant cell could produce following a shift from the permissive to the restrictive temperature before the arrest of development at the cell cycle block. In many of the mutants that have been examined, a mutant cell forms only one or two cells at the restrictive temperature before arrest. Furthermore, this response is correlated with the position of a cell in the cell division cycle at the time of the temperature shift, in that those cells that are early in the cycle arrest in the first cell cycle and those cells that are late in the cycle arrest in the second cell cycle. We interpret this observation to signify that at a particular time in the cell cycle, while the cell is growing at the permissive temperature, it completes the thermolabile event and acquires the capacity to complete the present cell cycle following a shift to the restrictive temperature. This time is called the execution point and has been calculated in fractions of a cell cycle for many of the mutants (Table 3). The execution points exhibited by a set of mutants appear to be cistron- and not allele-specific (Tables 3 and 5).

Other mutants produce more than two cells at the restrictive temperature. These mutants might be thermolabile for the synthesis (assembly) of the gene product or they might harbor leaky alterations in the function of the gene product. In either case, when such mutants have been found in a complementation

group, their existence suggests that the gene product is normally present in excess. If this is the case, then the finding of a mutant in the same complementation group that exhibits an execution point implies that the latter mutant is thermolabile for the function of the gene product. Thus, the execution points determined for genes *cdc* 1, 2, 14, 16, 17, 20, 21, and 23 (all of which have members exhibiting the two types of responses) may represent the time of function of the respective gene products.

It was of interest to determine whether different complementation groups represented distinct genes and whether genes that produce the same phenotype were closely linked. The mapping studies have provided some insight into both of these questions. Of the 31 cell cycle mutants analyzed, 14 have been assigned to specific locations on the genetic maps of *Saccharomyces cerevisiae* (Figure 2). Two others, *cdc 1* and *cdc 17* are tentatively located on CHR V. Most of the unmapped genes have been analyzed in only a preliminary fashion. While none of this latter group is closely linked to a centromere, some may show significant centromere linkage if studied further. No clear pattern emerges from the map positions of the cell cycle mutants. There are three possible "clusters," *cdc 7*, *cdc 2*, and *cdc 9* on CHR IV, *cdc 4* and *cdc 14* on CHR VI, and *cdc 8* and *cdc 11* on CHR X. However, the genes in these sets are not closely linked and the clustering most likely represents only chance associations. Of more interest is the close linkage of three of the cell-division cycle mutants to tyrosine-inserting ochre suppressors: *cdc 4—SUP 11*, *cdc 14—SUP 6*, and *cdc 8—SUP 4* (Table 8). However, the significance of these associations is not yet apparent. Beyond these linkages, the remaining cell division cycle mutants that have been mapped are dispersed over the genome.

In total with 32 complementation groups there are 496 possible cases of pairwise linkage. The mapping studies have ruled out 157 of these possibilities and have uncovered no case of linkage that is close enough to suggest that the two complementation groups are adjacent on the chromosome. We consider it likely, therefore, that these thirty-two complementation groups represent thirty-two distinct cistrons and that few if any of these cistrons are organized into operons with a common promotor. They may or may not be under common control.

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