THE SELECTION OF AMBER MUTATIONS IN GENES REQUIRED FOR COMPLETION OF START, THE CONTROLLING EVENT OF THE CELL DIVISION CYCLE OF *S. CEREVISIAE*

STEVEN I. REED*

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

Manuscript received October 23, 1979
Revised copy received March 14, 1980

ABSTRACT

Using a modification of a procedure developed for the isolation of temperature-sensitive mutants defective in the start event of cell division, amber mutations were obtained for two Class-I start genes, *cdc28* and *cdc37*. Genetic analysis demonstrated that co-segregation of an amber suppressor with such alleles was required for viability of spores subsequent to meiosis. These mutations are expected to be useful in the identification of the molecular products of the genes *cdc28* and *cdc37*.

THE cell division cycle of yeast, *Saccharomyces cerevisiae*, has been analyzed genetically by means of the isolation and characterization of temperature-sensitive mutants (HARTWELL *et al.* 1973). *cdc* (cell division cycle) mutants were identified by phenotypes indicative of stage-specific interruptions of the cell division cycle, suggesting that the latter consists of steps arranged in a dependent pathway, each requiring the expression of specific genes (HARTWELL *et al.* 1974). Subsequent genetic and physiological investigations on the organization of the yeast cell cycle have confirmed the dependent pathway hypothesis (HEREFORD and HARTWELL 1974; HARTWELL 1976). In the course of further analyzing the events of cell division, the identification of the macromolecular products of the *cdc* genes and the elucidation of their roles must be undertaken. A strategy that has been employed successfully in certain prokaryotic systems to match genes identified mutationally with the polypeptides encoded by them is the analysis of nonsense mutations (HENDRIX 1971; MURIALDO and SIMINOVITCH 1971; STUDIER 1972; HENDRIX and TSUI 1978). Premature translation termination and its resultant products (nonsense fragments) made conditional by the presence of suppressor tRNA species provide a direct means of identifying gene products. For this reason, nonsense alleles of cell division cycle genes were sought. Yeast has well-characterized amber and ochre suppressor systems (HAWTHORNE and LEUPOLD 1974; LIEBMAN, SHERMAN and STEWART 1976), a feature not shared by many eukaryotic organisms. The major difficulty confronting such an undertaking, however, is the intractability of nonsense suppression to short-term conditionality and the implications that this has for mutations in essential genes.

* Present address: Department of Biological Sciences, University of California, Santa Barbara, California 93106.

For cell division cycle genes, the problem is acute since cells cannot survive for long in the absence of gene function; yet, mutations must be identified and characterized precisely under these circumstances. I have devised a selection procedure for the isolation of nonsense alleles of a specific class of cell cycle genes known as Class-I start genes (see preceding article). These genes are thought to function in the first and controlling step in the cell division pathway, start, and a comprehensive program to elucidate the nature and physiological roles of their products, of which these studies are a part, has been initiated. The selection procedure utilizes a unique property of Class-I start mutants, specifically their ability to conjugate after absence of gene function has caused them to arrest in division. I report here the isolation of amber (UAG) mutations for two of the Class-I start genes, *cdc28* and *cdc37*.

MATERIALS AND METHODS

Strains and media: The genotypes of the strains employed are shown in Table 1. All media used were standard for yeast culture and have been previously described (HARTWELL 1967).

Genetic techniques: Techniques of genetic analysis were as described in MORTIMER and HAWTHORNE (1969).

Strategy for the isolation of mutants: The rationale employed here for the isolation of nonsense Class-I start mutations is analogous to that presented in the preceding article for the isolation of temperature-sensitive mutations. Briefly, selection begins with a strain (Figure 1, A) bearing a cell cycle mutation known to confer an inability to conjugate after preincubation at the restrictive temperature. Secondary mutations are superimposed by chemical mutagenesis (Figure 1, B) and those (Class-I start mutants) that specifically cause cells to arrest at the step in the cell cycle where they can conjugate, rather than at the infertile *cdc* arrest point, can be selected (Figure 1, D and E). *cdc15* has been substituted for the *cdc7* mutation previously

TABLE 1

Genotypes of strains

Strain designation	Genotype
SR607–1	*mata, trp1–1†, met8–1†, ile1, ilv2, cdc15–1*
SR611–1	*matα, trp1–1, met8–1, met2, leu2, ade1, cdc28–1*
SR664–1	*mata, trp1–1, met8–1, arg4, aro1, hom2, cdc15–1*
SR656–1	*matα, trp1–1, met8–1, leu2, ade1, cdc37–1*
SRas9–3	*mata, trp1–1, met8–1, met2, ile1, ade1, SUPA1*, cdc28–21*
SRas1–1	*mata, trp1–1, met8–1, leu2, ilv2, ade1, SUPA1*, cdc28–22*
SR653–1	*matα, trp1–1, met8–1, tyr1, ile1, cdc28–4*
SRas18–1–2	*matα, trp1–1, met8–1, aro1, hom2, ade1, SUPA4*, cdc37–11*
SRas14–1–2	*matα, trp1–1, met8–1, arg4, leu2, aro1, hom2, ade1, SUP6*, cdc37–12*
SR675–1	*mata, trp1–1, ade1, leu2, cdc37–2*
SRas12–3–1	*mata, trp1–1, met8–1, aro1, hom2, arg4, ade1, cdc37–2*

* Suppressors specified in these genotypes are Class-X amber suppressors (HAWTHORNE and LEUPOLD 1974) that were acquired independently by spontaneous mutation and subsequent selection on medium requiring suppression of known amber mutations for growth. The numerical designations given the suppressors in the genotypes above are arbitrary and merely for the purpose of indexing within the context of this work. Except in the case of *SUPA1*, which could be identified in several isolates because of tight linkage to *ilv2*, numerical designations do not imply identity or lack of identity of these amber suppressors with respect to one another.

† *trp1–1* and *met8–1* are both amber alleles (HAWTHORNE and LEUPOLD 1974).

Figure 1.

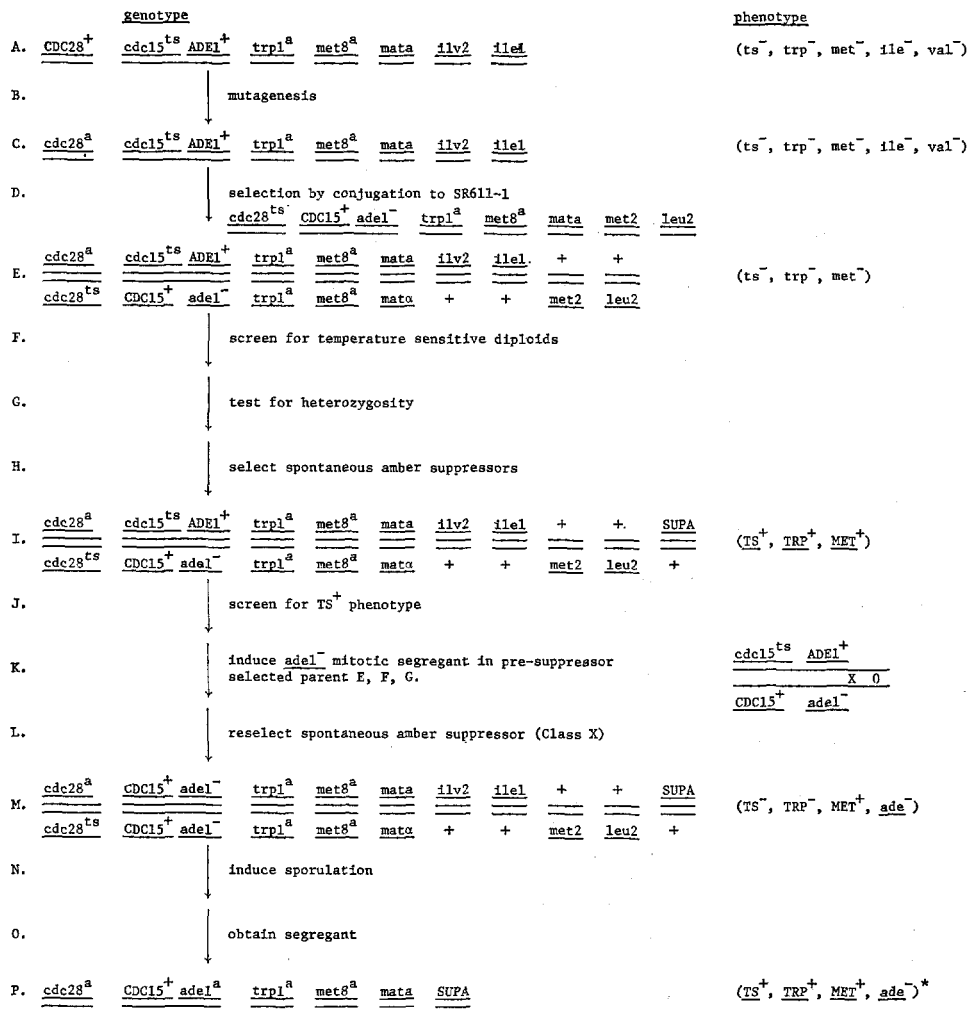

FIGURE 1.—Summary of procedure for selection of amber alleles of *cdc28*. The diagram provides the genotypes and phenotypes consistent with an amber allele had it resulted from the mutagenesis at the first step and been followed through the entire procedure. Underlined phenotypic characteristics are those that distinguish the appropriate strain at a critical point in the isolation procedure and therefore are used for screening. The superscript (a) designates an amber allele.

**SUPA* was found to be recessive in its ability to suppress *trp1-1* and *cdc28a* at 38°.

employed (REED 1980). The chromosomal location of *cdc15* facilitates its removal at a subsequent step (Figure 1, K). After mutagenesis, cells are allowed a total of only 2 generation equivalents to recover at the permissive temperature and to incubate at the restrictive temperature prior to demanding that they conjugate. The rapid succession of mutagenesis and conjugation is necessary in this case because the nonsense mutations sought cannot be made conditional on the time scale required for selection. Termination of division is expected upon exhaustion of premutagenesis excess of the product of the mutagenized gene. Conveniently,

the termination phenotype of the mutations sought (cell cycle arrest to start) is amenable to selection.

As outlined above, this is a general procedure for the selection of Class-I start mutations, quite analogous to that presented in the preceding article (REED 1980). An analytical strategy however, has been incorporated through the inclusion of diagnostic mutations in the starting strains of the procedure that permits the efficient detection of amber mutations in previously identified Class-I start genes by simple screening procedures. Predetermination of the gene for which mutant alleles are sought, or "targeting," is achieved by inclusion of a temperature-sensitive mutation in a known Class-I start gene in the other parental strain of the selection (Figure 1, D). A new defect in the particular gene carried by the mutagenized parent, then, is signalled by temperature sensitivity of the resultant diploid (Figure 1, E). One need screen only diploid progeny of the conjugation selection for temperature sensitivity to obtain what, in theory, should be a heterogeneous set of mutant alleles of a specific target gene, including the amber alleles being sought.

Amber mutations were identified among the new uncharacterized alleles of the target gene after selection of nonsense suppressors of the known amber mutations, *trp1–1* and *met8–1*, by requiring growth in the absence of methionine and tryptophen (Figure 1, H). Two amber mutations each were identified for *cdc28* and *cdc37*.

Isolation of mutations incapable of complementing cdc28–1 (see Figure 1): Ten ml of a culture of strain SR607–1 (Figure 1, A: *mata*, *trp1–1*, *met8–1*, *ile1*, *leu2*, *cdc15–1*) was grown to stationary phase on supplemented minimal medium and then chemically mutagenized by treatment with ethyl methanesulfonate (EMS), as described in the preceding article (REED 1980). Immediately after inactivation of residual EMS by incubation with sodium thiosulfate (10 ml), 1 ml aliquots of mutagenized cells (Fig.1, C) were diluted 20-fold into rich medium (YM-1), allowed to recover from EMS treatment for 3 hr at 23° and then incubated at 36° for an additional 3 hr. Approximately 3×10^6 cells from each of these cultures were mixed with an equal number of cells of strain SR611–1 (Figure 1, D: *matα*, *trp1–1*, *met8–1*, *met2*, *leu2*, *ade1*, *cdc28–1*) and allowed to conjugate on millipore filters, as described in the preceding article except that the 35° incubation was carried out for 3 instead of 4 hr. Each conjugation filter was prepared in duplicate, and at the end of the 3-hr incubation, duplicate filters were placed together in 250 ml Erlenmeyer flasks each containing 100 ml of minimal medium supplemented with tryptophan and methionine. Cultures were allowed to grow to stationary phase at 23° and were then stored at 4° prior to screening.

Selected diploids (Figure 1, E) were screened for temperature sensitivity (Figure 1, F) by dispersing cells on plates containing minimal medium supplemented with tryptophan and methionine and replica plating at 38°. By this procedure, temperature-sensitive colonies were found (in this experiment) to make up between 1 and 2% of the diploid population.

A concern with this scheme is that since only a short interval is possible between mutagenesis and conjugation, an induction of mitotic recombination caused by the mutagen (ROMAN 1973) might result in homozygosis of the temperature-sensitive allele of the parent strain. Homozygous temperature-sensitive segregants, however, are distinguishable from heterozygotes because they are incapable of yielding wild-type mitotic segregants when treated with a recombinogen. Therefore, in order to eliminate *cdc28–1* homozygotes induced by treatment with EMS, all temperature-sensitive isolates were transferred to YEPD medium by streaking with a toothpick, allowed to grow into streaks and then replica-plated to 2 plates, 1 of which was immediately subjected to a 6000 rad X-ray treatment to induce mitotic recombination. After 4 hr, both replicas were placed at 38° and incubated for approximately 2 days. The appearance of wild-type papillations on an X-rayed replica indicated heterozygosity at the *cdc28* locus. Strains unable to produce papillations under these conditions were discarded (approximately 90% of those tested). The remaining 10% were assumed to be heterozygotes containing new alleles (Figure 1, G).

Isolation of mutations incapable of complementing cdc37–1: The techniques employed above were used except that strain SR664–1 (*mata*, *trp1–1*, *met8–1*, *hom2*, *aro1*, *arg4*, *cdc15–1*) was

treated with EMS and conjugated to strain SR656-1 (*mata*, *trp1-1*, *met8-1*, *leu2*, *ade1*, *cdc37-1*). In this experiment, only 0.1 to 0.2% of selected diploids were found to be temperature sensitive.

Identification of amber mutations: Cultures of temperature-sensitive diploid strains found to be heterozygous at the *cdc28* or *cdc37* locus were grown to stationary phase in rich medium. Approximately 2×10^8 cells were dispersed per plate of methionineless medium. Usually 5 to 100 colonies appeared in several days. These, assumed to contain amber suppressors resulting from spontaneous mutation, were replica-plated to tryptophanless medium and complete medium at 38°. Co-reversion of methionine and tryptophan auxotrophies was considered evidence of the presence of an amber suppressor in the strain (Figure 1, H and I). Loss of temperature sensitivity in such strains as indicated by growth at 38°, strongly suggested the presence of a suppressible allele of *cdc28* or *cdc37*, depending on the experiment (Figure 1, J).

Isolation of haploid segregants containing suppressible alleles: After identifying strains thought to contain amber mutations, genetic analysis had to be performed for verification. The presence of 2 temperature-sensitive mutations in each diploid strain rendered such analysis needlessly complex and, since the *cdc15-1* mutation was now superfluous, eliminating it would be advantageous. This could be accomplished by inducing mitotic recombination to obtain segregants (Figure 1, K) homozygous for the wild type allele of *cdc15*. A mutation in *ade1*, a locus tightly linked to *cdc15* on chromosome *I* (HARTWELL *et al.* 1973), was included on the arm to be rendered homozygous. Adenine auxotrophy could then serve as an indicator for the event.

0.5 ml of stationary culture of the pre-suppressor containing diploid isolate was subjected to 6000 rad of X rays in a glass petri dish, diluted to 5 ml (10-fold) in rich medium and allowed to grow back to stationary phase at 23°. Cells were dispersed on plates containing rich medium to give 200 colonies per plate, and these were allowed to incubate until stationary. Pink colonies and sectors, indicating adenine auxotrophy, were considered to have arisen by mitotic recombination on chromosome *I*. In a control experiment, 80% of the adenine auxotrophs were found to have lost the *cdc15 mutation* (data not shown).

Spontaneous suppressors were then selected in the induced adenine auxotrophic strains, as described above (Figure 1, L). In theory, it should have been possible to observe the meiotic segregation pattern of the selected amber suppressors and the putative amber Class-I start alleles by inducing the screened diploid isolates to sporulate and performing tetrad analysis at this stage. Several complications arose in the present study that precluded this direct analysis. For both the *cdc28* and *cdc37* projects, the temperature-sensitive alleles employed in the selection (*cdc28-1* and *cdc37-1*) were leaky at temperatures lower than 38°. However, it was found that, of the spontaneous amber suppressors isolated, those capable of suppressing the new alleles at 38° presented problems when genetic analysis was undertaken. For *cdc28*, segregation of these suppressors in meiosis was always linked to lethality. These results suggested that the suppressor isolated in these cases was *SUP61*, known to be haplolethal (HAWTHORNE and LEUPOLD 1974). Similar results were obtained in the *cdc37* experiment except that, in addition, a second class of suppressors was also effective at 38°. These conferred a petite phenotype (respiratory deficient) and prevented sporulation altogether. Such properties have been attributed to the Class-IX amber suppressors (HAWTHORNE and LEUPOLD 1974). However, it was found that diploids containing suppressors that were capable of suppressing *met8-1* but that did not confer the ability to grow at 38°, could be induced to sporulate. Presumably, these are the Class-X amber suppressors that have been shown to be less efficient, but also less detrimental to sporulation (HAWTHORNE and LEUPOLD 1974). Recessive suppression of specific alleles has been described for such weak suppressors (HAWTHORNE and LEUPOLD 1974), and it was hoped that such was the case for these *cdc28* ambers at the stressfully high growth temperature (38°). Colonies (Figure 1, M) that could grow on methionineless medium, but that showed no or poor growth on tryptophanless medium and no growth at 38° (considered to have Class-X amber suppressors), were induced to sporulate (Figure 1, N) by plating 0.2 ml of stationary liquid culture on potassium acetate plates (1% potassium acetate, 0.25% yeast extract, 0.1% dextrose). After approximately 1 week, segregants (Figure 1, O and P) containing putative suppressible alleles of *cdc28* and *cdc37* were obtained from asci containing 4 viable spores, 2 of which were temperature sensitive for division and lacked the amber suppressor. The latter 2 spores must

not contain the putative amber *cdc* lesion or they would be inviable; hence, the remaining 2 temperature-insensitive and amber-suppressor-bearing spores must contain the amber *cdc* lesion.

Genetic analysis of suppressible alleles: Segregants obtained as described above were crossed to more tractable temperature-sensitive strains for comprehensive segregation studies. Tetrad analysis was performed as has been described (MORTIMER and HAWTHORNE 1969) (see Tables 2 through 5). It was found that the suppressors previously isolated were capable of suppressing the *cdc* amber mutations in haploid segregants at 36°, the restrictive temperature for the temperature-sensitive alleles used. Thus, segregation at the *cdc28* or *cdc37* locus was directly scorable.

Screening for temperature-sensitive alleles of cdc37: Temperature-sensitive *cdc37* heterozygotes obtained as described above were screened for new temperature-sensitive alleles by subjecting 0.5 ml of stationary liquid culture to 6000 rad of X rays, diluting 10-fold in rich medium, regrowing to stationary phase, plating to give 100 colonies per plate and then replica-plating to phenylalanineless and threonineless medium. Phenylalanine auxotrophs were screened for threonine auxotrophy. The appearance of such double auxotrophs was considered evidence of a new temperature-sensitive allele (for reasons described in RESULTS. Strains that yielded them were induced to sporulate and subjected to tetrad analysis to confirm this and to obtain haploid segregants.

RESULTS

Segregation analysis of new Class-I start alleles confirms that they contain amber mutations: The segregation behavior of suppressible Class-I start alleles and selected amber suppressors was examined by tetrad analysis (MORTIMER and HAWTHORNE 1969). Second generation crosses were made between amber-containing segregants and appropriate temperature-sensitive *cdc* strains containing the most tractable alleles available. The results for two suppressible alleles each of *cdc28* and *cdc37* are shown in Tables 2 through 5, respectively. The results conform well to theoretical expectation. For segregation of suppressor and start loci, parental ditypes usually gave four viable spores, tetratypes gave three and nonparental ditypes gave two. In most cases, spore mortality is ac-

TABLE 2

Segregation of cdc28–21 *and* SUPA1*

Ascus type†	Number	Viability (spores per ascus) 4	3	2	1	Fraction mortality attributable to lack of co-segregation	Fraction viable *cdc28–21* segregants also containing *SUPA1*
Parental ditype	3	3	0	0	0	none	1.0 (6/6)
Nonparental ditype	3	0	0	3	0	1.00	none
Tetratype	15	0	14	1	0	0.94	1.0 (15/15)
Indeterminate‡	1	0	0	0	1	—	—

* Tetrad analysis performed as in MORTIMER and HAWTHORNE (1969). Strains crossed were SRas9–3 and SR653–1.

† Ascus type was assigned on the basis of the segregation of the *SUPA1* mutation, which could be determined by suppression of homozygous suppressible nutritional markers in the cross, and the segregation of *cdc28–21*, which could be determined by temperature insensitivity, as well as by segregation of the closely linked heterozygous *tyr1* marker.

‡ Tetrads scored as indeterminate lacked sufficient information to be assigned. For example, an ascus containing two viable spores, one of each parental type, could have resulted from either parental ditype or tetratype segregation.

TABLE 3

Segregation of cdc28–22 *and* SUPA1*

Ascus type†	Number	Viability (spores per ascus) 4	3	2	1	Fraction mortality attributable to lack of co-segregation	Fraction viable *cdc28–22* segregants also containing *SUPA1*	
Parental ditype	2	1	0	1	0	0	1.0	(4/4)
Nonparental ditype	1	0	0	1	0	1.00	none	
Tetratype	9	0	7	2	0	0.82	1.0	(8/8)
Indeterminate	7	0	0	2	5	—	—	

* Tetrad analysis performed as in MORTIMER and HAWTHORNE (1969). Strains crossed were SRas1–1 and SR653–1.

† Ascus type was assigned on the basis of the segregation of the *SUPA1* mutation, which could be determined by suppression of homozygous suppressible nutritional markers in the cross, and the segregation of *cdc28–22*, which could be determined by temperature insensitivity, as well as by segregation of the closely linked heterozygous *tyr1* marker.

TABLE 4

Segregation of cdc37–11 *and* SUPA4*

Ascus type†	Number	Viability (spores per ascus) 4	3	2	1	Fraction mortality attributable to lack of co-segregation	Fraction viable *cdc37–11* segregants also containing *SUPA4*	
Parental ditype	2	2	0	0	0	none	1.0	(4/4)
Nonparental ditype	2	0	0	2	0	1.0	none	
Tetratype	10	0	6	4	0	0.71	1.0	(9/9)
Indeterminate	4	0	0	1	3	—	—	

* Tetrad analysis performed as in MORTIMER and HAWTHORNE (1969). Strains crossed were SRas18–1–2 and SR675–1.

† Ascus type was assigned on the basis of the segregation of the *SUPA4* mutation, which could be determined by suppression of homozygous suppressible nutritional markers in the cross, and the segregation of *cdc37–11*, which could be determined by temperature insensitivity, as well as by segregation of the closely linked heterozygous *hom2* marker.

TABLE 5

Segregation of cdc37–12 *and* SUPA6*

Ascus type†	Number	Viability (spores per ascus) 4	3	2	1	Fraction mortality attributable to lack of co-segregation	Fraction viable *cdc37–12* segregants also containing *SUPA6*	
Parental ditype	4	3	1	0	0	0	1.0	(7/7)
Nonparental ditype	2	0	0	2	0	1.0	none	
Tetratype	8	0	4	4	0	0.67	1.0	(6/6)
Indeterminate	5	0	0	1	4	—	—	

* Tetrad analysis performed as in MORTIMER and HAWTHORNE (1969). Strains crossed were SRas14–1–2 and SR675–1.

† Ascus type was assigned on the basis of the segregation of the *SUPA6* mutation, which could be determined by suppression of homozygous suppressible nutritional markers in the cross, and the segregation of *cdc37–12*, which could be determined by temperature insensitivity, as well as by segregation of the closely linked heterozygous *hom2* marker.

counted for by failure of the suppressible Class-I start allele and the amber suppressor to co-segregate. Deviations from this are attributable to somewhat reduced spore viabilities in all crosses containing amber suppressors (REED, unpublished).

The identification of new temperature-sensitive alleles: The procedure for isolating amber mutations employs a screening system designed to identify these from a pool of mutations in a target gene. It is conceivable that the isolation of other classes of mutations from this pool might be advantageous. A case in point is *cdc37*, for which only one temperature-sensitive allele had been previously isolated. Additional alleles of this gene, therefore, were sought. Uncharacterized alleles of *cdc37* were rendered homozygous by treating heterozygous diploid strains with X rays. The region of chromosome *IV* containing *cdc37* is marked by two centromere-proximal genes, *aro1* and *hom2*, the latter being only two meiotic map units from *cdc37*. A mitotic crossover proximal to *aro1* results in homozygosis for *aro1*, *hom2* and *cdc37* and is detected by the acquisition of auxotrophy for phenylalanine and threonine. Such homozygotes should be viable only if the *cdc37* locus contains a new temperature-sensitive allele, since all other types of mutation would be absolute under the conditions of the experiment and would therefore deprive the segregant of an essential function. The heteroallelic diploid strain (equivalent to Figure 1, I) was known to be heteroallelic and not just homoallelic for the input temperature-sensitive allele because it gave temperature-insensitive cells after X-ray-induced mitotic recombination (MATERIALS AND METHODS). When such a viable double auxotroph was found in the course of screening and the parent strain was induced to sporulate, all meiotic segregants were found to be temperature sensitive, and a new allele (*cdc37–2*) was obtained; *cdc37–2* is more temperature sensitive than *cdc37–1* and has proven useful in genetic and physiological studies.

DISCUSSION

Nonsense mutations can play a significant role in the identification of gene products and ultimately in their characterization. In bacteriophage systems, use of nonsense alleles has been one of the primary approaches for assigning genes identified by mutational analysis to polypeptides induced upon infection (HENDRIX 1971; MURIALDO and SIMINOVITCH 1971; STUDIER 1972). Simply, correlations between nonsense mutations in specific genes and loss of specific polypeptide bands from SDS polyacrylamide gel patterns have been used to establish correspondence between phage genes and gene products. Recently, a host gene product essential for virion assembly in several phage systems, the *Escherichia coli groE* protein, has been identified using a similar approach in conjunction with recombinant DNA techniques (HENDRIX and TSUI 1978). Both wild-type and amber *groE* genes were isolated on recombinant lambda phages, and polypeptide analysis performed during the course of infections by these phages identified the gene product. Current advances in yeast recombinant DNA technology, notably the development of procedures for introducing DNA sequences directly into yeast cells by transformation (HINNEN, HICKS and FINK

1978; BEGGS 1978), now permit the isolation of virtually any yeast gene on recombinant plasmids (NASMYTH and REED 1980; V. WILLIAMSON, personal communication). The availability of nonsense mutations for genes so isolated should provide a direct means of identifying their products. Messenger RNA species from wild-type and amber strains could be purified by complementarity to the cloned gene, and *in vitro* translation products could then be subjected to SDS polyacrylamide gel analysis. The isolation of *cdc28* and *cdc37* on recombinant plasmids has already been achieved (NASMYTH and REED 1979; REED, unpublished) and with the nonsense alleles now available for these genes, the strategy outlined above should prove feasible.

Finally, it is hoped that in addition to their intended roles in the identification of gene products, the amber mutations reported here will prove useful in the investigation of gene function. Critical to this endeavor is the discovery of a means of making suppression more amenable to conditional manipulation. A temperature-sensitive suppressor has been reported (RASSE-MESSENGUY and FINK 1973) but has not proven useful with the alleles reported here due to its inefficiency, even at the permissive temperature. It is probable that the constraints inherent in tRNA structure preclude the isolation of a truly effective temperature-sensitive suppressor. Other possible avenues to conditional suppression are currently being explored.

I would like to acknowledge the support of American Cancer Society Postdoctoral Fellowship #PF-1218 and Public Health Service Grant GM17709 awarded to LELAND H. HARTWELL. I would like also to thank TRESA NAUJACK for excellent technical assistance in initial phases of this work and LELAND HARTWELL for numerous valuable discussions during the course of this work and for a critical reading of this manuscript.

LITERATURE CITED

BEGGS, J. D., 1978 Transformation of yeast by a replicating hybrid plasmid. Nature **275**: 104–108.

HARTWELL, L. H., 1967 Macromolecule synthesis in temperature sensitive mutants of yeast. J. Bacteriol. **93**: 1662–1670. ——, 1976 Sequential function of gene products relative to DNA synthesis in the yeast cell cycle. J. Mol. Biol. **104**: 803–817.

HARTWELL, L. H., J. CULOTTI, J. R. PRINGLE and B. J. REID, 1974 Genetic control of the cell division cycle in yeast: a model. Science **163**: 46–51.

HARTWELL, L. H., R. K. MORTIMER, J. CULOTTI and M. CULOTTI, 1973 Genetic control of the cell division cycle in yeast. V. Genetic analysis of *cdc* mutants. Genetics **74**: 267–286.

HAWTHORNE, D. C. and U. LEUPOLD, 1974 Suppressor mutations in yeast. Curr. Top. Microbiol. Immunol. **64**: 1–47.

HENDRIX, R. W., 1971 Identification of proteins coded in phage lambda. pp. 355–370. In: *The Bacteriophage Lambda.* Edited by A. D. HERSHEY. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

HENDRIX, R. W. and L. TSUI, 1978 Role of the host in virus assembly: cloning of the *Escherichia coli groE* gene and identification of its protein product. Proc. Natl. Acad. Sci U.S. **75**: 136–139.

HEREFORD, L. M. and L. H. HARTWELL, 1974 Sequential gene function in the initiation of *S. cerevisiae* DNA synthesis. J. Mol. Biol. **84**: 445–461.

HINNEN, A., J. B. HICKS and G. H. FINK, 1978 Transformation of yeast. Proc. Natl. Acad. Sci. U.S. **75**: 1929–1933.

LIEBMAN, S. W., F. SHERMAN and J. W. STEWART, 1976 Isolation and characterization of amber suppressors in yeast. Genetics **82**: 251–272.

MORTIMER, R. K. and D. C. HAWTHORNE, 1969 Yeast Genetics. pp. 385–460. In: *The Yeast*, Vol. I. Edited by A. A. ROSE and J. S. HARRISON. Academic Press, New York.

MURIALDO, H. and L. SIMINOVITCH, 1971 The morphogenesis of bacteriophage lambda. III. Identification of genes specifying morphogenetic proteins. pp. 711–723. In: *The Bacteriophage Lambda*. Edited by A. D. HERSHEY. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

NASMYTH, K. and S. REED, 1980 The isolation of genes by complementation in yeast: The molecular cloning of a cell cycle gene. Proc. Natl. Acad. Sci. U.S. (in press).

RASSE-MESSENGUY, F. and G. R. FINK, 1973 Temperature-sensitive nonsense suppressors in yeast. Genetics **75**: 459–464.

REED, S., 1980 The selection of *S. cerevisiae* mutants defective in the start event of cell division. Genetics **95**: 561–577.

ROMAN, H., 1973 Studies of recombination in yeast. Stadler Symp. **5**: 35–48.

STUDIER, F. W., 1972 Bacteriophage T7. Science **176**: 367–376.

Corresponding editor: E. JONES