Genetic Analysis of *Saccharomyces cerevisiae* Chromosome *I*: On the Role of Mutagen Specificity in Delimiting the Set of Genes Identifiable Using Temperature-Sensitive-Lethal Mutations

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ABSTRACT

In a previous attempt to identify as many as possible of the essential genes on *Saccharomyces cerevisiae* chromosome *I*, temperature-sensitive (Ts^-) lethal mutations that had been induced by ethyl methanesulfonate or nitrosoguanidine were analyzed. Thirty-two independently isolated mutations that mapped to chromosome *I* identified only three complementation groups, all of which had been known previously. In contrast, molecular analyses of segments of the chromosome have suggested the presence of numerous additional essential genes. In order to assess the degree to which problems of mutagen specificity had limited the set of genes detected using Ts^- lethal mutations, we isolated a new set of such mutations after mutagenesis with UV or nitrogen mustard. Surprisingly, of 21 independently isolated mutations that mapped to chromosome *I*, 17 were again in the same three complementation groups as identified previously, and two of the remaining four mutations were apparently in a known gene involved in cysteine biosynthesis. Of the remaining two mutations, one was in one of the essential genes identified in the molecular analyses, and the other was too leaky to be mapped. These results suggest that only a minority of the essential genes in yeast can be identified using Ts^- lethal mutations, regardless of the mutagen used, and thus emphasize the need to use multiple genetic strategies in the investigation of cellular processes.

CHROMOSOME *I* of *Saccharomyces cerevisiae* has provided a vivid example of the "gene number paradox" (KABACK *et al.* 1984; PRINGLE 1987). That is, although the chromosome appears to contain >100 transcribed regions, a significant fraction of which are essential (DIEHL and PRINGLE 1991; D. KABACK, personal communication), only ~15 genes have been identified by classical genetic analyses of all types (MORTIMER *et al.* 1989). Moreover, an intensive effort to identify essential genes using temperature-sensitive (Ts^-) lethal mutations found only three complementation groups, all of which were known previously (KABACK *et al.* 1984).

One factor that might be contributing to the discrepancy between classical and molecular genetic analyses in this system is mutagen specificity. The 32 mutations analyzed by KABACK *et al.* (1984) were all induced by ethyl methanesulfonate (EMS) or *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NG). These mutagens are known to induce predominantly G:C to A:T transitions and to exhibit pronounced hotspots and coldspots for mutagenesis (PRAKASH and SHERMAN 1973; MILLER 1983; LÖRINCZ and REED 1986; KOHALMI and KUNZ 1988; G. DAS and F. SHERMAN, personal communication). Conceivably, for many of the essential genes, no one of the mutations that is induced with appreciable frequency by these mutagens suffices to produce a Ts^- gene product. To explore this possibility, we recapitulated the study of KABACK *et al.* (1984) but used UV and nitrogen mustard as mutagens. As the available evidence indicates that the specificity of these mutagens is quite different from that of EMS and NG (MILLER 1983; HAMPSEY, DAS and SHERMAN 1986; KUNZ *et al.* 1987; LEE *et al.* 1988; KUNZ and MIS 1989), we anticipated that additional essential genes would be identified. To our surprise, nearly all of the mutations obtained again fell into the same few genes.

MATERIALS AND METHODS

Strains, media and growth conditions: The principal strains used in this study are described in Table 1. Cells were grown routinely on rich medium, either YM-P liquid medium (LILLIE and PRINGLE 1980) or YEPD solid medium (SHERMAN, FINK and HICKS 1986). Media used for the analysis of auxotrophic markers were as previously described (SHERMAN, FINK and HICKS 1986). When necessary, media were supplemented with cysteine at 30 mg/liter. Liquid cultures were grown in test tubes on a roller drum. Permissive and restrictive temperatures for growth of Ts^- mutants were 21–22° and 36–37°, respectively.

Mutagenesis and isolation of mutants: All mutants were isolated in strain X1221a-7C, which is monosomic for chromosome *I* (BRUENN and MORTIMER 1970; KABACK *et al.* 1984). For UV mutagenesis, cultures were grown in YM-P to a cell density of 4–8 × 10^7 cells/ml, then diluted with YM-P and spread on YEPD plates at a density of approximately 200 cells per plate. These plates were immediately subjected to mutagenesis using a germicidal UV lamp (Ultra-Violet Products, Inc., San Gabriel, California) at a distance of 12 cm for 24 sec. Preliminary experiments demonstrated

TABLE 1

***S. cerevisiae* strains used in this study**

Strain	Relevant genotype[a]	Source/Reference
X1221a-7C	**a**/α *ade1/0 arg4/+ leu1/+ trp1/+*	BRUENN and MORTIMER (1970)
X1221a-7C-2A	**a** *ade1*	This study[b]
X3402-15C	**a** *ADE1/ADE1 ura3 leu1*	MORTIMER and HAWTHORNE (1973)
C276-4A	**a** (prototrophic)	WILKINSON and PRINGLE (1974)
C276-4B	α (prototrophic)	WILKINSON and PRINGLE (1974)
DC5	**a** *his3 leu2*	BROACH, STRATHERN and HICKS (1979)
JPDC5D-1A	α *his3 leu2*	JOHNSON *et al.* (1987)
X2928-3D-1C	α *ade1 ura3 his2 leu1 met14 trp1 gal1*	YGSC[c]
17017	**a** *cdc15-1*	HARTWELL *et al.* (1973)
JPT161BD1-1A	**a** *cdc15-28*	This study[d]
JPT19	**a** *cdc24-4*	SLOAT, ADAMS and PRINGLE (1981)
Y128	α *cdc24-1 leu2*	A. BENDER
Y135	**a** *cdc24-1 leu2*	A. BENDER
Y143	α *cdc24-4 leu2*	A. BENDER
Y148	**a** *cdc24-4 leu2*	A. BENDER
DK144-22D	**a** *pyk1-101*[e] *ade1*	KABACK *et al.* (1984)
RW1105	**a** *mak16-1 ade1*	WICKNER and LEIBOWITZ (1979)
VG42	**a**/α *fun24::ARG4/+ ade1/+ arg4/arg4*	D. KABACK
S554	α *tsv115 ade1 arg4*	This study[f]
S519	α *tsv115 ade1 trp1*	This study[f]
S519BD1-25A	**a** *tsv115*	This study[g]
BD2631T5-1A	**a** *fun11::URA3 ura3 leu2 trp1*	B. DIEHL[h]
S290BD1-8A	α *tsv11 ade1 ura3*	This study[i]

[a] Some strains have nutritional markers other than those listed here.
[b] A haploid segregant isolated by tetrad dissection of X1221a-7C.
[c] Yeast Genetics Stock Center, Berkeley, California 94720.
[d] Segregant from the cross of JPT161 (KIM, HAARER and PRINGLE 1991) to C276-4B.
[e] *pyk1-101* was initially referred to as *tsb35*.
[f] Segregant from a cross of the original haploid *tsv115* isolate (see text) to C276-4A.
[g] Segregant from a cross of S519 to C276-4A.
[h] Contains a *URA3* insertion in the *FUN11* transcribed region. See DIEHL and PRINGLE (1991) for further details on the construction of this strain.
[i] A segregant from a cross of the original haploid *tsv11* isolate (see text) to BD2631T5-1A.

that this dosage yielded 70–80% viable cells for strain X1221a-7C and 15–25% viable cells for its haploid derivative X1221a-7C-2A. For mutagenesis with nitrogen mustard, cells from a stationary-phase culture in YM-P were washed and resuspended in the same volume of phosphate-glucose buffer (0.2 M sodium phosphate, 0.11 M glucose, pH 8), then treated with 100 μg/ml of nitrogen mustard (Aldrich Chemical Co., Milwaukee, Wisconsin) for 30 min at 25°. Nitrogen mustard was dissolved in phosphate-glucose buffer immediately before use. After mutagenic activity was terminated by diluting the cells 10-fold into 6% sodium thiosulfate, the cells were washed and diluted further in YM-P and spread onto YEPD plates at a density of approximately 200 cells per plate. Preliminary experiments demonstrated that this dosage caused no detectable loss of viability for strain X1221a-7C while yielding 50% viable cells for its haploid derivative X1221a-7C-2A.

Following mutagenesis, plates were incubated at 21–22° (in the dark for UV mutagenesis experiments) until colonies formed. Temperature-sensitive clones were identified by replica plating to YEPD plates at 36° and verified by streaking onto YEPD plates at 22° and 36°. Haploids carrying the Ts^- mutations were recovered by sporulating these clones and dissecting tetrads. UV-induced and nitrogen mustard-induced mutants were given *tsv* and *tsn* isolation numbers, respectively.

Genetic methods: Complementation tests, linkage analyses and "disome exclusion mapping" were performed as described previously (KABACK *et al.* 1984; SHERMAN, FINK and HICKS 1986). All mutants were crossed at least once (to strain C276–4A, C276–4B, DC5 or JPDC5D-1A) prior to further genetic analysis. Some mutants displaying poor spore viability were subjected to additional crosses (using the same strains) until good spore viability was obtained.

The *fun24* rescue experiment was based on the observation that spores containing a lethal disruption of *FUN24* undergo three to eight divisions before dying (V. GAUCCI and D. KABACK, personal communication). A diploid heterozygous for the *FUN24* disruption (VG42) was sporulated and tetrads were dissected. A single cell from a *MATα arg4* strain carrying *tsv115* (S554) was then placed adjacent to each spore on the dissection slab in order to allow mating of the *MAT***a** spores to occur. As the disruption is marked by *ARG4*, the only viable Arg^+ clones that could be present were the diploids generated by mating between a dying spore and an S554 cell. If all the Arg^+ colonies were Ts^-, then the *tsv115* mutation must be in *FUN24*. Alternatively, if the Arg^+ colonies were uniformly Ts^+, then *tsv115* must not be in *FUN24*.

RESULTS

We searched for UV-induced Ts^- lethal mutations on chromosome *I* in the expectation of identifying genes not found when EMS or NG was used as mutagen (KABACK *et al.* 1984). Approximately 10^6 mutagenized clones of the monosomic strain were

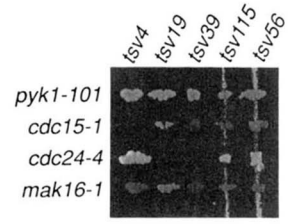

FIGURE 1.—Complementation behavior of strains carrying chromosome *I* mutations. The following strains were used as testers (see also Table 1): JPT19 (*cdc24–4*), 17017 (*cdc15–1*), DK144–22D (*pyk1–101*), and RW1105 (*mak16–1*). The grid was constructed by stamping from lawns onto a YEPD plate using sawed-off tongue depressors. This plate was incubated for 1 day at 23°, then replicated to a second YEPD plate, which was then incubated for 2 days at 36° before being photographed. Note the leakiness of the *tsv115* and *tsv56* strains, as demonstrated by the residual growth of the haploid cells at 36°.

screened (see MATERIALS AND METHODS), and 135 independently isolated mutants capable of growth at 22° but not at 36° were isolated. Of this group, 97 mutants sporulated and yielded viable segregants. The presumably haploid segregants from 54 of these mutants exhibited excessive leakiness and/or reversion (see DISCUSSION) and were not analyzed further. The segregants from the majority of the remaining 43 mutants were uniformly Ts^-. In some cases, the segregants from a particular mutant ranged in phenotype from clear-cut Ts^- to leaky and/or extensively reverting. However, in subsequent crosses of appropriate segregants, each of these mutants segregated 2:2 for temperature sensitivity, indicating that a single mutation was responsible for the original Ts^- phenotype. Each of the 43 Ts^- mutations was recessive, as the heterozygous diploids constructed for the crosses all grew at 36°.

Screening of approximately 5×10^4 nitrogen mustard-mutagenized clones yielded two additional independently isolated Ts^- lethal mutants. The viable haploid segregants from each of these mutants were uniformly Ts^-, and subsequent crosses demonstrated that the Ts^- mutations segregated 2:2 and were recessive in both cases.

Complementation analyses (Figure 1) indicated that 13 of the 45 mutations (including one of the two nitrogen mustard-induced mutations) were in *CDC24* and four were in *CDC15*; these are two of the previously known essential genes on chromosome *I* that had also been identified repeatedly using EMS-induced or NG-induced Ts^- lethal mutations. This conclusion was confirmed by linkage analyses. All of the putative *cdc24* mutations tested exhibited tight linkage to the *cdc24–1* and *cdc24–4* markers (Table 2), whereas the four putative *cdc15* mutations were all tightly linked to *ade1* (Table 2).

In the course of analyzing the crosses of the mutants to C276–4A and C276–4B, we observed that the Ts^- segregants from two of the remaining 28 mutants (*tsv11* and *tsv33*) were incapable of growing on defined media at permissive temperature unless it was supplemented with cysteine. Further investigation revealed that the Ts^- defect on rich medium could also be corrected for most *tsv11* and *tsv33* segregants by the addition of cysteine. *tsv11* and *tsv33* failed to complement each other at 36° and tetrad analysis revealed no Ts^+ segregants among 48 viable spores from 12 tetrads. Crosses to a disomic strain demonstrated that both mutations are on chromosome *I* (Table 3). Genetic mapping (Table 4) was consistent with these mutations' being in either *CYS1* or *CYS3*, two linked genes on chromosome *I* whose products are involved in cysteine metabolism (ONO *et al.* 1984).

The disome exclusion test also indicated that two of the remaining 26 mutations (*tsv56* and *tsv115*) are on chromosome *I* (Table 3). Attempts to map the *tsv56* mutation to a specific locus on chromosome *I* have been subverted by the leakiness and reversion associated with this mutation. However, it is clear that *tsv56* is not tightly linked to *ade1*, and thus that it is distant from the centromere. In contrast, the *tsv115* mutation was easily scored in crosses and showed tight linkage to both *ade1* and *cdc15* as well as an almost complete lack of second division segregation as judged using the centromere marker *trp1* (Table 5). Thus *tsv115* is very tightly linked to the centromere of chromosome *I*.

This tight centromere linkage suggested that *tsv115* might be in *FUN24*, a transcribed region immediately adjacent to the centromere on the left arm of chromosome *I*, which had been identified and shown to be essential in recent molecular analyses (V. GAUCCI, H. Y. STEENSMA and D. KABACK, personal communication) but had not previously been identified in clas-

TABLE 2

Linkage analyses for new mutations in *CDC24* and *CDC15*

Mutation	Complementation group	Test marker: *cdc24*[a] PD	NPD	T	*ade1* PD	NPD	T
tsv1	*cdc24*	24	0	0		ND	
tsv19	*cdc24*	23	0	0		ND	
tsv39	*cdc24*	19	0	0		ND	
tsv94	*cdc24*	29	0	1[b]		ND	
tsv113	*cdc24*	15	0	0		ND	
tsv136	*cdc24*	21	0	2[b]		ND	
tsv2	*cdc15*		ND		7	0	1
tsv4	*cdc15*		ND		7	0	0
tsv119	*cdc15*		ND		6	0	0
tsv123	*cdc15*		ND		9	0	1

Data were obtained from crosses of Ts^- *ade1 tsv* haploid segregants to tester strains Y128, Y135, Y143, Y148, C276-4A, or C276-4B (Table 1). Crosses to *cdc24-1* and *cdc24-4* testers yielded similar results, which are combined in the table.

[a] PD was 0 Ts^+:4 Ts^-, NPD was 2 Ts^+:2 Ts^-, and T was 1 Ts^+:3 Ts^-.

[b] These Ts^+ segregants appeared bona fide, suggesting an unusual amount of recombination within the *CDC24* gene (*cf.* COLEMAN *et al.* 1986).

TABLE 3

Disome exclusion tests for chromosome *I* linkage of new mutations

	No. of tetrads segregating					
	Ts^+:Ts^-			Ade^+:ade^-		
Mutation	4:0	3:1	2:2	4:0	3:1	2:2
tsv11	11	4	5	10	2	8
tsv33	5	3	2	7	0	3
tsv56	8	7	0	6	1	8
tsv115	5	0	7	5	1	6
tsv5	0	0	21	10	6	4
tsv35	0	0	23	16	4	3
tsv42[a]	0	1	22	12	7	4
tsv47[a]	1	0	9	7	1	2
tsv51[a]	1	5	18	15	4	5
tsv52	0	0	21	11	5	6
tsv55[a]	0	2	22	11	6	5
tsv62	0	0	9	6	0	3
tsv71[a]	0	1	15	10	6	1
tsv78	0	0	14	8	2	4
tsv89[a]	0	6	10	11	1	4
tsv97	0	0	14	9	0	5
tsv99	0	0	15	12	0	3
tsv105[a]	0	1	12	6	3	5
tsv106	0	0	11	6	2	3
tsv107	0	0	10	7	2	1
tsv112[a]	0	6	5	7	2	2
tsv116	0	0	9	6	2	1
tsv121	0	0	11	7	1	3
tsv126	0	0	11	6	2	3
tsv127	0	0	12	5	3	4
tsn1	0	0	8	4	0	6

Each mutation that complemented the previously known Ts^- lethal mutations on chromosome *I* was subjected to disome exclusion mapping (MORTIMER and HAWTHORNE 1973; KABACK *et al.* 1984) using strain X3402-15C. This strain is disomic for chromosome I and homozygous *ADE1/ADE1* (Table 1). As each strain to be tested was Ts^- *ade1*, the resulting trisomic strains yielded a mix of 4:0, 3:1, and 2:2 (Ade^+:ade^-) ratios for the segregation of *ADE1*. If the mutation of interest was also on chromosome *I*, a similar mix of ratios for Ts^+:Ts^- was observed. If the mutation was on another chromosome, ratios of 2 Ts^+:2 Ts^- predominated. Additional markers not located on chromosome I also segregated in these crosses and almost always yielded ratios of 2^+:2^-.

[a] Some mutations that appeared not to map to chromosome *I* but were leaky and prone to reversion yielded occasional tetrads segregating 4^+:0^- and 3^+:1^- for temperature sensitivity. Crosses of strains carrying these mutations to C276-4A (Table 1) were conducted in parallel to the crosses to the disomic strain, and also revealed occasional tetrads segregating 4^+:0^- and 3^+:1^- for the Ts^- mutation. Moreover, the extra "Ts^+" segregants did not grow as well at 36° as did the two apparently wild-type segregants per tetrad, suggesting that the former arose as a result of intragenic or extragenic suppression of the Ts^- mutations.

sical genetic analyses. To test this possibility, we attempted to rescue spores dying as a result of a lethal disruption of *FUN24* by mating them to cells carrying *tsv115* (see MATERIALS AND METHODS). A total of 14 Arg^+ diploid colonies were obtained and all were Ts^-, implying that *tsv115* and the *FUN24* disruption were noncomplementing mutations. Tetrads derived from two of these diploids segregated uniformly 2:2 for viability and all viable segregants were Ts^- and Arg^-.

TABLE 4

Linkage analysis for *tsv11*

Marker pair	Spore type[a] P	Spore type[a] R	Map distance[b] (cM)
tsv11-ade1	152	24	14
tsv11-fun11::URA3	128	48	27
ade1-fun11::URA3	114	62	35

Data were obtained from a cross of S290BD1-8A to BD2631T5-1A. The latter strain is marked by insertion of *URA3* in *FUN11*, a gene ~17 cM (11.5 kb) centromere-proximal to *CDC24* (COLEMAN *et al.* 1986; DIEHL and PRINGLE 1991) and thus ~38 cM from *CYS1/CYS3*, which is in turn ~11 cM from *ade1* (MORTIMER and SCHILD 1985). The dissected tetrads were analyzed on a random spore basis because of the poor spore viability associated with *tsv11*, which could not be corrected by growth of the dissected spores on YEPD supplemented with cysteine. The segregation of *tsv11* was monitored by scoring the Ts^- phenotype and the associated cysteine auxotrophy. Dropout plates used to score the *ade1* and *URA3* markers were supplemented with cysteine. This allowed us to identify unambiguously the Ade^- and Ura^- segregants.

[a] P, parental spore genotype; R, recombinant spore genotype.

[b] Map distances were calculated by the formula R/P + R.

Thus, *tsv115* is a temperature-sensitive mutation in *FUN24*.

For the remaining 24 mutants, the disome exclusion test indicated that the mutations were not on chromosome *I* (Table 3). Presumably, these recessive mutations had become hemizygous (by chromosome loss) or homozygous (by mitotic recombination) during growth of the mutagenized cells (see DISCUSSION).

DISCUSSION

In a previous study, 32 independently isolated Ts^- lethal mutations that mapped to chromosome *I* were found to fall into only three genes, all of which had been known previously (KABACK *et al.* 1984). In contrast, molecular analyses indicate that the chromosome contains numerous additional essential genes (DIEHL and PRINGLE 1991; D. KABACK, personal communication). Thus, we wished to explore the reasons for the identification of so few genes using Ts^- lethal mutations. It seemed possible that the specificity of the mutagens used imposed a major limitation. The mutations analyzed by KABACK *et al.* (1984) were all induced by EMS or NG, two mutagens that are known to induce predominantly (≥97% in yeast studies) G:C to A:T transitions and to exhibit pronounced hotspots for mutagenesis (PRAKASH and SHERMAN 1973; MILLER 1983; LÖRINCZ and REED 1986; KOHALMI and KUNZ 1988; G. DAS and F. SHERMAN, personal communication). The combined effect of these twin constraints would be to limit severely the amino acid substitutions that would be produced at an appreciable frequency in any given gene product. If none of these substitutions happened to be among the (possibly small) set that could render the gene product thermolabile over an appropriate temperature range, then no Ts^- mutations in the corresponding gene would

TABLE 5

Linkage analysis for *tsv115*

Cross	Marker pair	Tetrad type PD	NPD	T
S519 × C276-4A	*tsv115-ade1*	78	0	6
	tsv115-trp1	39	44	0
	ade1-trp1	37	39	6
S519BD1-25A × X2928-3D-1C	*tsv115-ade1*	133	1	17
	tsv115-trp1	66	84	1
	ade1-trp1	60	73	18
S519 × JPT161BD1-1A	*tsv115-ade1*	20	0	1
	tsv115-cdc15	17	0	4

ade1 and *cdc15* are on the right arm of chromosome *I* in the order *CENI-ade1-cdc15* (MORTIMER *et al.* 1989). *trp1* is very tightly linked to the centromere of chromosome *IV* (MORTIMER and SCHILD 1980); thus, any cross-overs between *tsv115* or *ade1* and *CENI* would be revealed as tetratypes for the *tsv115-trp1* or *ade1-trp1* marker pairs.

be found. If mutagen specificity were indeed a major constraint in the chromosome *I* studies, this would have important implications in other areas; for example, the large collections of Ts⁻ *cdc* and *sec* mutants were mostly obtained after mutagenesis with EMS or NG (HARTWELL *et al.* 1973; NOVICK, FIELD and SCHEKMAN 1980; PRINGLE 1987).

The constraints of mutagen specificity should be less severe when UV light is used as mutagen. Among 17 UV-induced missense mutations of *CYC1*, nine were G:C to A:T transitions, but six were A:T to G:C transitions and two were G:C to T:A transversions (HAMPSEY, DAS and SHERMAN 1986). Similarly, examination of the UV-induced mutations in a plasmid-borne *SUP4-o* gene in yeast revealed that while G:C to A:T transitions were the most common lesion (66% of total events), A:T to G:C transitions (19% of total events) and all four types of transversions (15% of total events) were also observed (KUNZ *et al.* 1987). Although hotspots for UV-induced mutations were detected at several sites in these studies, these hotspots did not correspond to those found in the same systems when EMS and NG were used as mutagens (KOHALMI and KUNZ 1988; G. DAS and F. SHERMAN, personal communication). In addition, transitions and transversions were found to occur with equal frequencies among UV-induced forward mutations at the *URA3* locus (LEE *et al.* 1988). Thus, the use of UV rather than EMS or NG would approximately quadruple the number of amino acid substitutions obtained with appreciable frequency, as well as altering their spatial distribution. Similarly, analysis of the spectrum of mutations induced by nitrogen mustard in the *SUP4-o* gene indicates that it also has a mutational specificity different from that of EMS or NG (KUNZ and MIS 1989). Thus, it seemed likely that mutagenesis by UV or nitrogen mustard would allow us to obtain Ts⁻ lethal mutations in other essential genes on chromosome *I*.

Our screen was conducted using a strain that is monosomic for chromosome *I*. As the vast majority of Ts⁻ lethal mutations are recessive (*e.g.*, 45/45 mutations analyzed in this study; *cf.* also HARTWELL *et al.* 1973; NOVICK, FIELD and SCHEKMAN 1980; KABACK *et al.* 1984), we anticipated that the majority of the mutants isolated would have mutations on this chromosome, as was in fact observed in the earlier study of mutations induced by EMS or NG (KABACK *et al.* 1984). However, of the 45 mutants that could be analyzed, only 21 had mutations mapping to chromosome *I*. Presumably, the other 24 mutations had become hemizygous through chromosome loss or homozygous through mitotic recombination; both types of events are induced by UV irradiation (PARRY *et al.* 1979; KUNZ and HAYNES 1981). We were also surprised by the high proportion of mutants (54 of the 99 that sporulated and yielded viable segregants) for which all of the presumably haploid segregants were substantially more leaky and/or prone to reversion than the monosomic strain from which they were derived. We speculate that in these mutants, the amount of active gene product remaining at restrictive temperature is sufficient to allow some continuing growth of the haploid cells but not of the larger cells of the near-diploid monosomic strain; this leakiness would then provide the opportunity for selection of intragenic or extragenic suppressors.

Contrary to our expectations, of the 21 mutations mapping to chromosome *I*, 17 were in two of the same genes that had been identified previously using EMS and NG. Moreover, two of the remaining mutations, while presenting some residual puzzles (*e.g.*, why not all mutant segregants are rescued by cysteine at restrictive temperature), appear to be in one of the known genes *CYS1* or *CYS3*. Thus, only two mutations were in newly defined genes, and only one of these was tight enough to analyze in detail; it proved to be in a transcribed region that had been identified and shown to be essential in parallel molecular studies by V. GAUCCI, Y. STEENSMA and D. KABACK (personal communication). As extrapolation from the available molecular data suggests that there are likely to be >20 essential genes on chromosome *I* (DIEHL and PRINGLE 1991), it appears that only a minority of the essential genes can be identified using Ts⁻ lethal mutations generated *in vivo*, even when mutagens with differing specificities are used.

Two factors may contribute to this situation. First, it seems clear that some proteins are inherently insusceptible to Ts⁻ mutations. The requirement that a protein retain more or less normal function at 23° while being more or less nonfunctional at 36° is rather stringent, and may sometimes be difficult to satisfy by replacing a single amino acid (HECHT, STURTEVANT and SAUER 1984; ALBER *et al.* 1988). In such a case, it might be possible to generate a thermolabile gene

product by replacing two or more amino acids, but the chances of detecting such mutants *in vivo* would be small, given the background of mutants involving single substitutions. Even with *in vitro* mutagenesis, where mutants involving multiple substitutions could presumably be obtained, some proteins have proved remarkably refractory to yielding Ts^- mutations (HAN *et al.* 1987; NAUMOVSKI and FRIEDBERG 1987; HUFFAKER, THOMAS and BOTSTEIN 1988; SCHATZ, SOLOMON and BOTSTEIN 1988). Second, there could be major constraints on *in vivo* mutagenesis and/or repair that would apply regardless of the mutagen used. For example, there is evidence for differential repair in the genomes of yeast, human, and Chinese hamster ovary cells (BOHR *et al.* 1985; MELLON *et al.* 1986; TERLETH *et al.* 1990); in particular, DNA sequences that are transcriptionally active appear to be more efficiently repaired in these systems (MELLON, SPIVAK and HANAWALT 1987; LEADON and SNOWDEN 1988; TERLETH *et al.* 1990). It is conceivable that the complex interactions between the different DNA repair pathways in yeast (GAME 1983) may result in particular regions of chromosome *I* being differentially susceptible to *in vivo* mutagenesis. It should be possible to assess the relative contributions of the two factors just discussed by mutagenizing some of the cloned essential genes *in vitro* and then screening for Ts^- mutations using the plasmid-shuffle technique (BOEKE *et al.* 1987). In this regard, it is of interest that REED and co-workers could obtain Ts^- mutations by *in vitro* mutagenesis of *CDC28* that were at sites different from the few sites at which *in vivo* generated mutations fell (LÖRINCZ and REED 1986).

Whatever the explanation(s), our results appear to have important implications for attempts to study various cellular processes genetically. In particular, it appears that the use of Ts^- mutants is unlikely to identify more than a minority of the nonredundant essential genes involved in any particular process. Thus, for example, the *cdc* mutations known to block particular steps in the cell cycle (HARTWELL *et al.* 1973; PRINGLE and HARTWELL 1981) and the *sec* mutations known to block particular steps in secretion (NOVICK, FIELD and SCHEKMAN 1980; SCHEKMAN 1985) are likely to be only the tips of their particular icebergs. Moreover, it seems unlikely that this situation will be easily remedied by using mutagens other than those used in the earlier studies (see also CROSBY and MEYEROWITZ 1986), although it remains possible that the use of other mutagens (DAS, STEWART and SHERMAN 1986), or perhaps of mutator strains (HOEKSTRA and MALONE 1987), would allow additional essential genes to be identified using Ts^- mutations. However, it seems more likely that emphasis should instead be placed on avoiding overreliance on Ts^- mutations. In this regard, it is important to note that other types of conditional mutations, such as cold-sensitive mutations (MOIR *et al.* 1982; A. HEALY, A. STAPLETON and J. PRINGLE, unpublished results), suppressible ochre mutations (RILES and OLSON 1987; K. CORRADO, A. FAREWELL and J. PRINGLE, unpublished results), and D_2O-sensitive mutations (BARTEL and VARSHAVSKY 1988) appear to identify sets of genes that overlap only partially with the set identified by Ts^- mutations.

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