TEMPERATURE-SENSITIVE LETHAL MUTATIONS ON YEAST CHROMOSOME *I* APPEAR TO DEFINE ONLY A SMALL NUMBER OF GENES

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ABSTRACT

A method was developed for isolating large numbers of mutations on chromosome *I* of the yeast *Saccharomyces cerevisiae*. A strain monosomic for chromosome *I* (*i.e.*, haploid for chromosome *I* and diploid for all other chromosomes) was mutagenized with either ethyl methanesulfonate or *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and screened for temperature-sensitive (Ts^-) mutants capable of growth on rich, glucose-containing medium at 25° but not at 37°. Recessive mutations induced on chromosome *I* are expressed, whereas those on the diploid chromosomes are usually not expressed because of the presence of wild-type alleles on the homologous chromosomes. Dominant *ts* mutations on all chromosomes should also be expressed, but these appeared rarely. —— Of the 41 *ts* mutations analyzed, 32 mapped on chromosome *I*. These 32 mutations fell into only three complementation groups, which proved to be the previously described genes *CDC15*, *CDC24* and *PYK1* (or *CDC19*). We recovered 16 or 17 independent mutations in *CDC15*, 12 independent mutations in *CDC24* and three independent mutations in *PYK1*. A fourth gene on chromosome *I*, *MAK16*, is known to be capable of giving rise to a *ts*-lethal allele, but we recovered no mutations in this gene. The remaining nine mutations isolated using the monosomic strain appeared not to map on chromosome *I* and were apparently expressed in the original mutants because they had become homozygous or hemizygous by mitotic recombination or chromosome loss. —— The available information about the size of chromosome *I* suggests that it should contain approximately 60–100 genes. However, our isolation in the monosomic strain of multiple, independent alleles of just three genes suggests that only a small proportion of the genes on chromosome *I* is easily mutable to give a Ts^--lethal phenotype. —— During these studies, we located *CDC24* on chromosome *I* and determined that it is centromere distal to *PYK1* on the left arm of the chromosome.

STUDIES of the amounts of DNA and of the numbers of genes in various eukaryotic cells have led to two related, but distinct, paradoxes. The first

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of these, the well-known "C value paradox" (CAVALIER-SMITH 1978, 1980; GALL 1981; PRESCOTT 1983), refers to the wide variations in C value (DNA content per haploid genome) among related organisms, to the frequent lack of any detectable correlation between the C values and the apparent phenotypic complexities of the organisms and to the general gross discrepancy between the C values observed and those required to account for the numbers of RNA and protein species estimated (by either genetic or molecular methods—see following data) to be encoded by the genomes. The second paradox, which is our concern in this paper, can be called the "gene number paradox." It has arisen in the course of attempts to answer the following questions: How many genes are there altogether in various eukaryotic genomes? How many of these genes encode functions essential for the survival and reproduction of individual cells of the organism in question? How many of these genes encode functions essential for cell differentiation, development or other functions at the level of the whole organism (in multicellular organisms)? The paradox is that the answers to these questions suggested by formal genetic (mutational) analyses are fourfold to tenfold lower than the answers suggested by molecular analyses.

The gene number paradox can be illustrated by data from a variety of sources. For example, many elegant genetic studies in Drosophila have suggested that this organism contains a total of about 5000 genes, or about one gene per band of the polytene chromosomes (JUDD, SHEN and KAUFMAN 1972; LEFEVRE 1974, 1981; SPRADLING and RUBIN 1981; RIPOLL and GARCIA-BELLIDO 1979; GAUSZ *et al.* 1981; NICKLAS and CLINE 1983), that most of these genes are essential for survival of the organism as a whole (LEFEVRE 1974, 1981; RIPOLL and GARCIA-BELLIDO 1979), but that only about 10–12% of these essential genes (or about 500–600 genes) are essential for the survival and reproduction of individual somatic cells (RIPOLL 1977; RIPOLL and GARCIA-BELLIDO 1979). Similarly, genetic studies in Caenorhabditis suggest a total of 2000–4000 essential genes in this nematode (BRENNER 1974; ROGALSKI, MOERMAN and BAILLIE 1982), and genetic analyses of development in Dictyostelium suggest that only about 300 genes are essential specifically for development in this slime mold (LOOMIS 1978). In addition, recent attempts to identify new *CDC* genes (genes whose products function in specific steps of the cell cycle) in Saccharomyces using temperature-sensitive-lethal (*ts*-lethal) mutations have yielded primarily mutants carrying alleles of already-known genes (PRINGLE 1981; PRINGLE and HARTWELL 1981); at first glance, this suggests that the 50 known *CDC* genes may be a majority of the total. These various genetic estimates give the impression that eukaryotic cells and organisms, and their constituent processes, are relatively simple in terms of the numbers of gene products involved.

In contrast, a very different impression is given by a variety of molecular studies at both the protein and nucleic acid levels. For example, surprisingly large numbers of polypeptides have been reported in such seemingly simple structures as the flagellar axoneme of Chlamydomonas (≥280 polypeptides: LUCK, HUANG and PIPERNO 1982; R. SEGAL and D. LUCK, personal commu-

nication) and the silk moth eggshell (≥186 polypeptides: REGIER, MAZUR and KAFATOS 1980). It is difficult to reconcile these numbers with the notion that only 500–600 gene products are necessary altogether for the survival and reproduction of a eukaryotic cell or that only 50–60 gene products are necessary for all of the specific steps of the cell cycle. Indeed, vegetatively growing yeast and Dictyostelium amoebas both appear to contain about 4000–5000 distinct mRNA species (HEREFORD and ROSBASH 1977; KABACK, ANGERER and DAVIDSON 1979; MANGIAROTTI *et al.* 1983); the macronuclear genome of the ciliate Oxytricha, which presumably contains only genes essential for vegetative growth, appears to contain about 24,000 distinct genes (PRESCOTT 1983); and a variety of arguments suggests strongly that the known yeast *CDC* genes are but a minority of the total (PRINGLE 1981). Moreover, an additional 2000–3000 mRNA species have been reported to appear during Dictyostelium development (MANGIAROTTI *et al.* 1983), in striking contrast to the genetic data cited earlier. The numbers of distinct sequences apparently utilized by the unicellular yeast, slime mold and ciliate are themselves difficult to reconcile with the genetic estimates of the total numbers of genes in the metazoans Drosophila and Caenorhabditis (see preceding data). Thus, it is not surprising to find that sea urchins appear to express at least 35,000 distinct mRNA species in the course of development (GALAU *et al.* 1976; LEE *et al.* 1980; DAVIDSON, HOUGH-EVANS and BRITTEN 1982), whereas adult mouse brain appears to express some 150,000 different mRNA species (VAN NESS, MAXWELL and HAHN 1979; OUELLETTE 1980). Indeed, recent studies of mRNA sequence complexity in Drosophila itself have apparently detected ≥17,000 distinct mRNA species (LEVY and MANNING 1981), whereas the spacing of transcribed regions in a variety of cloned Drosophila chromosome segments is much closer than that expected from a hypothesis of one gene per polytene-chromosome band (SPRADLING and RUBIN 1981; ISH-HOROWICZ *et al.* 1979; SIROTKIN and DAVIDSON 1982; GRIFFIN-SHEA, THIREOS and KAFATOS 1982; SINA and PELLEGRINI 1982; STORTI and SZWAST 1982; SNYDER and DAVIDSON 1983).

As resolution of the gene number paradox would have both philosophical significance and practical implications for research strategies, we have approached this paradox directly by a combined mutational and molecular analysis of chromosome *I* of the yeast *S. cerevisiae*. These studies are facilitated by the small size of this chromosome. It contains only about 100 cM of recombination distance between its two most distal known markers (MORTIMER and SCHILD 1980, 1982); this is about 1/50 of the total known recombination distance for the yeast genome. In addition, chromosome *I* appears to contain only about 200 ± 50 kilobase pairs (kbp) of DNA (D. SCHWARTZ, C. CANTOR, Y. STEENSMA and D. KABACK, unpublished results); this is about 1/70 of the total for the yeast haploid genome (LAUER, ROBERTS and KLOTZ 1977). In the work reported here, we have attempted to identify as many as possible of the "essential genes" (*i.e.*, genes necessary for vegetative growth on rich medium) on chromosome *I* by analyzing *ts*-lethal mutations that map to this chromosome. Isolation of such mutations was facilitated by use of a parent strain that is diploid for other chromosomes but haploid for chromosome *I*. As most *ts*-

lethal mutations are recessive, most Ts$^-$-lethal mutants isolated in this strain carry mutations on chromosome *I*. Genetic analysis of 32 independently isolated mutations revealed that they fell into only three genes. This number is much smaller than that expected given the size of the chromosome, the typical spacing of one transcribed region per 2–3 kb observed in yeast (HEREFORD and ROSBASH 1977; LAUER, ROBERTS and KLOTZ 1977; KABACK, ANGERER and DAVIDSON 1979; HEREFORD *et al.* 1979; SHALIT *et al.* 1981; ST. JOHN and DAVIS 1981; SHERMAN *et al.* 1983) and the common assumptions that most genes are essential and that most can be identified by *ts* mutations (EDGAR, DENHARDT and EPSTEIN 1964; HARTWELL 1967; PRINGLE 1975). Extrapolation to the whole genome would suggest that only some 250 genes were essential for vegetative growth in yeast. As this conclusion is almost certainly wrong (see DISCUSSION), the results appear to provide another vivid example of the gene number paradox but in a relatively simple and tractable system that should allow clarification of the reason(s) for the paradox (see DISCUSSION).

MATERIALS AND METHODS

Strains, media and growth conditions: The principal strains used as parents and/or testers in this study are listed in Table 1. All other strains used were derived from these using either standard procedures (SHERMAN, FINK and HICKS 1982) or the procedures described explicitly in this section. Cells were grown routinely on YEPD liquid (per liter: 10 g of Difco yeast extract + 20 g of Difco Bacto peptone + 20 g of glucose) or solid (20 g/liter agar added) medium. Selective media for analysis of auxotrophic markers were as described elsewhere (SHERMAN, FINK and HICKS 1982). Liquid cultures were incubated in flasks with vigorous rotary shaking or in test tubes in a roller drum. Permissive and restrictive growth temperatures for Ts$^-$ mutants were 24–25° and 36–37°, respectively. Some Ts$^+$ cultures were grown at 30° to achieve more rapid growth rates.

Mutagenesis and isolation of mutants: Mutants were isolated in strain X1221a-7C, a strain that appears to be monosomic for chromosome *I* (*i.e.*, haploid for chromosome *I* and diploid for all other chromosomes; see BRUENN and MORTIMER 1970; KABACK and HALVORSON 1977). For mutagenesis with ethyl methanesulfonate (EMS), cells from a 3-day-old stationary-phase culture in YEPD were washed and resuspended in sodium phosphate-glucose buffer (0.2 M phosphate, 0.11 M glucose, pH 8), then treated with 3% (v/v) EMS (Sigma) for 60 min at 25°. After mutagenic activity was terminated by diluting the cells 50-fold into 6% sodium thiosulfate, the cells were centrifuged, resuspended in water and plated on YEPD medium at 25°. Temperature-sensitive clones were detected by replica plating to YEPD plates at 37°, rechecked by streaking on YEPD at 25° and 37° and given *tsb* isolation numbers. The EMS treatment produced no detectable loss of viability in the monosomic strain. Because nonproliferating cells were mutagenized and plated with no intervening period of growth, all of the *tsb* mutations must be independent in origin.

Mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NG) was carried out under conditions that allowed the majority of monosomic cells to undergo sporulation and produce viable haploid spores. Stock solutions containing 2.5 mg/ml of NG (Sigma) in sterile distilled H_2O were prepared immediately before use. Cultures growing exponentially in YEPD medium at 30° (cell density $<2 \times 10^7$ cells/ml) received 2 μl of NG stock solution per ml of culture and were incubated an additional 25 min at 30°. The cultures were then diluted 10- to 100-fold with 6% sodium thiosulfate, diluted further with YEPD medium and spread on YEPD plates. Preliminary experiments had shown that this NG treatment yielded 75–85% viable cells for strain X1221a-7C and about 50% viable cells for its haploid derivative X1221a-7C-6B. Ts$^-$ mutants were then identified as described for the EMS-treated cultures and given *tsd* isolation numbers. Note that, because some of the cells in the population divided between the time of addition of NG and the time of plating, it was possible, although unlikely, for a single mutational event to have been represented in two different colonies, *i.e.*, the mutants recovered from the NG-treated population cannot all be assumed to be independent.

TABLE 1

S. cerevisiae *strains used in this study*

Strain	Relevant Genotype[a]	Source	Reference
X1221a-7C[b]	**a**/α *ade1/0 leu1/+ trp1/+*	J. BRUENN	KABACK and HALVORSON (1977)
X1221a-7C-6B	α *ade1*	This study[c]	
X3402-15C[d]	**a** *ADE1/ADE1 leu1 ura3*	R. MORTIMER	MORTIMER and HAWTHORNE (1973)
α ade5	α *ade5*	H. HALVORSON	
DK71-4C	**a** *ade5*	This study[e]	
C276-4A	**a** (prototrophic)	J. PRINGLE	WILKINSON and PRINGLE (1974)
C276-4B	α (prototrophic)	J. PRINGLE	WILKINSON and PRINGLE (1974)
17017	**a** *cdc15-1*	L. HARTWELL	HARTWELL *et al.* (1973)
H127-6-2	α *cdc15-1*	L. HARTWELL	HARTWELL *et al.* (1973)
DK25-4C	**a** *cdc15-1 ade1 lys2*	This study[f]	
DK25-5B	α *cdc15-1 ade1 ade2*	This study[f]	
JPT10	**a** *cdc15-9*	J. PRINGLE[g]	
JPTA1438	α *cdc15-10*	J. PRINGLE[g]	
5011-D6-J2D	**a** *cdc24-1 ade1 ade2 ura1*	J. PRINGLE	SLOAT, ADAMS and PRINGLE (1981)
5011-D6-J2A	α *cdc24-1 ade1 ade2 ura1*	J. PRINGLE	SLOAT, ADAMS and PRINGLE (1981)
JPT19	**a** *cdc24-4*	J. PRINGLE	SLOAT, ADAMS and PRINGLE (1981)
JPT19α	α *cdc24-4*	J. PRINGLE	SLOAT, ADAMS and PRINGLE (1981)
DK17-4B	**a** *cdc24-5 ura1*	This study[h]	
DK17-2B	α *cdc24-5 trp5*	This study[h]	
DK17-3A	**a** *cdc24-5 ade1*	This study[h]	
DK17-2C	α *cdc24-5 ade1*	This study[h]	
395	**a** *cdc19-1*[i] *ade1 ade2 ura1*	L. HARTWELL	HARTWELL *et al.* (1973)
LH395BD1-1A	α *cdc19-1*[i] *ade1 ura1*	This study[j]	
DK210-1A	**a** *cdc19-1*[i] *ade1 ade2 trp5*	This study[k]	
DK210-8A	α *cdc19-1*[i] *trp5*	This study[k]	
JW4-5C	**a** *cys1*	YGSC[l]	MORTIMER and SCHILD (1980)
POD17-5A	**a** *cdc24-4 cys1*	This study[m]	
POD18-71C	**a** *cdc19-1*[i] *cdc24-4*	This study[m]	
RW1105	**a** *mak16-1 ade1*	R. WICKNER	WICKNER and LEIBOWITZ (1979)
RW1770	α *mak16-1 ade1 trp1 leu2*	R. WICKNER	WICKNER and LEIBOWITZ (1979)
DK136-1B	**a** *ade1 tsb71*	This study[n]	See Table 3
DK136-7D	α *ade1 tsb71*	This study[n]	See Table 3
DK144-22D	**a** *ade1 tsb35*	This study[o]	See Tables 3 and 4
DK144-9D	α *ade1 tsb35*	This study[o]	See Tables 3 and 4
DBY746	α *leu2-3 leu2-112 trp1-289*	D. BOTSTEIN[p]	
DK303-8A	α *leu2-3 leu2-112 tsb35*	This study[q]	

[a] Some strains have nutritional markers other than those listed here.

[b] This strain appears to be monosomic for chromosome *I*; *i.e.*, haploid for this chromosome and diploid for all other chromosomes.

[c] A haploid segregant isolated by dissection of an ascus from X1221a-7C.

TABLE 1—Continued

[d] This strain appears to be disomic for chromosome *I*; *i.e.*, diploid for this chromosome and haploid for all other chromosomes.

[e] α *ade5* was crossed to Y185-21A, an **a** *his2* or *his8* segregant from Y185 (TINGLE, KÜENZI and HALVORSON 1974), and an **a** *ade5* His^+ segregant was named DK71-4C.

[f] Segregants from the cross of 17017 × DK8(Ade^+)-6B; the latter strain is itself a segregant from DK8(Ade^+) (KABACK, BHARGAVA and HALVORSON 1973).

[g] These strains were isolated from C276-4A and C276-4B, respectively, and shown to carry *cdc15* mutations in a recent screening of EMS-induced Ts-lethal mutants (A. ADAMS and J. PRINGLE, unpublished results).

[h] Segregants from strain DK17 (KABACK and HALVORSON 1978). Identification of the *ts* mutation in these strains as a *CDC24* allele is described in RESULTS.

[i] Although it now appears that *CDC19* should be known by the more informative name of *PYK1* (KAWASAKI 1979; PRINGLE and HARTWELL 1981; FRAENKEL 1982), this mutant allele is still referred to by its original *cdc19* designation (HARTWELL *et al.* 1973).

[j] A segregant from the cross of 395 × C276-4B.

[k] These segregants from the cross of 395 × DK17-2B were shown to be *cdc19 CDC24* by complementation.

[l] Yeast Genetics Stock Center, Berkeley, California 94720.

[m] POD17-5A is a segregant from the cross of JW4-5C × JPT19α. POD18-71C is a *CYS1* segregant from the cross of POD17-5A × LH395BD1-1A.

[n] Segregants from the cross of the original haploid *tsb71* isolate × DK71-4C.

[o] Segregants from the cross of α *ade5* × DK139-8A; the latter strain is itself a segregant from the cross of the original haploid *tsb35* isolate × α *ade5*.

[p] DBY746 is a segregant from the same tetrad as DBY747 (BOTSTEIN *et al.* 1979).

[q] A segregant from the cross of DK144-22D × DBY746.

Genetic methods: Standard methods were used for complementation and linkage analyses (SHERMAN, FINK and HICKS 1982). Complementation was usually tested by replicating *MAT***a** *vs.* *MAT*α grids from 25° to 37° on YEPD medium (*cf.* Figure 1), but in some cases the possibility of apparent noncomplementation because of poor mating was eliminated by testing the growth at 36°–37° of isolated diploid clones or of populations known to be diploid by virtue of the complementation of parental auxotrophic markers.

Meiotic linkage analyses were complicated by the poor spore viability observed in some cases when the Ts^- haploids derived from the monosomic mutants were used as parents in subsequent crosses. To overcome this problem, the Ts^- haploids were backcrossed one to three times against α ade5, DK71-4C, DK17-4B or DK17-2B (Table 1), until a diploid giving good spore viability was obtained. Appropriate haploid segregants were then used in the analysis of meiotic linkage as described in RESULTS.

For "disome exclusion mapping" (or "trisomic analysis," MORTIMER and HAWTHORNE 1973), haploid segregants (obtained as just described) carrying the *ts* mutations of interest and *ade1* were crossed to X3402-15C (Table 1), a strain disomic for chromosome *I* (*i.e.*, diploid for chromosome *I* and haploid for all other chromosomes) and homozygous for *ADE1*. Analysis of tetrads from the resulting trisomic strains yields a mix of 4:0, 3:1 and 2:2 (Ade^+:Ade^-) ratios for the segregation of *ADE1* and a corresponding mix of ratios for Ts^+:Ts^- if the mutation of interest is on chromosome *I*. If the mutation of interest is on another chromosome, ratios of 2 Ts^+:2 Ts^- are usually observed.

Transformation of *pyk1* (*cdc19*) mutants was carried out with minor modifications of the procedure described elsewhere (ITO *et al.* 1983), using treatment with 0.5 M LiCl and 10 μg of plasmid DNA. Plasmid YEp13-PYK1 (KAWASAKI and FRAENKEL 1982) was generously provided by G. KAWASAKI and D. FRAENKEL. Transformants were selected and analyzed as described in RESULTS.

RESULTS

Known essential genes on chromosome I: The gene *LET1*, which maps very near the centromere of chromosome *I*, is known from an amber-suppressible, re-

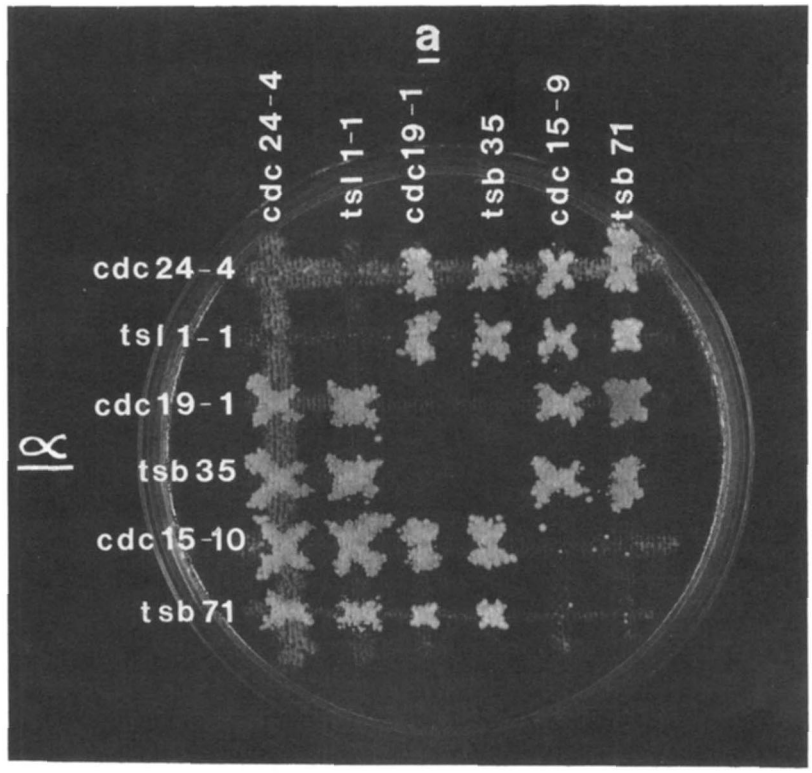

FIGURE 1.—Complementation behavior of strains carrying mutations on chromosome *I*. The strains used were as follows (see also Table 1): *cdc24-4*, JPT19 and JPT19α; *ts11-1* (*i.e.*, *cdc24-5*), DK17-4B and DK17-2B; *cdc19-1*, 395 and LH395BD1-1A; *tsb35*, DK144-22D and DK144-9D; *cdc15-9*, JPT10; *cdc15-10*, JPTA1438; *tsb71*, DK136-1B and DK136-7D. The grid was constructed by stamping from lawns using sawed-off tongue depressors, then incubated 2 days on YEPD at 24° before being replicated to YEPD at 36.5°. The photograph was taken after 2 days at 36.5°.

cessive-lethal allele (MORTIMER and HAWTHORNE 1973); to the best of our knowledge, no *ts*-lethal allele of this gene has been isolated. The genes *CDC15* (PRINGLE and HARTWELL 1981), *PYK1* (or *CDC19*; PRINGLE and HARTWELL 1981; FRAENKEL 1982) and *MAK16* (WICKNER and LEIBOWITZ 1979) also map on chromosome *I* (MORTIMER and SCHILD 1980) and give rise to *ts* mutations that are lethal on YEPD medium. In addition, two indendently isolated *ts*-lethal mutations in a fifth gene were isolated from strain X1221a-7C in a preliminary phase of this study and mapped to the vicinity of *PYK1* on chromosome *I* (KABACK and HALVORSON 1978; MORTIMER and SCHILD 1980). (These mutations were originally designated *tse* and *tsh*, and subsequently *ts11-1* and *ts11-2*, respectively.) Independently, efforts to map *CDC24*, a gene whose product is involved in the morphogenetic events of the cell cycle (PRINGLE and HARTWELL 1981; SLOAT, ADAMS and PRINGLE 1981), led to its localization near *PYK1* on chromosome *I* (see following data). The following data then demonstrated that *ts11-1* and *ts11-2* are, in fact, alleles of *CDC24*. (1) *ts11-1* and *ts11-2* neither complement nor recombine with each other (KABACK and

HALVORSON 1978). (2) *ts11-1* and *ts11-2* do not complement *cdc24-1* or *cdc24-4* testers, although all strains involved complement strains carrying other *ts* lethals on chromosome *I* (Figure 1). (3) Dissection of asci from the *ts11-1* × *cdc24-4* diploid yielded 24 complete tetrads, all of which were 0 Ts^+:4 Ts^- for growth at 36.5°. (4) Both *ts11-1* and *ts11-2* strains displayed the characteristic terminal cell morphology of *cdc24* mutants (SLOAT, ADAMS and PRINGLE 1981) after growth at 37°. Because four independently isolated *cdc24* mutants have been described previously (HARTWELL *et al.* 1973; SLOAT, ADAMS and PRINGLE 1981), the *ts11-1* and *ts11-2* mutations have been renamed *cdc24-5* and *cdc24-6*, respectively.

The map order of *PYK1* and *CDC24* relative to more centromere-proximal genes was determined by the crosses shown in Table 2. Together with previously published data (MORTIMER and SCHILD 1980, 1982; ROTHSTEIN and SHERMAN 1980), these data establish the map for chromosome *I* that is shown in Figure 2.

Isolation and identification of new ts*-lethal mutations mapping on chromosome* I: With the expectation of identifying additional essential genes on chromosome *I*, we undertook the isolation of *ts*-lethal mutations in a strain monosomic for chromosome *I* (see MATERIALS AND METHODS). After screening approximately 10^6 colonies from the EMS-treated population, 163 clones capable of growth on YEPD at 25°, but not at 37°, were isolated. Approximately 120 of these clones sporulated and yielded viable, presumably haploid spores, all of which were Ts^-; 47 of these mutants have been analyzed further. In 16 cases, ratios of 3:1 and 4:0 (Ts^+:Ts^-) were observed in subsequent crosses, suggesting that more than one mutation was responsible for the original Ts^- phenotype. These mutants were not analyzed further simply because of the difficulties of such an analysis. In one case (isolate *tsb23*), ratios of 1:3 and 0:4 (Ts^+:Ts^-) were observed in the subsequent cross, indicating the presence of two *ts*-lethal mutations in the original mutant (see following data), whereas in the remaining 30 cases, only ratios of 2 Ts^+:2 Ts^- were observed. These 31 mutants (32 *ts* mutations) were included in the genetic analyses described here.

After approximately 2.4×10^5 colonies from the NG-treated population were screened, 18 Ts^- clones were isolated; 17 of these clones sporulated and yielded at least one viable spore, but in ten cases, ratios of 3:1 and 4:0 (Ts^+:Ts^-) were observed in subsequent crosses. Only the remaining seven isolates, which consistently yielded ratios of 2 Ts^+:2 Ts^- in subsequent crosses, were included in the genetic analyses described here.

Prior to complementation analyses, the mutations of interest were tested for dominance by crossing to appropriate Ts^+ haploids. In all but one case, the mutations were recessive, since the heterozygous diploids grew normally at 37°. The one exceptional case was isolate *tsb68*, whose mutation appeared at least partially dominant in some, but not all, crosses to other haploids.

The complementation analyses indicated that 30 of the 39 mutations analyzed fell into three of the known essential genes that map on chromosome *I* (Figure 1; Table 3). One isolate (*tsb23*) appeared to carry mutations in two of these three genes. Additional evidence supporting the gene assignments sug-

TABLE 2

Linkage data establishing the order of chromosome I *markers*

Cross	Marker Pair	PD	NPD	T	Map distance (cM)
POD18-71C × RW1770[a]	*ade1* × *mak16*	27	2	33	37
	ade1 × *pyk1*	18	4	34	58
	ade1 × *cdc24*	16	2	44	49
	mak16 × *pyk1*	41	0	19	16
	pyk1 × *cdc24*	41	0	19	16
	mak16 × *cdc24*	26	0	40	30
	ade1 × *CEN1*	48		4[b]	4
	mak16 × *CEN1*	27		27	32
POD17-5A × LH395BD1-1A[c]	*ade1* × *cys1*	179	0	56	12
	ade1 × *pyk1*	60	14	173	58
	ade1 × *cdc24*	50	15	188	63
	cys1 × *pyk1*	67	11	144	51
	pyk1 × *cdc24*	196	0	45	9.4
	cys1 × *cdc24*	56	12	162	56
JW4-5C × RW1770[d]	*ade1* × *cys1*	22	0	6	11
	cys1 × *mak16*	15	1	11	32
	ade1 × *mak16*	12	2	19	50
	ade1 × *CEN1*	29		4[e]	6
	cys1 × *CEN1*	23		5[e]	9
	mak16 × *CEN1*	16		16	32

For each cross, diploids were obtained and sporulated. Asci were dissected by conventional techniques. The haploid parents are described in Table 1. Abbreviations are PD, parental ditype; NPD, nonparental ditype; T, tetratype. For the linkage of markers to the chromosome *I* centromere (*CEN1*), the data given are for first-division segregations (FDS; under PD) and second-division segregations (SDS; under T); the segregation patterns for the centromere were deduced from the segregation patterns for *trp1*, *leu2* and *ade1*. Map distances were estimated from the tetrad data using Figures 2 and 3 of MORTIMER and SCHILD (1980). Apparently because of the presence in these crosses of the *cys1* mutation, of multiple *ts*-lethal mutations, or both, the frequency of uncertainties in scoring was somewhat higher than usual. Not only was it frequently difficult to distinguish *cys1* from *CYS1*, but *cys1* segregants often grew sufficiently poorly (even on cysteine-containing plates) that scoring of other markers (particularly the *ts* markers) was difficult. To minimize problems in the scoring of *cys1*, all relevant plates were scored independently by two observers before any of the other data were available. Moreover, each segregant was scored both in a comparison of a "complete" plate (containing minimal medium plus all commonly used nutritional supplements, including cysteine) to a "complete-minus-cysteine" plate and in a comparison of a "minimal + cysteine" plate to a "minimal" plate (with both of the minimal plates containing also a minimum set of other supplements relevant to the cross in question). Unequivocally better growth on the cysteine-containing plate in either comparison was regarded as sufficient to score a segregant as *cys1*. The comparison of complete plates usually, but not always, provided the more convincing scoring. To ensure that the residual uncertainties in the scoring of *cys1* and the other markers had not perturbed the conclusions drawn, all data from all crosses were assigned during the initial analyses to one of five reliability classes based both on our confidence in the particular tetrad as a whole (which was sometimes reduced by our having recovered only three spores or by one or more markers' having failed to segregate 2:2) and on our confidence in the scoring of the particular pair of markers under consideration. As the conclusions about map order were identical, and the map distances similar, regardless of which reliability classes were included in the analyses, data from all classes but that of lowest reliability have been combined for presentation in the Table and for estimation of map distances.

[a] The three *ts*-lethal markers in this cross were scored by complementation (see MATERIALS AND METHODS); scoring was clear in nearly all cases. *trp1*, *leu2* and *MAT* were also scored in most tetrads and segregated 2:2 with only a few exceptions. Analysis of individual tetrads from this

TABLE 2—Continued

cross strongly supports the map order of *MAK16-PYK1-CDC24* that is suggested by the estimated map distances. Excluding the 24 tetrads in which no crossovers were detected in the interval bounded by these three markers, and the six tetrads in which one of these markers (*pyk1* in each case) did not segregate 2:2, the available tetrads can be explained by one crossover in this interval (34 cases) or two crossovers in this interval (two cases, both three-strand doubles) if the order is *MAK16-PYK1-CDC24*. In contrast, to explain the same tetrads requires one crossover in the interval (17 cases) or two crossovers in the interval (19 cases, including 17 two-strand doubles and two three-strand doubles) if the order is either *PYK1-MAK16-CDC24* or *MAK16-CDC24-PYK1*. Similarly, both of the estimated map distances and the analysis of individual tetrads from this cross support the previously reported (MORTIMER and SCHILD 1980) map order of *ADE1-MAK16-PYK1*. With this order, the available tetrads can be explained by zero crossovers in the interval bounded by these three markers (16 cases), one crossover in this interval (29 cases) or two crossovers in this interval (12 cases, including three two-strand doubles, five three-strand doubles and four four-strand doubles). In contrast, if the order were *ADE1-PYK1-MAK16*, the tetrads with crossovers detected in the relevant interval would need to be explained by one crossover in this interval (23 cases), two crossovers in this interval (16 cases, including nine two-strand doubles, five three-strand doubles and two four-strand doubles) or three crossovers in this interval (two cases). Given the order *MAK1-PYK1-CDC24*, an order of *ADE1-PYK1-MAK16* would also require that *CDC24* be rather close to *ADE1*, which it clearly is not.

[b] Of these four tetrads, two were NPD for *ade1* × *mak16*, and two were T, with both of the latter showing FDS for *mak16*. Thus, these data support the previous conclusion (MORTIMER and SCHILD 1980; ROTHSTEIN and SHERMAN 1980) that the centromere is between *ADE1* and *MAK16*.

[c] The two *ts*-lethal markers in this cross were scored by complementation (see MATERIALS AND METHODS); scoring was usually clear. *ura1* and *MAT* were also scored in nearly all tetrads and segregated 2:2 with only a few exceptions. Analysis of individual tetrads from this cross strongly supports the map order of *CYS1-PYK1-CDC24* that is suggested by the estimated map distances. Of the 45 tetrads that were T for *pyk1* × *cdc24*, five were either 3:1 or seriously uncertain for *cys1*. The remaining 40 tetrads were distributed as follows: 15 were PD for *pyk1* × *cys1* and T for *cdc24* × *cys1*; four were T for *pyk1* × *cys1* and PD for *cdc24* × *cys1*; four were T for *pyk1* × *cys1* and NPD for *cdc24* × *cys1*; two were NPD for *pyk1* × *cys1* and T for *cdc24* × *cys1*; and 15 were T for both intervals.

[d] *mak16* was the only *ts*-lethal marker segregating in this cross and was easily scored by replica plating to YEPD at 36°. *trp1* and *leu2* were also scored in all tetrads and segregated 2:2 with only one exception. Analysis of individual tetrads from this cross supports the map order suggested by the estimated map distances and by previously published data (MORTIMER and SCHILD 1980). Excluding the 11 tetrads in which no crossovers were detected in the interval bounded by *ADE1*, *CYS1* and *MAK16*, and the seven tetrads in which one of these markers appeared not to segregate 2:2 (six somewhat questionable cases for *cys1* and one case for *mak16*), the available tetrads can be explained by one crossover in this interval (14 cases) or two crossovers in this interval (two cases, both four-strand doubles) if the order is *ADE1-CYS1-MAK16*. In contrast, to explain the same tetrads would require one crossover in the interval (ten cases), two crossovers in the interval (five cases, including four two-strand doubles and a four-strand double) or three crossovers in the interval (one case), if the order were *CYS1-ADE1-MAK16*.

[e] Analysis of these individual tetrads supports the conclusion that the centromere is between *ADE1* and *CYS1* (MORTIMER and SCHILD 1980; ROTHSTEIN and SHERMAN 1980). Of the four tetrads showing SDS for *ade1*, two show FDS for both *cys1* and *mak16*, one has *cys1* apparently segregating 3:1 but *mak16* showing FDS and only one has *cys1* and *mak16* both PD with respect to *ade1*. Of the five tetrads showing FDS for *cys1*, two showed FDS for *ade1* whereas *mak16* was PD with respect to *cys1*, one showed FDS for *ade1* and a 3:1 segregation for *mak16*, one showed FDS for *ade1*, whereas *mak16* was T with respect to *cys1* and only the one tetrad showed *ade1* and *mak16* both PD with respect to *cys1*.

gested by complementation analysis came from analysis of the linkage relationships of the mutations. Each putative *cdc15* mutation examined showed the expected tight linkage to *ade1* (Table 4); in addition, crosses between strains carrying the *tsb40* and *tsb43* mutations, between a *tsb43* strain and a *cdc15-1* tester, and between a *tsb76* strain and a *cdc15-1* tester yielded exclusively Ts^-

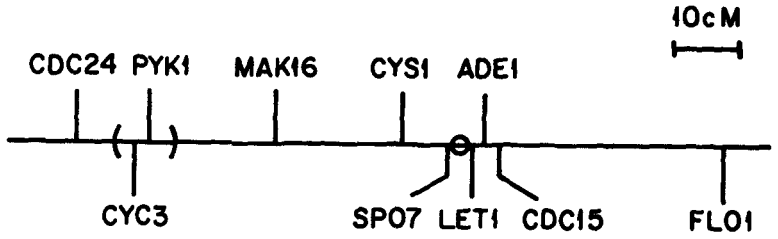

FIGURE 2.—Genetic map of *S. cerevisiae* chromosome *I*. This map is based on the data given in Table 2 (for the genes shown above the line) and those collected by MORTIMER and SCHILD (1980, 1982) and ROTHSTEIN and SHERMAN (1980). The order of *PYK1* and *CYC3* with respect to the flanking markers has not been determined by linkage analyses. However, molecular studies suggest that *CYC3* lies between *PYK1* and *CDC24* (A. TZAGALOFF and R. ROTHSTEIN, personal communication). The map distances shown should be taken as approximate, as there appear to be significant variations in different crosses in the map distances determined for various intervals on chromosome *I* (Table 2; Table 1 of MORTIMER and SCHILD 1980; KAWASAKI 1979).

TABLE 3

Complementation behavior and terminal cell morphologies of newly isolated mutants

Mutant isolate	Complementation when tested against[a]							Terminal cell morphology[b]
	cdc15-1	*tsb19*	*tsb43*	*cdc24-5*	*cdc24-4*	*cdc19-1*	*mak16*	
tsb19[c]	−	−	−	+	+	+	+	*cdc15*
tsb30	−			+		+	+	*cdc15*
tsb40[c]	−	−	−	+		+	+	*cdc15*
tsb43[c]	−	−	−	+	+	+	+	*cdc15*
tsb56	−			+		+	+	*cdc15*
tsb67[c]	−	−	−	+	+	+	+	*cdc15*
tsb71[c]	−	−	−	+	+	+	+	*cdc15*
tsb76[c]	−	−	−	+	+	+	+	None[d]
tsb80	−			+		+	+	*cdc15*
tsb92	−			+		+	+	*cdc15*
tsb104	−			+		+	+	*cdc15*
tsb109	−			+		+	+	*cdc15*
tsb110[c]	−	−		+	+	+	+	None[d]
tsd4	−			+		+	+	*cdc15*
tsd5	−			+		+	+	*cdc15*
tsd12	−			+		+	+	*cdc15*
tsb23	−			−		+	+	*cdc24*
tsb11[e]	+	+	+	−	−	+	+	*cdc24*
tsb37	+			−		+	+	None[d]
tsb58[e]	+	+		−/+	+	+	+	*cdc24*
tsb61[e]	+	+		−	−	+	+	*cdc24*
tsb84	+			−		+	+	*cdc24*
tsb87	+			−		+	+	*cdc24*
tsb93	+			−		+	+	*cdc24*
tsb108	+			−		+	+	*cdc24*
tsd14	+			−		+	+	*cdc24*
tsb35[f]	+	+	+	+	+	−	+	ND
tsb72[f]	+			+		−	+	ND
tsb105	+			+		−	+	ND
tsb5	+			+		+	+	ND

TABLE 3—Continued

Mutant isolate	Complementation when tested against[a]							Terminal cell morphology[b]
	cdc15-1	*tsb19*	*tsb43*	*cdc24-5*	*cdc24-4*	*cdc19-1*	*mak16*	
tsb27	+			+		+	+	ND
tsb44[g]	+	+		+	+	+	+	None
tsb53[g]	+	+	+	+	+	+	+	None
tsb68[h]	+	+	+	+	+	−/+	+	None
tsb99	+			+		+	+	ND
tsd1	+			+		+	+	None
tsd9	+			+		+	+	None
tsd18	+			+		+	+	None

[a] Complementation tests were normally done in grids such as that of Figure 1. Because some pairs of strains appeared not to mate well, most tests were done in both arrangements with respect to mating type (*e.g.*, a *MATα tsb19* was tested against a *MAT***a** *cdc15-1*, and a *MAT***a** *tsb19* was tested against a *MATα cdc15-1*). Clear evidence of complementation in either arrangement was taken as sufficient to justify an entry of + in the Table. In a few cases in which questionable results in the complementation grids seemed potentially significant, the complementation tests were repeated using isolated diploid clones. Blank spots in the table imply that the corresponding pairs of mutations were not tested against each other.

[b] Cellular morphologies were determined by phase-contrast microscopy on cells removed from YEPD plates after 24 hr at 37°. The distinctive morphologies of *cdc15* and *cdc24* cells have been described by HARTWELL *et al* (1973) and SLOAT, ADAMS and PRINGLE (1981). ND means that the cells were not examined microscopically; "none" means that the cells were examined, but no distinctive morphological phenotypes were observed.

[c] These mutants were also tested against *cdc15-9* (*tsb43* and *tsb110*), *cdc15-10* (*tsb40*), or both (*tsb19*, *tsb67*, *tsb71* and *tsb76*); clear noncomplementation was observed in all cases (*e.g.*, the *tsb71* results shown in Figure 1). Clear noncomplementation was also observed between *tsb67* and *tsb71* and between *tsb76* and *tsb110*. *tsb40* and *tsb71* clearly complemented the additional *pyk1* tester *tsb35* (see Figure 1 and footnote *f*). In contrast, *tsb67* showed only weak complementation with *tsb35*. The reason for this behavior is unknown, as *tsb67* complemented well with *cdc19-1* in several independent tests, and Ts^+ segregants (two among 16 viable spores) were recovered in a cross of *tsb67* × *tsb35*.

[d] Various possible explanations for such aberrant morphological phenotypes have been discussed and exemplified by HARTWELL *et al.* (1973) but were not explored further here (note, however, the case of *tsb23*). Note that linkage data (see text) also support the conclusion that *tsb76* carries a *cdc15* mutation.

[e] *tsb11* and *tsb61* also clearly failed to complement both each other and a *cdc24-1* tester, whereas *tsb11* clearly complemented the additional *pyk1* tester *tsb35* (see footnote *f*). In contrast, *tsb58* appeared to complement (to a variable extent) *cdc24-1*, *cdc24-4*, *cdc24-5*, *tsb11* and *tsb61* testers in some matings. It is not yet clear if this represents genuine interallelic complementation.

[f] *tsb35* also clearly complemented several additional *cdc15* testers (see Figure 1 and footnote *c*), but failed to complement with *tsb72*. The noncomplementation of *tsb35* with *cdc19-1* (Figure 1) was confirmed on a known diploid.

[g] *tsb44* and *tsb53* also clearly complemented *tsb76* (*cdc15*), *tsb11* (*cdc24*) and *tsb35* (*pyk1*).

[h] The *tsb68* mutation appeared dominant in some, but not all, crosses. For example, complementation with both *cdc19-1* and *tsb35* testers was variable and sometimes weak or undetectable, although the linkage data (Tables 4 and 6) appeared to rule out the possibility that *tsb68* carries a *pyk1* mutation (See also Table 5.)

segregants in nine, 18 and 11 tetrads, respectively. The putative *cdc24* mutations examined showed the expected linkage to *cdc24-5* and lack of linkage to *ade1* (Table 4). The putative *pyk1* mutations examined (*tsb35*, *tsb72*) appeared linked both to *ade1* and to *cdc24-5* (Table 4). In addition, a cross of a *tsb35* strain with a *cdc19-1* tester (DK210-8A) yielded seven 0 Ts^+:4 Ts^- tetrads and one apparent 1 Ts^+:3 Ts^- tetrad. (The one Ts^+ segregant was presumably either a revertant or a recombinant.)

TABLE 4

Tests for linkage between newly isolated mutations and chromosome I markers

Mutation tested	Complementation group	Test marker: *ade1* PD	*ade1* NPD	*ade1* T	*cdc24-5* PD	*cdc24-5* NPD	*cdc24-5* T
tsb40	*cdc15*	20	0	6	5	7	16
tsb43	*cdc15*	28	0	1	4	1	23
tsb56	*cdc15*	25	0	1	4	2	21
tsb92	*cdc15*	30	0	8	3	5	30
tsd4[a]	*cdc15*	7	1	1		ND	
tsd5[a]	*cdc15*	5	0	0		ND	
tsb23(cdc15)[b]	*cdc15*	14	0	1	2	1	12[c]
tsb23(cdc24)[b]	*cdc24*	2	1	13		ND	
tsb61	*cdc24*		ND		23	1	11[d]
tsb93	*cdc24*		ND		8	0	0[d]
tsb35	*pyk1*	17	2	26	27	1	18
tsb72	*pyk1*	6	1	18	14	0	11
tsb5[e]	New	12	10	9	2	9	23
tsb27	New	11	8	38	11	10	35
tsb44	New	2	4	24	6	6	20
tsb53	New	11	8	34	5	6	17
tsb68[f]	New	30	13	16	13	3	38
tsb99[g]	New	6	4	17	5	6	17
tsd1[h]	New	12	10	23	5	6	33
tsd9[h]	New	2	6	13	4	4	11
tsd18	New	11	6	3	9	11	43

Except where otherwise noted, data were obtained from a cross of DK17-4B or DK17-2B by a *ts ade1* haploid segregant from the particular monosomic mutant strain or from a backcross of the mutant as described in MATERIALS AND METHODS. The two *ts* markers segregating in these and related crosses (footnotes *h* and *i*) were scored by complementation (see MATERIALS AND METHODS), using *MAT***a** and *MAT*α testers for *cdc24-5* and for the *tsb* or *tsd* mutation in question. Where necessary for these and other crosses, *ade1* was also scored by complementation. ND means that these data could not be determined in the cross in question.

[a] Data were obtained from crosses of DK71-4C or α *ade5* by *ts ade1* haploid segregants from the particular monosomic mutant strains.

[b] Data were obtained from a cross of α *ade5* by a *MAT***a** *ade1* haploid segregant that carried both of the *ts* mutations present in the original monosomic *tsb23* mutant. The two *ts* mutations segregating in the cross were then scored by complementation using *MAT***a** and *MAT*α testers for *cdc15-1* and *cdc24-5*.

[c] These data refer to the linkage between the two different *ts* mutations in *tsb23*.

[d] PD was 0 Ts^+:4 Ts^-, NPD was 2 Ts^+:2 Ts^- and T was 1 Ts^+:3 Ts^-. The "Ts^+" segregants in the *tsb61* cross appeared bona fide, suggesting an extraordinary amount of recombination within the *CDC24* gene. (The data in Table 3 on complementation behavior and morphological phenotype, together with the linkage data, make it almost certain that *tsb61* is really an allele of *CDC24*.) However, some crosses involving *cdc24* mutations have given misleading results because of high rates of reversion of the *ts* mutations, and this possibility has not yet been ruled out in the present case.

[e] In this same cross, *tsb5* gave 24 PD and NPD, and only 12 T, with respect to *trp1*, consistent with the apparent centromere linkage of *tsb5*.

[f] The suggestion from these data of linkage of *tsb68* to chromosome *I* markers was apparently refuted (see also Table 6) by data from two other, similar crosses, in which *tsb68* × *ade1* yielded 6 PD, 5 NPD and 0 T, and 1 PD, 10 NPD and 4 T, respectively. Note that these data also support the apparent centromere linkage of *tsb68*.

[g] A *MAT***a** *ade5 cdc24-5 tsb99* segregant obtained after several backcrosses was crossed by a *MAT*α *ade1* Ts^+ tester.

[h] *tsd9* and *ade5 tsd1* segregants obtained after several backcrosses were crossed by DK17-3A or DK17-2C (Table 1).

Further evidence supporting the gene assignments for the new mutations came from examination of the phenotypes of the mutants. In every case examined but three (Table 3), the putative *cdc15* and *cdc24* mutants developed at 36° the distinctive cellular morphologies described previously for such mutants (HARTWELL *et al.* 1973; PRINGLE and HARTWELL 1981; SLOAT, ADAMS and PRINGLE 1981). The three exceptional cases probably have trivial explanations (Table 3, footnote *d*). In addition, the putative *pyk1* mutation *tsb35* was Ts^- for growth on glucose but Ts^+ for growth on acetate or lactate, consistent with its possessing a temperature-labile pyruvate kinase, as do *ts pyk1* strains carrying the *cdc19-1* allele (see Table 1; KAWASAKI 1979; FRAENKEL 1982).

Finally, we showed that a *leu2 tsb35* strain (DK303-8A; Table 1) could be transformed simultaneously to Leu^+ and Ts^+ using the autonomously replicating plasmid YEp13-PYK1. This plasmid contains about 10.7 kb of yeast DNA inserted into the *LEU2*-containing vector YEp13 (BROACH, STRATHERN and HICKS 1979). The inserted DNA has been shown to contain the pyruvate kinase structural gene (*PYK1*) from chromosome *I* (KAWASAKI and FRAENKEL 1982; FRAENKEL 1982; BURKE, TEKAMP-OLSON and NAJARIAN 1983; Y. STEENSMA and K. COLEMAN, unpublished results). After transformation, a Leu^+ clone was isolated on leucine-free plates at 25° and shown to be Ts^+ on YEPD at 37°. When this clone was grown under nonselective conditions (YEPD at 25°), about half of the colonies tested were still Leu^+ and Ts^+, whereas about half were Leu^- and Ts^-. This result suggests that *tsb35*-complementing activity was indeed carried on the unstably maintained plasmid, consistent with the conclusion that *tsb35* is an allele of *PYK1*.

In summary, 30 of the 39 mutations characterized here [or, counting *ts11-1* and *ts11-2* (KABACK and HALVORSON 1978), 32 of the 41 mutations isolated from the strain monosomic for chromosome *I*] fell into three previously known genes on chromosome *I*. There were 16–17 independent isolates in *CDC15* (recall that only two of the three NG-induced *cdc15* mutations can be assumed to be independent; see MATERIALS AND METHODS), 12 independent isolates in *CDC24* (counting *ts11-1* and *ts11-2*) and three independent isolates in *PYK1*. No isolates appeared to carry mutations in *MAK16* or *LET1*.

Analysis of the mutations not in CDC15, CDC24 *or* PYK1: The remaining nine mutations complemented all available *ts* testers for chromosome *I* genes (Table 3) and each other (Table 5), except for pairings involving the sometimes dominant *tsb68*. Do these mutations then define new essential genes on chromosome *I*? Linkage analysis suggests that none of these mutations is linked to *ade1* or *cdc24* (Table 4), but there may be regions of chromosome *I*, distal to all presently mapped genes (Figure 2), that do not show linkage to these markers. As *tsb5*, *tsb68* and *tsd18* appear centromere linked (Table 4), but not linked to *ade1*, they must be on other chromosomes. As a further test of possible linkage to chromosome *I*, a disome exclusion test was used (see MATERIALS AND METHODS). All seven mutations tested showed exclusively 2:2 segregations in these crosses (Table 6), whereas the *ade1* control markers showed the expected 4:0, 3:1 and 2:2 segregation ratios (Table 6). Thus, it appears that none of these mutations is on chromosome *I*.

TABLE 5

Complementation behavior of newly isolated mutations not in CDC15, CDC24 or PYK1

*MAT***a** strains	MATα strains								
	tsb5	*tsb27*	*tsb44*	*tsb53*	*tsb68*	*tsb99*	*tsd1*	*tsd9*	*tsd18*
tsb5	−	+	+	+	+	+	+	+	+
tsb27	+	−	+	+	+/−	+	+	+	+
tsb44	+	+	−	+	+/−	+	+	+	+
tsb53	+	+	+	−	+/−	+	+	+	+
tsb68	+	+/−	+/−	+/−	−	+	+/−	+	+
tsb99	+	+	+	+	+	−	+	+	+
tsd1	+	+	+	+	+/−	+	−	+	+
tsd9	+	+	+	+	+	+	+	−	+
tsd18	+	+	+	+	+	+	+	+	−

Complementation was scored as described in MATERIALS AND METHODS. An entry of "+/−" indicates that complementation was variable and sometimes weak in crosses between these pairs of mutants, apparently because of the dominance of the *tsb68* mutation (*cf.* also Table 3, footnote *h*).

TABLE 6

Disome exclusion tests for chromosome I *linkage of newly isolated mutations*

	No. of tetrads segregating					
	$Ts^+:Ts^-$			*ADE1*$^+$*:ade1*		
Mutation	4:0	3:1	2:2	4:0	3:1	2:2
tsb27	0	0	8	3	2	5
tsb44	0	0	8	3	4	1
tsb53	0	0	20	17	3	0
tsb68	0	0	7	2	5	0
tsd1	0	0	23	13	6	4
tsd9	0	0	18	9	2	7
tsd18	0	0	11	7	2	2

In each case, a *MATα ade1* haploid containing the *tsb* or *tsd* mutation was crossed to X3402-15C, a strain that appears to be disomic for chromosome *I* and homozygous *ADE1/ADE1* (Table 1). Asci were dissected and the segregants analyzed for growth at 37° and for growth in the absence of adenine. No attempt was made to analyze *tsb5*, and crosses of several different *tsb99* strains to X3402-15C did not yield sufficiently good spore viability to make the analysis feasible.

DISCUSSION

Chromosome *I* of the yeast *S. cerevisiae* is a very small eukaryotic chromosome. It contains only about 100 cM of recombination distance between its most distal known markers (MORTIMER and SCHILD 1980, 1982), and it appears to contain only about 200 kb of DNA (D. SCHWARTZ, C. CANTOR, Y. STEENSMA and D. KABACK, unpublished results). This small size and the availability of powerful methods of formal and molecular genetic analysis for yeast

suggest that it should be feasible to analyze the structural and functional properties of this chromosome in considerable detail. Among other benefits, such detailed studies of a single chromosome should contribute to resolution of the gene number paradox (see Introduction) and thus help to answer also the related question of how many genes are essential for the survival, growth and reproduction of eukaryotic cells.

To begin the detailed investigation of yeast chromosome *I*, we attempted to identify as many as possible of the essential genes on this chromosome by the use of *ts*-lethal mutations. Such mutations are easier to work with than cold-sensitive or suppressible nonsense mutations and were thought (see Introduction and points 2 and 4 in the following data) to be capable of identifying the majority of genes. To facilitate this analysis, we developed a method for efficiently isolating large numbers of *ts*-lethal mutations on chromosome *I*. A strain monosomic for this chromosome was mutagenized with EMS or NG and screened for Ts^- mutants incapable of growth on rich, glucose-containing medium at the restrictive temperature of 37°. As dominant *ts*-lethal mutations are rare, and as recessive mutations on the diploid chromosomes *II–XVII* should, in general, not be expressed because of the presence of wild-type alleles on the homologous chromosomes, we expected that most of the mutants isolated would carry mutations on the haploid chromosome *I*. This expectation was realized: of the 41 single-gene *ts*-lethal mutations analyzed, 32 mapped to chromosome *I*. None of the remaining nine mutations appears to map to chromosome *I* (see text and Tables 4 and 6); the only residual uncertainty involves *tsb99*, for which no disome exclusion data were obtained (*cf.*, however, Table 4). As these nine mutations were all recessive (with the partial exception of *tsb68*, see Tables 3 and 5), we presume that they were expressed phenotypically in the original mutants because they had become either hemizygous by chromosome loss or homozygous by mitotic recombination. Consistent with this presumption is the observation that all viable haploid spores recovered from these nine mutants carried the *ts* mutations. Not surprisingly, none of these nine mutations was allelic to any other (Table 5).

In contrast, the 32 mutations mapping to chromosome *I* fell into only three complementation groups, all of which were known previously. The recovery of multiple, independently isolated mutations in each of these three genes suggests that chromosome *I* contains few other genes that give rise readily to *ts*-lethal mutations. This conclusion was surprising, as other considerations suggest that chromosome *I* contains many more than three genes (see following data), and it was thought (see Introduction and DISCUSSION points 2 and 4) that the majority of those genes could be identified using *ts*-lethal mutations. At least five factors may be contributing to the apparent discrepancy.

1. Chromosome *I* may contain an unexpectedly small number of genes. The total known genetic map length of *S. cerevisiae* is about 5000 cM (MORTIMER and SCHILD 1982), and the haploid genome contains about 14,000 kbp of DNA (LAUER, ROBERTS and KLOTZ 1977). Thus, chromosome *I* appears to represent about $1/50$–$1/70$ of the haploid genome. Measurements both of the kinetic complexity of mRNA populations and of the amount of genomic DNA

complementary to these populations have suggested that vegetatively growing *S. cerevisiae* cells express about 4000–5000 distinct mRNA sequences (HEREFORD and ROSBASH 1977; KABACK, ANGERER and DAVIDSON 1979). Thus, chromosome *I* would be expected to contain about 60–100 genes that would be expressed in vegetatively growing cells. Although it is conceivable that genes are unusually sparse on chromosome *I*, the available data do not support this interpretation. The ten mapped genes in 100 cM of total map distance on chromosome *I* (Figure 2) are comparable to the average density of mapped genes in the genome as a whole (413 mapped genes in 5000 cM of total map distance: MORTIMER and SCHILD 1982). Moreover, in the one region for which data are so far available (the *PYK1-CDC24* region), five transcribed segments have been located in about 13.5 kbp of DNA (K. COLEMAN, Y. STEENSMA and J. CROWLEY, unpublished results), a packing density comparable to those observed in other regions of the genome (KABACK, ANGERER and DAVIDSON 1979; HEREFORD *et al.* 1979; ST. JOHN and DAVIS 1981; SHALIT *et al.* 1981; SHERMAN *et al.* 1983). Finally, observations similar to those reported in this paper have also been made on yeast chromosome *III* (G. FINK, personal communication): a deletion covering the region from *HIS4* to *LEU2* (a distance of some 40 kbp) did not produce inviability in haploid strains, and *ts*-lethal mutations isolated as failing to complement longer, lethal deletions defined only a few genes.

2. Only a small fraction of the genes on chromosome *I* may encode products that are essential for vegetative growth on rich medium. Of the ten known genes on this chromosome (Figure 2), five (*ADE1*, *CYS1*, *FLO1*, *SPO7* and *CYC3*) appear to code for products that are not essential in this sense (BROACH 1981; ROTHSTEIN and SHERMAN 1980), although the *ADE1* and *CYS1* gene products are necessary for growth on media lacking adenine or cysteine, respectively. Thus, we did not expect to find mutations in these genes in our search. However, it is possible that *FLO1*, *SPO7* and *CYC3* code for essential products and that the nonlethal phenotypes associated with known mutations in these genes (flocculence, inability to sporulate and reduced cytochrome levels leading to an inability to grow on nonfermentable carbon sources, respectively) reflect partial retention of activity by the mutant gene products. In this case, our failure to obtain *ts*-lethal mutations in these genes would presumably be explained by one of the other factors discussed later. More generally, there has been a rather pervasive belief that the majority of gene products in an organism are essential for survival and/or reproduction under laboratory conditions. However, we think that there is no very good experimental basis for this belief. Even in the bacteriophage T4, it now appears that the majority of genes are nonessential for reproduction on standard hosts under standard laboratory conditions (MOSIG 1983). It is easy to imagine that in cellular systems, many genes might encode functions that increase the fidelity or efficiency of cellular processes without being absolutely essential for those processes to proceed well enough for survival and reproduction of the cells. The ability to detect such nonessential genes by classic mutational analysis is severely limited by the ingenuity and patience with which mutants with subtle phenotypes can

be sought; they would certainly have been missed with the screening procedure used in this study.

3. Some genes on chromosome *I* that encode essential products may be difficult to detect mutationally because they are duplicated (on chromosome *I* or elsewhere in the genome). It is now clear that a significant number of yeast protein-coding genes are present in two or three functional copies (or near copies) per haploid genome (HEREFORD *et al.* 1979; HOLLAND and HOLLAND 1980; FRIED *et al.* 1981; MCALISTER and HOLLAND 1982; SHERMAN *et al.* 1983) and that in at least some of these cases one of the copies is sufficent to allow essentially normal growth of a haploid cell (KOLODRUBETZ, RYKOWSKI and GRUNSTEIN 1982; MCALISTER and HOLLAND 1982). Cleary, loss-of-function mutations in such genes will not normally be detected. Thus, one possible explanation (see also following data) for our failure to detect *ts*-lethal mutations in the known essential genes *MAK16* (WICKNER and LEIBOWITZ 1979) and *LET1* (MORTIMER and HAWTHORNE 1973), as well as in other possible essential genes on chromosome *I*, is that these genes are functionally duplicated in strain X1221a-7C. (We must presume, then, that *MAK16* and *LET1* were not duplicated in the strains in which the *mak16* and *let1* mutations were previously isolated and characterized.) A variation of this idea would be that a whole section of chromosome *I* is duplicated (*e.g.*, by nonreciprocal translocation) in strain X1221a-7C. It should be noted, however, that we did detect genes on both arms of the chromosome, including genes rather distal on the left arm. Thus, such a duplication might plausibly account for our failure to detect genes in the distal region of the right arm but appears less likely to account for the results as a whole.

4. Some single-copy genes on chromosome *I* that encode essential products may be difficult or impossible to mutate to temperature sensitivity. It is clear that many genes can mutate to *ts* alleles (EDGAR, DENHARDT and EPSTEIN 1964; HARTWELL 1967; PRINGLE 1975) and that some genes can do so at many sites (SMITH, BERGET and KING 1980). However, it is also clear that the requirement for an acceptably high level of function at one temperature, together with little or no function at another temperature only 10°–13° higher, is a rather stringent one (GRÜTTER, HAWKES and MATTHEWS 1979). Thus, it is not surprising that different genes differ greatly in their apparent susceptibilities to *ts* mutations (Table 3; HARTWELL *et al.* 1973; PRINGLE 1975; REED 1980; PRINGLE 1981). [In this context, it is worth noting that the frequency of recovery of mutations in the known genes in this study is not obviously a function of gene size. The *PYK1* and *CDC24* transcripts are approximately 1.6 and 2.1 kb, respectively (K. COLEMAN, Y. STEENSMA and J. CROWLEY, unpublished results), or only a little larger than the average yeast transcript (KABACK, ANGERER and DAVIDSON 1979). Thus, it does not appear either that the isolation of mutations in these genes and not in others is a function of these genes' being exceptionally large or that the fourfold difference in the frequencies of recovery of mutations in these two genes (Table 3; HARTWELL *et al.* 1973) is a simple reflection of their relative sizes.] By extension, it is conceivable that there are many genes in yeast whose products simply cannot be rendered Ts^- over the

usual temperature range by single amino acid substitutions. This idea is supported by the observation that, despite the extensive searches for cell cycle (*cdc*) mutants among Ts^- lethals (HARTWELL *et al.* 1973; PRINGLE and HARTWELL 1981; PRINGLE 1981), six of seven *cdc* complementation groups identified using cold-sensitive mutations had not previously been identified using *ts* mutations (MOIR *et al.* 1982). Thus, another possible explanation for our failure to detect mutations in *MAK16* and *LET1*, as well as in other possible essential genes on chromosome *I*, is that these genes are refractory to mutation to temperature sensitivity. This explanation is clearly plausible for *LET1*, for which only a nonsense-suppressible allele is known, but is more problematic for *MAK16*, as a *ts*-lethal allele of this gene is already available. However, this *mak16-1* allele was isolated by screening mutagenized cells for the inability to maintain "killer factor" (the additional Ts^--for-growth phenotype was discovered subsequently), it was the only mutant allele of *MAK16* found and it proved extremely difficult to revert, only one Ts^+, Mak^+ revertant's being recovered (WICKNER and LEIBOWITZ 1979). These facts are consistent with the speculation that special circumstances (*e.g.*, a possible requirement for two separate nucleotide changes) may be necessary to produce a *ts*-lethal allele of *MAK16*. In any case, it is highly likely that additional mutant hunting in the monosomic strain using cold-sensitive mutants or some system for working with suppressible nonsense mutations would reveal additional genes. However, the number of additional genes that would be revealed in this way depends on the relative contributions of the various factors discussed here to the gene number paradox as observed to data for chromosome *I*.

5. It is possible that the apparent near saturation of the chromosome *I* map with *ts*-lethal mutations is an illusion due to the presence of several "hot spots" for the mutagens used. It seems unlikely *a priori* that the concentration of recovered mutations into just three genes on a 200-kbp chromosome could be due solely to this factor, but we cannot rule this out. Our failure to recover mutations in *MAK16* could be taken as evidence for the significance of this factor. However, there are other possible reasons for this failure (see preceding data), and it should be noted that the *mak16-1* allele itself was recovered after mutagenesis with EMS (WICKNER and LEIBOWITZ 1979). Similarly, the different frequencies of recovery of mutations in *CDC15*, *PYK1* and *CDC24* might also reflect the presence of mutational hot spots but seem at least as likely to reflect differences in the susceptibilities of the gene products to *ts* mutations. The finding that the NG-induced mutations occurred in the same genes as the EMS-induced mutations (Table 3) does not argue strongly against the importance of hot spots in generating our results, as the mutational spectra of these mutagens appear very similar (COULONDRE and MILLER 1977). Analysis of a set of UV-induced mutations would probably be informative.

In summary, the results of a focused search for essential genes on yeast chromosome *I* appear at first glance to suggest that this chromosome may contain only the five already-known essential genes *CDC15*, *LET1*, *MAK16*, *PYK1* and *CDC24*. Extrapolation of this estimate to the genome as a whole would suggest that only some 250–350 genes were essential for the vegetative

growth and reproduction of *S. cerevisiae* cells in rich medium. However, molecular studies appear to demonstrate that vegetatively growing yeast cells express at least 15 times this many distinct mRNA species. This discrepancy is representative of the general discrepancy between genetic and molecular estimates of the numbers of genes (the gene number paradox), as observed in a variety of systems (see Introduction). Our molecular cloning studies of chromsome *I* should allow us to determine the density of transcribed sequences along this chromosome and the fraction of such sequences that are duplicated elsewhere in the genome. In addition, we should be able to determine the fraction of such sequences that are essential for vegetative growth, as judged by the use of *in vitro* mutagenesis procedures to generate null mutants (KOLODRUBETZ, RYKOWSKI and GRUNSTEIN 1982; MCALISTER and HOLLAND 1982; SHORTLE, HABER and BOTSTEIN 1982). Thus, we should be able to evaluate the relative contributions of the factors discussed earlier to the gene number paradox in this system; presumably, this evaluation will provide some guidance for the resolution of this paradox in other systems as well.

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