Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 4156–4160, June 1987
Genetics

SSC1, a member of the 70-kDa heat shock protein multigene family of *Saccharomyces cerevisiae*, is essential for growth

(gene disruption/cell viability)

ELIZABETH A. CRAIG, JULIE KRAMER, AND JUGANA KOSIC-SMITHERS

Department of Physiological Chemistry, University of Wisconsin, Madison, WI 53706

Communicated by Mary Lou Pardue, February 2, 1987

ABSTRACT **The genome of the yeast *Saccharomyces cerevisiae* contains a family of genes related to the HSP70 genes (encoding the 70-kDa heat shock protein) of other eukaryotes. Mutations in two of these yeast genes (*SSC1* and *SSD1*), whose expression is increased a few fold after temperature upshift, were constructed *in vitro* and substituted into the yeast genome in place of the wild-type alleles. No phenotypic effects of the mutation in *SSD1* were detected. However, a functional *SSC1* gene is essential for vegetative growth. This result, in conjunction with experiments involving mutations in other members of this multigene family, indicates that at least three distinct functions are carried out by genes of the HSP70 family.**

The 70-kDa heat-inducible protein (HSP70) has been highly conserved in evolution; related proteins have been identified in plants, animals, and bacteria. The bacterium *Escherichia coli* has a single HSP70-related gene, *dnaK*, whereas eukaryotes have evolved families of related genes (1, 2). The complexity and number of genes composing the families differ among species. The *Drosophila* HSP70 family contains at least three genes that are expressed during normal development (3), as well as a single copy of the heat-inducible HSP68 gene and five to six copies of the inducible HSP70 genes (4). The regulation of expression of these related genes in eukaryotes is complex, some being expressed only after a temperature upshift or other stress, while others are expressed under normal growth conditions.

The HSP70 multigene family of *Saccharomyces cerevisiae* contains at least eight genes. These genes, originally named *YG100–107*, have been renamed, on the basis of structural and functional similarities, *SSA1–4* (*s*tress *s*eventy subfamily *A*; *YG100, YG102, YG106*, and *YG107*, respectively), *SSB1* and *-2* (*YG101* and *YG103*, respectively); *SSC1* (*YG104*), and *SSD1* (*YG105*). The sequence relationship among the members of this family is complex, with nucleotide sequence similarity ranging from about 50% to 96% (5). The expression of the family members is modulated differentially in response to changes in growth temperature. For example, *SSA3* and *SSA4* are expressed at very low levels during steady-state growth at 23°C, but their expression is greatly enhanced upon an upshift to 37°C (5). Transcripts of *SSB1* and *SSB2* are abundant during steady-state growth but rapidly decrease upon an upshift in temperature (6). Expression of other family members change little or only severalfold after a heat shock.

A major question concerning multigene families is whether the members of a family perform identical or distinct functions. In our laboratory, strains containing mutations in members of the HSP70 family have been constructed, in an attempt to determine the number of distinct functions carried out by members of this family. Previous reports (6, 7) described the effects of mutations in four genes of this family. We report here the effects of mutations in two other members of the HSP70 family, one of which (*SSC1*) is an essential gene.

MATERIALS AND METHODS

Strains, Culture Conditions, Transformations, and Hybridization Analysis. The *S. cerevisiae* strains used were T87 (**a**/α *ade2-101/ADE2 lys2/lys2 ura3-52/ura3-52 Δtrp1/Δtrp1 leu2-3,112/leu2-3,112 HIS4/his4-713*), JKX21 {**a**/α *leu2-3,112/leu2-3,112 ade2/ade2-101 lys2/lys2 ura3-52/URA3 Δtrp1/TRP1 HIS3/his3-11,15* [SSC1:LEU2(G)]}, and JKX40 {**a**/α *leu2/leu2 cyc1-72/CYC1 ade2/ade? MET3/met3 TRP1/Δtrp1 URA3/ura3-52 LYS2/lys2* [SSC1:LEU2(G)]}.

As previously described, transformations were carried out using LiOAc (7). The yeast culture media and classical mapping procedures used in this study have been described (7, 8). DNA isolation from yeast cells, blotting of DNA to nitrocellulose, and hybridization with nick-translated probes were done as described (7). RNA was isolated (9) and analyzed by hybridization after electrophoresis in denaturing (formaldehyde-containing) gels (6).

Plasmid Construction. Initial clones containing *SSC1* and *SSD1* were isolated by M. Ellwood in this laboratory. Size-fractionated *Hin*dIII-digested S288C DNA was ligated into the *Hin*dIII site of pBR322, and the resulting clones were screened by hybridization with a portion of the protein-coding region of *SSA2* (9). The isolated homologous clones, SSC1H and SSD1H, contained *Hin*dIII fragments of 3.0 kilobases (kb) and 1.2 kb, respectively. To construct plasmid SSC1:LEU2(S) (Fig. 1 *Left*), SSC1H was partially digested with *Sal* I and ligated with a 2.2-kb *Xho* I–*Sal* I fragment [isolated from YEp13 (10)], carrying the *S. cerevisiae LEU2* gene. A clone containing the *LEU2* gene inserted into the *Sal* I site at amino acid codon 318 of *SSC1* was designated SSC1:LEU2(S). To construct SSC1:LEU2(G), a 3.0-kb *Bgl* II fragment carrying the *LEU2* gene (isolated from YEp13) was inserted into the single *Bgl* II site of SSC1H.

To obtain a clone containing additional 5′ flanking DNA of *SSC1*, a library of *Bgl* II genomic DNA fragments cloned into the *Bgl* II site of pMT11 (obtained from H. Huang, Washington University) was constructed and screened, using as a probe the isolated *Hin*dIII fragment of SSC1H. A 6.1-kb *Bgl* II fragment containing *SSC1* was subcloned into the *Bam*HI site of YCp50, which contains autonomously replicating sequence ARS*1*, centromere *CEN4*, and the *URA3* gene (11), forming YCp50:SSC1. YCp50:SSC1ΔS was constructed by digesting YCp50:SSC1 with *Sal* I and religating, thus removing the 3′ end and flanking regions of SSC1, as well as a portion of YCp50.

The *Hin*dIII fragment of SSD1H was used to screen the *Bgl* II genomic library described above, and a clone containing a

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: HSP*nn*, heat shock protein of *nn* kDa.

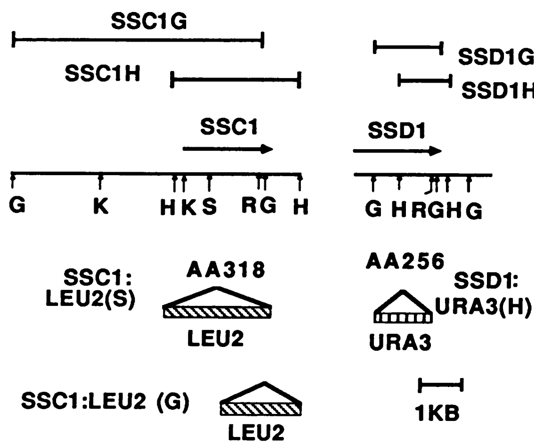

FIG. 1. *SSC1* (*Left*) and *SSD1* (*Right*) gene regions. Horizontal lines above the restriction maps represent the clones used as the starting material for construction of other plasmids used in this study. Horizontal arrows indicate the lengths of the transcribed regions of *SSC1* and *SSD1*, assuming that the genes contain no intervening sequences. The insertion mutations constructed are indicated below the restriction maps. Positions of insertions are indicated by amino acid codon numbers (AA318 and AA256). H, *Hin*dIII, G, *Bgl* II, R, *Eco*RI, K, *Kpn* I, S, *Sal* I.

1.6-kb fragment was isolated. This fragment was inserted into the *Bgl* II site of the vector pJRD (12) from which the original *Hin*dIII site had been eliminated. The *URA3* gene, contained on a 1.2-kb *Hin*dIII fragment [isolated from YEp24 (13)], was inserted into the single *Hin*dIII site at the codon for amino acid 256.

RESULTS

Isolation and Characterization of *SSC1* and *SSD1*. The *SSC1* and *SSD1* genes were isolated by screening plasmid genomic clones containing inserts of *Hin*dIII-digested *S. cerevisiae* DNA, using labeled DNA from the HSP70-related gene *SSA2* as a probe. To confirm the homology and determine the orientation of the genes, the DNA sequence from a site internal to the homologous region was determined. As diagrammed in Fig. 1, the orientation and placement of the transcription unit were predicted from the resulting data. The limited sequence data obtained were compared to those available for other genes in the family (5). *SSC1* and *SSD1* are 45–55% homologous to each other and to other members of the HSP70 multigene family.

We examined the expression of *SSC1* and *SSD1* under a number of temperature conditions by analyzing the level of RNA transcripts. Cellular RNAs that had been separated in denaturing gels and blotted to nitrocellulose filters were hybridized with a labeled *SSC1* or *SSD1* clone under conditions that did not allow cross-hybridization with other members of the HSP70 family. Both *SSC1* and *SSD1* RNAs are moderately abundant during steady-state growth at 23°C; the levels are a few fold higher during logarithmic growth at 30°C and 37°C. The levels increase a few fold after upshift to 37°C or 39°C from 23°C. In the experiments shown in Fig. 2, *SSC1* and *SSD1* transcripts increased 4.0- and 2.5-fold, respectively. In three separate experiments measuring RNA levels 30 min after heat shock, the increase of *SSC1* transcripts ranged from 1.5- to 4.0-fold. In the case of *SSD1*, no increase to a 2.5-fold increase was observed. The differences observed may be due to slight temperature variations between experiments and the rapidity of the temperature shift.

Construction and Analysis of *SSC1* and *SSD1* Mutations. Mutations in *SSC1* and *SSD1* were constructed *in vitro*. We then used the one-step gene-replacement method of Rothstein (14) to integrate the mutant genes into the yeast genome and simultaneously delete the wild-type gene. An insertion mutation of *SSD1* was constructed by inserting the *URA3* gene into the *Hin*dIII site present at the codon for amino acid 256. The resulting plasmid, SSD1:URA3(H), was cleaved with *Bgl* II to separate yeast sequences from vector sequences prior to transformation (Fig. 1 *Right*).

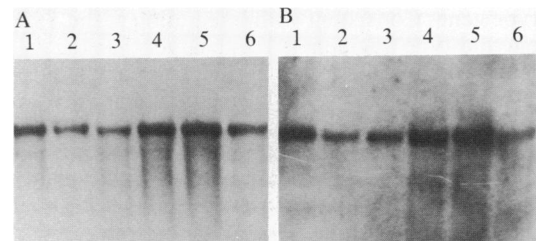

FIG. 2. Expression during steady-state growth and heat shock. Portions (3 μg) of the RNA preparations were electrophoresed in a 6% formaldehyde/1% agarose gel and then blotted to nitrocellulose. The RNA was hybridized with a ^{32}P-labeled probe prepared by nick-translation of the plasmid SSC1H (*A*) or SSD1H (*B*). RNA was isolated from cells growing logarithmically at 37°C (lanes 1), 30°C (lanes 2), or 23°C (lanes 3) and from cells 30 min after shift from 23°C to 39°C (lanes 4), 37°C (lanes 5), or 35°C (lanes 6).

An insertion mutation in *SSC1* was constructed by inserting the *LEU2* gene of *S. cerevisiae* carried on a 2.2-kb *Xho* I–*Sal* I fragment into the *Sal* I site at codon 318. Before transformation into yeast, the resulting plasmid, SSC1: LEU2(S), was cleaved with *Hin*dIII to separate vector from yeast sequences (Fig. 1 *Left*). In both cases, a diploid (strain T87) was used as a transformation recipient, since it was not known whether an *SSC1* or *SSD1* mutation would be lethal in the absence of a wild-type gene. Transformants in which one wild-type gene was replaced by a mutant allele were identified by hybridization to genomic DNA. When the 1.6-kb *Bgl* II fragment containing the *SSD1* gene was used to probe wild-type genomic DNA digested with *Bgl* II, a 1.6-kb fragment hybridized, as expected (data not shown). In analysis of an *SSD1* transformant (Fig. 3*B*), the probe hybridized to two fragments, a 2.8-kb fragment not present in the wild-type DNA and the 1.6-kb fragment derived from the

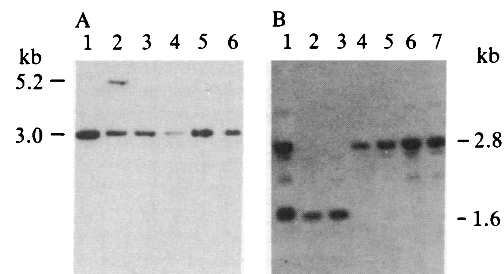

FIG. 3. Hybridization analysis of genomic DNA from diploids transformed with *SSC1* and *SSD1* insertion mutations. Strain T87 was transformed with *Hin*dIII-digested SSC1:LEU2(S) or *Bgl* II-digested SSD1:URA3(H) DNA. Genomic DNA was digested with *Hin*dIII (*SSC1* transformant, *A*) or *Bgl* II (*SSD1* transformant, *B*), electrophoresed in an agarose gel, and blotted to nitrocellulose. The DNA was hybridized with either a ^{32}P-labeled 3.0-kb *Hin*dIII fragment containing the 5′ portion of *SSC1* or a ^{32}P-labeled 1.2-kb *Bgl* II fragment containing the 5′ portion of *SSD1*. (*A*) Lanes: 1, wild type (T87); 2, *SSC1* transformant; 3–4 and 5–6, two viable haploids obtained from a single ascus (all are Leu$^-$ and contain only the wild-type *SSC1* fragment). (*B*) Lanes: 1, *SSD1* diploid transformant; 2–5, four haploid derivatives of diploid shown in lane 2, from a single ascus (lanes 2 and 3 are Ura$^-$); 6, a diploid strain derived from the two *ssd1* haploid strains shown in lanes 4 and 5. The wild-type strain (T87) showed a single 1.6-kb band (data not shown).

wild-type gene. Consistent with integration at the *SSD1* site, the non-wild-type fragment is 1.2 kb larger than the wild-type fragment, which is the expected increase in size due to the *URA3* insertion. Similarly, hybridization of *Hin*dIII-digested wild-type genomic DNA with an *SSC1* probe resulted in a single band at 3.0 kb, whereas transformant DNA yielded two bands, the wild-type 3.0-kb band and a 5.2-kb band that is consistent in size with the insertion of the 2.2-kb *LEU2*-containing fragment (Fig. 3*A*).

To determine the phenotype of haploid strains lacking a wild-type *SSD1*, haploid meiotic segregants were derived from the diploids transformed with the SSD1 insertion mutation. Spore viability was high and all tetrads in which four spores germinated displayed a normal Mendelian (2:2) segregation of Ura$^+$ vs. Ura$^-$. Hybridization analysis verified that the Ura$^+$ cells contained the mutant gene and Ura$^-$ cells the wild-type gene. As expected, Ura$^+$ cells contained only the larger, 2.8-kb *Hin*dIII fragment that hybridized to the *SSD1* probes, and the Ura$^-$ cells the smaller, 1.6-kb fragment found in wild-type cells (Fig. 3*B*). The recovery of the Ura$^+$ segregants from the diploids indicates that *SSD1* mutations are not lethal. No altered phenotype of the mutant strain has been observed. The strains grow at rates very similar to that of wild-type strains at several temperatures. To test whether the *SSD1* disruption affects mating or sporulation, a homozygous diploid strain was constructed by mating two haploid *ssdl* strains of opposite mating types. This homozygous diploid sporulated efficiently when starved for nitrogen, and >80% of the resulting spores germinated, indicating that *SSD1* product is not necessary for mating and germination.

The question of essentiality of *SSC1* was addressed in a similar manner. A diploid transformant heterozygous for the *SSC1* mutation was starved for nitrogen in order to induce sporulation. Nearly all tetrads had only two viable spores, both of which were Leu$^-$. DNA was isolated from the surviving spores from two of the tetrads and analyzed; consistent with the segregation of the leucine marker, all contained a wild-type gene but no mutant gene. Observation with a dissection microscope revealed that the mutant spores had germinated and small clusters of 8–30 cells had formed.

The failure to retreive haploids containing a mutant *SSC1* gene indicates that a functional *SSC1* product is necessary for viability. However, other interpretations are possible. For example, it is possible that the isolated *SSC1* clone contains an alteration outside the *SSC1* gene that occurred during cloning of the locus and that this alteration is responsible for the lethality observed. To confirm that the lethality was due to a mutant *SSC1* gene, two additional experiments were performed. First, an insertion mutation 3′ to the *SSC1* gene was constructed by inserting the *LEU2* gene into the *Bgl* II site immediately 3′ to *SSC1* (see Fig. 2). This mutant was transformed into diploids as described above and the resulting heterozygote was induced to sporulate. Spore viability was high, and all four-spore clones displayed a 2:2 segregation of Leu$^+$ vs. Leu$^-$. These results indicate that an insertion that is external to the *SSC1* gene does not affect viability, and that the lethality observed in the case of the insertion into the *Sal* I site is due to the disruption of *SSC1*.

Also, if the disruption of *SSC1* is responsible for the lethality, reintroduction of a wild-type *SSC1* gene should restore viability. An intact *SSC1* gene carried on a 6.1-kb *Bgl* II fragment was cloned into the centromere-containing vector YCp50, which carries the *URA3* gene (Fig. 4). This construct was transformed into the *SSC1/sscl* heterozygote and the resulting transformants were induced to sporulate. In the absence of the vector, 22 of 22 complete tetrads gave 2:2 segregation of normal colonies vs. clusters of cells. In the presence of the *SSC1*-containing vector, 28 of 48 tetrads showed 3:1 segregation of normal colonies vs. small clusters and 14 had four normal-sized colonies, while 6 showed 2:2 segregation. Each Leu$^+$ (*SSC1* mutant-containing) colony was also Ura$^+$, indicating that the presence of *SSC1* on the centromeric plasmid was permitting growth. This interpretation was strengthened by the inability of *SSC1* lacking its 3′ end (YCp50:SSC1ΔS) (Fig. 4) to rescue cells containing an *SSC1* mutation.

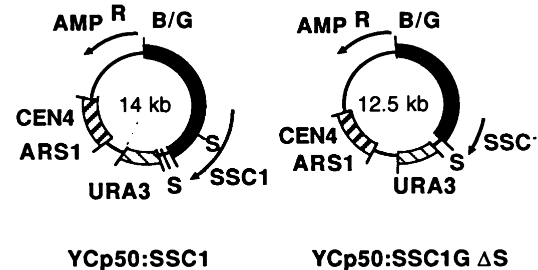

FIG. 4. *SSC1* plasmids used in "rescue" experiments. YCp50:SSC1 contains an intact *SSC1* gene. YCp50:SSC1ΔS lacks the information beyond the codon for amino acid 318. Details of plasmid construction are given in *Materials and Methods*. AMP^R, ampicillin-resistance gene; S, *Sal* I; B/G, *Bam*HI and *Bgl* II joined.

Mapping of *SSC1*. Since the results indicated that *SSC1* is an essential gene, it was of interest to determine whether *SSC1* represents a previously identified genetic locus. Initially, *SSC1* was localized to the right arm of chromosome 10 by the 2 μm plasmid integration technique of Falco and Botstein (15). Tetrad analysis indicated that *SSC1* was located ≈25 centimorgans from the centromere and tightly linked to *CYC1* (Table 1). In 58 complete tetrads that segregated 2:2 for SSC1:LEU2(G) and *CYC1*, no recombination events between these loci were detected. Hybridization analysis of genomic DNA from a set of *CYC1* deletion strains (generously provided by F. Sherman) suggested that the *Bgl* II fragment containing *SSC1* overlapped the regions covered by the deletions (data not shown). Therefore, the restriction enzyme maps of SSC1G and a clone containing ≈5 kb of DNA 5′ to the *CYC1* gene were compared (Fig. 5), and hybridization experiments were performed to determine if the two DNAs have sequences in common. The *Bgl* II fragment containing *SSC1* hybridized to a 3.6-kb *Hin*dIII–*Bgl* II fragment of clone AB183, 5′ to the *CYC1* gene. Comparison of the restriction maps indicated that the DNAs contain this 3.6-kb region in common, thus placing *SSC1* 4–5 kb centromere-proximal to *CYC1*.

Table 1. Chromosomal mapping of *SSC1*

	Number of asci					
	Segregation*		Ascus type†			Map distance,
Strain, marker	FD	SD	PD	NPD	T	centimorgans
Strain JKX21,‡						
CEN10–SSC1: LEU2(G)	12	12				24.5
Strain JKX40,§						
met3–SSC1:LEU2(G)			29	0	29	25
SSC1:LEU2(G)–*cycl-72*			58	0	0	0
met3–cycl-72			29	0	29	25

See *Materials and Methods* for genotypes of JKX21 and JKX40.
*FD, first-division segregation; SD, second-division segregation. The segregations were determined by examination of a marker relative to that of a known centromere-linked marker, in this case *TRP1*.
†PD, parental ditype; NPD, nonparental ditype; T, tetratype.
‡The *CEN10*–SSC1:LEU2(G) distance was calculated as in ref. 16.
§Distance in centimorgans equals 100(T + 6NPD)/2(PD + NPD + T).

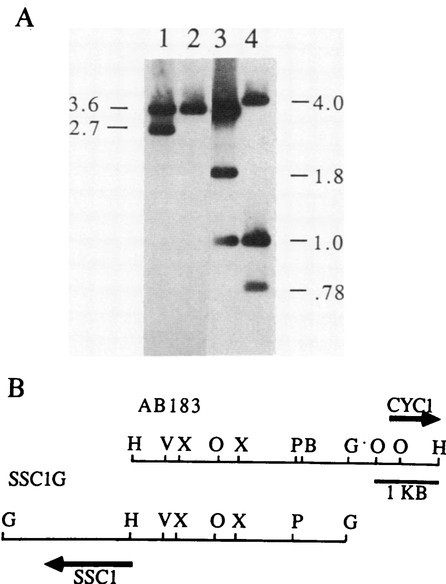

FIG. 5. Comparison of *SSC1*- and *CYC1*-containing clones. (*A*) DNAs from SSC1G and AB183 were cleaved with restriction endonucleases, electrophoresed in agarose gels, blotted to nitrocellulose, and hybridized with the 6.1-kb *Bgl* II fragment contained within *SSC1*. Lanes: 1, SSC1G digested with *Hin*dIII and *Bgl* II; 2, AB183 digested with *Hin*dIII and *Bgl* II; 3, SSC1G digested with *Bgl* II and *Xba* I; 4, AB183 digested with *Hin*dIII and *Xba* I. (*B*) The restriction maps of the inserts of SSC1 and AB183 are aligned to show overlap. H, *Hin*dIII; V, *Pvu* II; X, *Xba* I; O, *Xho* I; P, *Pst* I; G, *Bgl* II; B, *Bam*HI.

This placement of *SSC1* on the molecular map of the *CYC1* region (17) eliminates the possibility that *SSC1* is the same as any of the other loci molecularly mapped in the *CYC1* cluster; therefore, *SSC1* is probably a previously unidentified gene. Genetic analysis has placed the gene *REV5* proximal to *CYC1* in this molecularly mapped cluster. Rev5 mutants were isolated in a screen for mutations that impair induced mutagenesis (18). The viability of a $Rev5^-$ strain is reduced by a factor of 3 even after 30 J/m^2 irradiation, the highest fluence tested, and therefore *REV5* is thought not to be directly involved in the induced-mutagenesis pathway. At present, there is no evidence to support the idea that *SSC1* is identical to *REV5*.

DISCUSSION

The results show that a member of the HSP70 gene family is essential for growth. A spore lacking a wild-type *SSC1* gene is able to germinate but ceases growth after several divisions. It is likely that residual SSC1 protein in the spore permits this limited growth. Strains containing a mutant *SSD1* gene are viable, and we have observed no altered phenotype of the mutant strains. However, it is quite possible, since only a limited number of growth conditions were analyzed, that conditions exist under which the growth of *ssd1* strains would differ from the growth of wild-type strains.

The construction of the strains containing insertion mutations of *SSC1* and *SSC2* completes the preliminary genetic analysis of the eight isolated members of this gene family (Fig. 6). Of the eight genes, *SSD1* is the only member that has not been shown to be functional, in that no phenotype has been associated with the absence of the gene product. *SSC1* is the only member whose absence has been found to result in a phenotypic effect without the presence of a mutation in a second gene. The data presented here, in conjunction with the results of the analysis of the other members of the yeast HSP70 family, suggest that there are at least three genetically

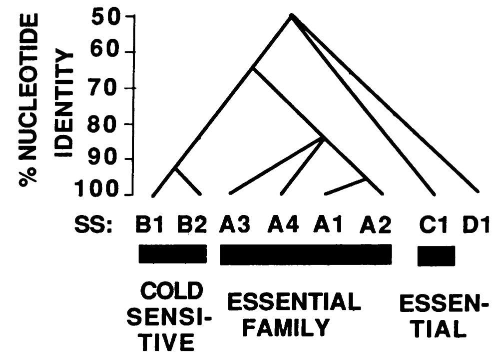

FIG. 6. HSP70 multigene family of *S. cerevisiae*. Approximate percent nucleotide identities are based on partial or complete sequence data (5). The phenotypes of strains containing mutations other than *ssc1* and *ssd1* have been described (6, 19).

identifiable functions that cannot be complemented by other members of the family. *SSC1* is a functionally unique member of the family. The most complex structural and functional subfamily includes *SSA1–4*. Each of the protein products of these genes can substitute, at least partially, for the absence of the other three. However, this intergenic complementation is complex in that the normal expression patterns of some of the genes must change in order for complementation to occur (20).

The *SSB1* and *SSB2* gene pair composes the third group. *ssb1* or *ssb2* strains appear wild type, but growth of a strain containing mutations in both genes is relatively cold-sensitive (6). The cold sensitivity cannot be alleviated by *SSA1*, even when *SSA1* is under the control of the *SSB1* promoter. Conversely, *SSB1* cannot rescue the temperature-sensitive *ssa1 ssa2* strain, even when fused to the *SSA2* promoter. These results indicate that the *SSB1/SSB2* gene pair encodes proteins whose functions or cellular locations are distinct from those of the *SSA1–4* product and the constitutively expressed *SSC1* product.

The nature of the functions performed by the different members of the HSP70 family is not clear. The structural similarities of the members seem to reflect functional similarities. However, this genetic analysis of the HSP70 family does not allow us to distinguish between function and location as a basis for phenotypic differences between subgroups. There is currently no information about the subcellular localization of the HSP70 and related proteins of yeast. However, for *Drosophila* and mammalian systems there is evidence that HSP70 and related proteins have different cellular locations. Both HSP70 (21) and a cognate protein (ref. 19; K. B. Palter, G. J. Gorbsky, G. G. Borisy, and E.A.C., unpublished results) in *Drosophila* appear to be located in the cytoplasm and move into the nucleus after a heat shock. An HSP70-related protein found in mouse cells has been localized to the endoplasmic reticulum (22). Therefore, it is possible that the inability of one protein to compensate for the absence of another is due to different cellular locations, even though the proteins can catalyze the same enzymatic reaction or perform essentially the same biochemical function. On the other hand, the related proteins may carry out very different functions. For example, one member of the family may be the clathrin-uncoating ATPase, as has been indicated by the ability of antibodies that react with purified bovine brain clathrin-uncoating ATPase to also react with members of the yeast HSP70 family (23). Other members of the family may have very different functions, even though they show structural similarities and some similar biochemical properties (such as a strong affinity for ATP).

An 8-member multigene family is unusually large for yeast, and until recently, higher eukaryotes were thought to have smaller HSP70 families than yeast. However, the sizes of higher eukaryotic HSP70 families appear to have been underestimated. Recently, 4 additional *Drosophila* genes homologous to HSP70 have been isolated (K. B. Palter and E.A.C., unpublished results), bringing to 13 the number of isolated family members. The human HSP70 gene family has been reported to contain at least 10 members (24). In light of these recent results, it appears that the size of the yeast multigene family is similar to that of other eukaryotes. It is likely that, like yeast, higher eukaryotes contain genetically distinct functional groups, although it remains to be seen whether the exact functions have been conserved between yeast and higher eukaryotes.

We thank J. Bardwell and M. Ellwood, who isolated the SSC1H and SSD1H plasmids; F. Sherman and L. Melnick for *CYC1* deletion strains and plasmids; Irv Edelman and Mike Culbertson for strains and advice on mapping; and M. Werner-Washburne for helpful suggestions on the manuscript. This work was supported by a National Institutes of Health grant to E.A.C., who was also supported by a Public Health Service Research Career Development Award. J.K. was supported by a Public Health Service training grant in molecular biology.

1. Craig, E. A. (1985) *Crit. Rev. Biochem.* **18,** 239–280.
2. Lindquist, S. (1986) *Annu. Rev. Biochem.* **55,** 1151–1191.
3. Craig, E. A., Ingolia, T. D. & Manseau, L. J. (1983) *Dev. Biol.* **99,** 418–426.
4. Southgate, R., Mirault, M.-E., Aymes, A. & Tissieres, A. (1985) in *Changes in Eucaryotic Gene Expression in Response to Environmental Stress*, eds. Atkinson, B. & Walden, D. B. (Academic, Orlando, FL), pp. 3–30.
5. Craig, E. A., Slater, M. R., Boorstein, W. R. & Palter, K. (1985) in *Sequence Specificity in Transcription and Translation*, UCLA Symposia on Molecular and Cellular Biology, eds. Calender, R. & Gold, L. (Liss, New York), Vol. 30, pp. 659–668.
6. Craig, E. A. & Jacobsen, K. (1985) *Mol. Cell. Biol.* **5,** 3517–3524.
7. Craig, E. A. & Jacobsen, K. (1984) *Cell* **38,** 841–849.
8. Sherman, F., Fink, G. R. & Hicks, J. B. (1982) *Methods in Yeast Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
9. Ingolia, T. D., Slater, M. R. & Craig, E. A. (1982) *Mol. Cell. Biol.* **2,** 1388–1398.
10. Broach, J. R., Strathern, J. N. & Hicks, J. B. (1979) *Gene* **8,** 121–133.
11. Johnston, M. & Davis, R. (1984) *Mol. Cell. Biol.* **4,** 1440–1448.
12. Davison, J., Heuterspeute, M., Merchez, M. & Brunel, F. (1984) *Gene* **28,** 311–318.
13. Botstein, D., Falco, S. C., Stewart, S. E., Breeman, M., Scherer, S., Stinchcomb, D. T., Struhl, K. & Davis, R. W. (1979) *Gene* **8,** 17–23.
14. Rothstein, R. J. (1983) *Methods Enzymol.* **101,** 202–211.
15. Falco, S. C. & Botstein, D. (1983) *Genetics* **105,** 857–872.
16. Mortimer, R. K. & Schild, D. (1981) in *The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance*, eds. Strathern, J. N., Jones, E. N. & Broach, J. R. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 13–16.
17. Barry, K., Stiles, J. I., Petris, D. F., Melnick, L. & Sherman, F. (1987) *Mol. Cell. Biol.* **7,** 632–638.
18. Lawrence, C. W., Krauss, B. R. & Christensen, R. B. (1985) *Mutat. Res.* **150,** 211–216.
19. Palter, K. B., Watanabe, M., Stinson, L., Mahowald, A. P. & Craig, E. A. (1986) *Mol. Cell. Biol.* **6,** 1187–1203.
20. Werner-Washburne, M., Stone, D. E. & Craig, E. A. (1987) *Mol. Cell. Biol.*, in press.
21. Velazquez, J. & Lindquist, S. (1984) *Cell* **36,** 655–662.
22. Munro, S. & Pelham, H. R. B. (1986) *Cell* **46,** 291–300.
23. Chapell, T. G., Welch, W. J., Schlossman, D. M., Palter, K. B., Schlesinger, M. J. & Rothman, J. E. (1986) *Cell* **45,** 3–13.
24. Mues, G. I., Munn, T. Z. & Raese, J. D. (1986) *J. Biol. Chem.* **26,** 874–877.