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Saccharomyces cerevisiae Contains a Complex Multigene Family Related to the Major Heat Shock-Inducible Gene of *Drosophila*

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Saccharomyces cerevisiae contains a family of genes related to the major heat shock-induced gene of *Drosophila* (hsp 70). Two members of the multigene family (YG100 and YG101) were isolated. The primary DNA sequences of more than one-half of the protein-encoding regions of YG100 and YG101 were determined and compared with the *Drosophila* hsp 70 gene sequence; the predicted amino acid sequences were 72 and 64% homologous to the sequence of the *Drosophila* hsp 70 protein, respectively. The predicted amino acid sequences of the yeast genes were 65% homologous. Our results demonstrate a striking sequence conservation of hsp 70-related sequences in evolution. Hybridization of the *S. cerevisiae* genes to total *S. cerevisiae* DNA indicated that the multigene family consists of approximately 10 members. Hybridization of labeled RNAs from heat-shocked and control cells suggested that, like transcription of the *Drosophila* hsp 70 gene, transcription of YG100 or a closely related gene is enhanced after heat shock. However, the amount of RNA sequences homologous to YG101 was reduced after heat shock. A multigene family related to the hsp 70 gene exists in *Drosophila*; transcription of some members is induced by heat shock, whereas transcription of others is not. Our results suggest that *S. cerevisiae*, like *Drosophila*, contains a multigene family of hsp 70-related sequences under complex transcriptional regulation and that the differential control, as well as the nucleotide sequence, has been highly conserved in evolution.

Heat shock and certain other stimuli result in a dramatic change in the pattern of gene expression in *Drosophila*. Transcription of most genes is suppressed, and expression of a small set of relatively inactive genes is greatly enhanced (2). The major polypeptide expressed after heat shock in *Drosophila melanogaster* is a 70,000-dalton protein (hsp 70). Furthermore, the most abundant species that accumulates after heat treatment is the mRNA encoding hsp 70 (25). In *Drosophila*, a multigene family related to the hsp 70 gene has been identified (14). Five distinct members of this family have been characterized. Two of the characterized members, the hsp 70 and hsp 68 genes, are induced by heat shock (2, 13). Three others, which are dispersed on chromosome 3, are transcribed at normal temperatures and not induced by heat treatment. A partial DNA sequence of the protein-encoding region has been determined for each gene. The predicted amino acid sequences are conserved about 75% among the five members of the multigene family (14; unpublished data).

A heat shock response has been observed in cells of taxa as diverse as *Saccharomyces cerevisiae* (24, 26), *Dictyostelium* (21), hamsters, chickens, humans (16), tobacco, and soybean (3, 18). Recovery from anoxia, as well as from agents which interfere with oxidative phosphorylation, elicits the heat shock response in a number of species (1, 21, 29, 34). Therefore, the alteration in gene expression is thought to be a general response to metabolic disturbance, not merely a response to an alteration in temperature.

When *S. cerevisiae* cells are subjected to a sudden increase in temperature, extreme alterations occur in the synthesis of particular proteins (20, 24, 26). Synthesis of some proteins is greatly enhanced, whereas synthesis of others is repressed. The major heat shock-inducible proteins of *S. cerevisiae* range in molecular weight from 70,000 to 85,000 (23). In this paper we report the identification in *S. cerevisiae* of a multigene family which is related to the *Drosophila* hsp 70 gene.

MATERIALS AND METHODS

General methods. Restriction enzyme digestion, agarose and acrylamide gel electrophoresis, selection of polyadenylic acid-containing RNA, blotting of genomic DNA to nitrocellulose, DNA-DNA hybridiza-

tion, plasmid DNA isolation, labeling of DNA by polynucleotide kinase and nick translation (7, 15), DNA sequence analysis (22), and cDNA extension and S1 nuclease digestion (4, 14) were carried out as described previously.

Isolation of YG100 and YG101. YG100 and YG101 were isolated from a library of *S. cerevisiae* strain S288C DNA. The DNA was partially digested with *Sau*IIIA, and the fragments were cloned by using the *Bam*HI arms of Charon 28 (28a). This *S. cerevisiae* library was screened by probing with a labeled plasmid (B8) which contains a copy of the *Drosophila* hsp 70 gene inserted into the *Bam*HI site of pBR322 (7). The hybridization solution contained 30% formamide, 0.75 M NaCl, 0.075 M sodium citrate, 0.1% sodium dodecyl sulfate, 1.0 mM EDTA, 10 mM Tris (pH 7.5), and 1× Denhardt solution (9) and was incubated at 37°C for 24 h. In the initial screening two phages (λ100 and λ101) which hybridized with B8 were isolated. The regions containing homology with B8 were subcloned into pBR322. An 8-kilobase (kb) *Bam*HI-*Hin*dIII fragment of λ100 was subcloned, and the resulting plasmid was designated YG100; and 3-kb *Hin*dIII fragment of λ101 was subcloned, and the resulting plasmid was designated YG101.

Growth of *S. cerevisiae* and heat shock procedure. *S. cerevisiae* was grown in YPD broth (2% peptone [Difco Laboratories], 2% glucose, 1% yeast extract [Difco]) at 23°C in 30-ml portions. Each culture to be heat shocked was placed in a 50°C bath until the temperature of the culture reached 37°C, usually within 2 min. The culture was then incubated with shaking for 20 min at 37°C before harvesting.

RNA isolation and labeling. The RNA used in the dot blot experiments was isolated essentially by the method of Lindquist (20). Briefly, *S. cerevisiae* cells suspended in 0.1 M Tris (pH 7.5)–0.1 M LiCl–0.01 M dithiothreitol were broken by blending in a Vortex mixer in the presence of glass beads, phenol, chloroform, and 0.5% sodium dodecyl sulfate. After several phenol extractions, the nucleic acid was precipitated with ethanol several times before use. RNA was partially degraded by alkali and labeled with ^{32}P by using T4 polynucleotide kinase, as described previously (5). The RNA used in the cDNA extension and S1 nuclease experiments was isolated by the guanidine thiocyanate extraction procedure (6) after spheroplasts had been formed by incubation in the presence of Zymolyase (Miles Biochemicals).

DNA dot blots. DNA was placed on nitrocellulose filters (pore size, 0.45 μm; Schleicher & Schuell Co.) essentially under the conditions described by Thomas (31). Before blotting, plasmid DNA was digested with *Hin*dIII, phenol extracted, and ethanol precipitated. The DNA was suspended in water, denatured by boiling, and adjusted to 10× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), and 0.5 μg was applied to the filter in a volume of 5 μl. Hybridization was carried out in a solution containing 50% formamide, 5× SSC, 0.2% sodium dodecyl sulfate, 10 mM EDTA, and 10 mM Tris-hydrochloride (pH 7.5) at 40°C.

RESULTS

Primary sequence of YG100 and YG101. We previously described the primary sequence of the protein-encoding region of one copy of the *Drosophila* hsp 70 gene (15). One reading frame is devoid of stop codons for more than 2,000 base pairs; the region between the first ATG and the first stop codon could encode a protein of 70,270 daltons. The recombinant plasmids YG100 and YG101 containing *S. cerevisiae* DNA were isolated on the basis of homology to a *D. melanogaster* hsp 70 gene (see above). Partial restriction maps of the clones are shown in Fig. 1. Primary sequences were determined by using the chemical cleavage method (22). The regions analyzed are indicated in Fig. 1 in the enlarged portions of the restriction maps.

Regions of homology among YG100, YG101, and the *Drosophila* hsp 70 gene were identified. The primary DNA sequences were determined for 76 and 56% of the protein-encoding regions of YG100 and YG101, respectively (Fig. 2). The predicted *Drosophila* hsp 70 protein contains 641 amino acids (Fig. 3). DNA sequences were determined in the following regions: in YG100, amino acids 1 through 342 and 393 through 541; in YG101, amino acids 1 through 73, 175 through 242, 256 through 397, and 438 through 529. Therefore, a sequence was obtained for both yeast genes in the following regions (using the predicted *Drosophila* hsp 70 amino acid sequence as a reference): amino acids 1 through 73, 175 through 242, 256 through 342, 393 through 397, and 438 through 529. This amounted to 50% of the coding region of the *Drosophila* gene. No termination codons, insertions, or deletions which would result in a change in the reading frame were found. However, several insertions or deletions of multiples of three bases were found in both genes. In the regions analyzed, YG100 contained four single amino acid deletions compared with the *Drosophila* hsp 70 gene, whereas YG101 contained four single amino acid deletions and one single and two double amino acid insertions compared with the *Drosophila* gene. The predicted amino termini differed among the three genes. YG100 had one additional amino acid, and YG101 had six additional amino acids. This type of heterogeneity in amino termini has been observed among *Drosophila* genes related to the *Drosophila* hsp 70 gene (14; unpublished data). Because of this heterogeneity, our analysis of homology began at amino acid 3.

The two *S. cerevisiae* genes had the same amino acids at 210 of the 322 positions analyzed (65%). In the regions analyzed, the predicted amino acid sequences of YG100 and YG101 were 72 and 64% homologous, respectively, to the *Drosophila* hsp 70 gene. If only the regions for which data were available for both *S. cerevisiae* genes were considered, the same values of homology to the *Drosophila* hsp 70 gene were found. Often, the amino acids substituted in the

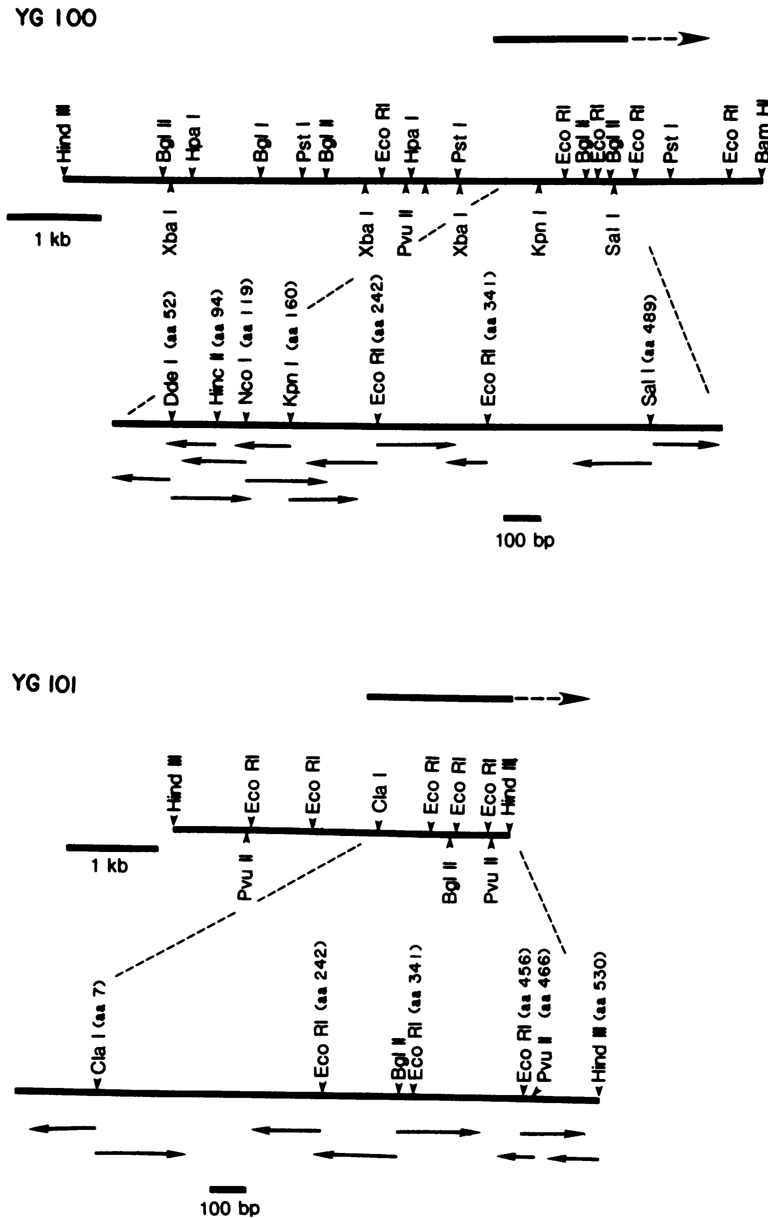

FIG. 1. Restriction map and strategy used to determine the nucleotide sequences of regions of YG100 and YG101. The restriction maps were deduced as previously described (7). The solid portions of the arrows above the maps indicate the regions which have been directly shown to be homologous to the *Drosophila* hsp 70 gene. In the case of YG100, the dashed region represents regions of homology predicted if an entire gene hsp 70-related gene exists in a continuous segment, and in the case of YG101, the dashed line indicates that the 3′ end of the gene is not contained within the fragment. DNA sequencing was carried out from each of the sites indicated by the arrows underneath the enlarged portions of the maps. The lengths of the arrows are proportional to the number of nucleotides actually sequenced from each start. In the analysis of YG100 and YG101, four restriction fragment junctures were not crossed; therefore, the sequences must be considered tentative. In YG100 these junctures were the *Eco*RI site at amino acid (aa) 241 and the *Bgl*II site at amino acid 309. The *Cla*I site at amino acid 7 and the *Bgl*II site at amino acid 324 are the junctures in YG101 which were not crossed.

FIG. 2. Comparison of the primary sequences of a *Drosophila* hsp 70 gene, YG100, and YG101. The DNA sequences are aligned with the *Drosophila* 87C hsp 70 sequence, which is taken from Ingolia et al. (15). Each dot indicates that the aligned DNA contains the same base as the *Drosophila* gene. Each dash indicates a deletion relative to the aligned DNA. The solid lines indicate that the DNA sequence was not determined. A triplet is underlined if the base change(s) results in an amino acid (aa) change. The first ATG of the *Drosophila* hsp 70 gene was designated amino acid 1.

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                                                                                                                              aa24
Dr. hsp70                               ATG CCT GCT ATT GGA ATC GAT CTG GGC ACC ACC TAC TCC TGC GTG GGT GTC TAC CAG CAT GGC AAG GTT GAG
YG100                               ATG TCA AAA ... G.C ..T ..T ... T.A ..T ..A ..A ... ..G ..T ..T .C. CAC .TT GCT A.. .AT CGT ..G ..C
YG101       ATG GCT GAA GGT GTT TTC CAA GG. ... ..C ..T ... ... T.A ..T ..A ... ... ..T ..T ..T .C. ACT ... G.A TCC TC. --- ... ..A

                                                                                                                      aa54
Dr. hsp70   ATT AAC GCC TAT GAC CAG GGC AAC CGC ACC ACG CCG TCC TAC GTG GCT TTC ACA GAC TCG GAA CGC CTC AAT GGT GAA CCG GCC AAG AAC
YG100       ... .TT ... A.C ..T ..A ..T ... A.A ... ..T ..A ..T .TT ..C ... ... ..T ... A.T ... A.A T.G .T. ... ..T G.T ..T ... ..T
YG101       ... .TT ... A.C ..A ..A ..T ... A.A GT. ..C ..A ..T .T. ..T ... ... ..T CCA GAA ... A.A T.G .T. ... ..T G.T ... ... ...

                                                                                                                      aa84
Dr. hsp70   CAG GTG GCC ATG AAC CCC AGA AAC ACA GTG TTT GAC GCC AAG CGA CTC ATC GGC CGA AAA TAC GAC GAT CCC AAA ATC GCA GAG GAC ATG
YG100       ..A .CT ..T ... ..T ..T TCG ..T ..C ..T ..C ... ..T ... ..T T.G ... ..T A.. ..C .T. A.. ..C ..A G.. G.G CAG .CT ... ...
YG101       ..A .CT ..T T.. ... ..A ... ... ..T ..C ..C ..T ..T ... ..T T.G ..T ..T A.. -------------------------------------------

                                                                                                                      aa114
Dr. hsp70   AAG CAC TGG CCT TTC AAA GTT GTA AGC GAT GGC GGA AAG CCC AAG ATC GGG GTG GAG TAT AAG GGT GAG TCC AAG AGA TTT GCT CCC GAG
YG100       ... ... .TC ..A ... ..G T.G A.C GAT .T. .A. ..T ... ..T C.A ..T CAA ..T ..A .T. ... ... ..A A.. ... .AC ... A.C ..A ..A
YG101       -----------------------------------------------------------------------------------------------------------------------

                                                                                                                      aa144
Dr. hsp70   GAG ATC AGT TCG ATG GTG CTG ACC AAG ATG AAG GAG ACG GCG GAG GCG TAT CTG GGC GAG AGC ATC ACG GAT GCA GTC ATC ACA GTT CCA
YG100       C.A ... TCC ..C ... ..C T.. GGT ... ... ... ..A ..T ..C ..A T.T ..C T.. ..A .CC .AG G.. .AT ..C ..T ... G.. ..T ..C ...
YG101       -----------------------------------------------------------------------------------------------------------------------

                                                                                                                      aa174
Dr. hsp70   GCT TAC TTC AAC GAC TCT CAG CGC CAG GCT ACC AAA GAC GCC GGT CAC ATC GCC GGC CTG AAT GTG CTC CGC ATC ATC AAT GAG CCC ACG
YG100       ... ... ... ... ..T ... ..A A.A ..A ... ... ..G ..T ..T ... AC. ..T ..T ..T T.. ... ..C T.G ..T ..T ..T ..C ..A ..T ..C
YG101       -----------------------------------------------------------------------------------------------------------------------

                                                                                                                      aa202
Dr. hsp70   GCG GCA GCA TTG GCC TAC GGA CTG GAC --- --- AAG AAT CTC AAG GGT GAG CGC AAT GTG CTT ATC TTC GAC TTG GGC GGC GGC ACC TTC
YG100       ..C ..T ..C A.T ..T ... ..T T.. ... --- --- ... ..G GGT ... .AA ..A .A. --- ..C T.G ..T ... ... ... ..T ..T ..T ..T ...
YG101       .TT .TT .TT A.T .TT ... ..T T.A .GT GTT GGT ... TTC GAA ... --- ..A A.A C.. ..T T.G ..T ... ..T ... ..T ..T ..T ..T ...

                                                                                                                      aa232
Dr. hsp70   GAT GTC TCC ATC CTG ACC ATC GAC GAG GGA TCT CTG TTC GAG GTG CGC TCC ACA GCC GGA GAC ACA CAC TTG GGC GGC GAG GAC TTT GAC
YG100       ... ... ..T T.G T.. TT. ..T ..A ..C ..T --- A.C ..T ..A ..T AAG G.. ..C ..T ..T ... ..C ..T ... ..T ..T ..A ..T ... ...
YG101       ... ..T ... T.G T.. CA. ..T .CT .GT ..T --- G.T .A. ACT ..T AAA ..T ..T T.. ..T A.. ..T ... ... ..T ..T C.A ..T ..C ...

                                                                                                                      aa261
Dr. hsp70   --- AAC CGG CTA GTC ACC CAC CTG GCG GAG GAG TTC AAG CGC AAG TAC AAG AAG GAT CTG CGC TCC AAC CCT CGC GCC CTA CGA CGC CTC
YG100       --- ... AGA T.G ... .A. ... T.C ATC CAA ..A ... ... A.A ... A.. ... ... ..C T.. TCT A.. ... .AA A.A ..T T.G A.. A.A T.A
YG101       ACC ... TT. T.G .AA CA. TT. AA. ..C ..A TTC --------------------------------------------------- A.A ..T T.G A.. A.A T.G

                                                                                                                      aa291
Dr. hsp70   AGA ACA GCA GCT GAA CGG GCC AAG CGC ACA CTC TCC TCT AGC ACG GAG GCC ACC ATC GAG ATC GAC GCA TTG TTT GAG GGC CAA GAC TTC
YG100       ... ..C ..T TG. ... A.C CAA G.. AA. TTT G.. ... ..C --- G.T C.A A.T T.. G.T ..A ..T ... T.. AA. AAC ..A ..T ATC ..T ...
YG101       ... ..T ..T ... ... A.A ..T ... A.A ..C T.A ..T ... GT. ..T C.A A.T ... G.T ..A G.T ... T.T ... ... ..C ..T G.. ..T ...

                                                                                                                      aa321
Dr. hsp70   TAC ACC AAA GTA AGC CGT GCC AGG TTT GAG GAG CTG TGC GCG AAC CTC TTC CGC AAC ACC CTG CAG CCT GTG GAG AAG GCC CTC AAC GAT
YG100       ... ..T TCC A.C .C. A.A ... ..A ..C ..A ..A T.. ..T ..T G.. T.G ... A.A TCT ..T T.. G.C ..A ..T ..A ... .T. T.G .GA ...
YG101       G.A T.. TCT T.G .CT A.A ..T ..A ... ..A ..C T.. AA. ..C GCA T.G ... AAG TCT ..T T.. G.A ... ..T ..A C.A .TT T.G ..G ...

                                                                                                                      aa351
Dr. hsp70   GCC AAG ATG GAC AAG GGT CAG ATC CAC GAC ATC GTG CTC GTC GGC GGA TCC ACT CGC ATT CCC AAG GTG CAA AGT CTG CTG CAG GAG TTC
YG100       ..T ..A T.. ... ..A TC. ..A G.. G.T ..A ..T ..C T.G ... ..T ..T ..T ..C A.A ... ---------------------------------------
YG101       ..T ... ..C TCT ... TC. ..A ... G.. ..A G.T ..C T.G ..T ..T ..T ... ..C A.A ... ..A ... ..C ... .AG T.. T.G TCT ..C ...

                                                                                                                      aa381
Dr. hsp70   TTC CAC GGC AAG AAC CTC AAC CTA TCC ATC AAC CCA GAC GAG GCA GTG GCA TAC GGA GCT GCT GTG CAG GCC GCT ATC CTC AGC GGA GAC
YG100       -----------------------------------------------------------------------------------------------------------------------
YG101       ..T G.. ..T ... C.A T.G G.A AA. ..T ..T ... ... ..T ..A ..T ..T ..T ... ..T ... ... ..T ..A .GT ... ... T.G .C. ..C ---

                                                                                                                      aa409
Dr. hsp70   CAG AGC --- --- GGC AAG ATC CAG GAC GTG CTG CTG GTG GAC GTG GCC CCA CTT TCA TTG GGA ATT GAG ACC GCT GGA GGT GTA ATG ACC
YG100       ---------------------------------------------------- .GT ..C ..T ... T.A ..C ... ..T ... ..A ..T ... --- ... ..C ... ...
YG101       ..A TC. ACA TCT .A. G.A .C. A.. ... T.. T.. T.. T.A ..T ..T ..T ... T.A -----------------------------------------------

                                                                                                                      aa439
Dr. hsp70   AAG CTG ATC GAG CGC AAC TGT CGC ATT CCG TGC AAG CAG ACT AAG ACG TTC TCC ACG TAC TCG GAC AAC CAG CCC GGA GTC TCC ATC CAG
YG100       ... T.. ..T TCA A.A ... .C. AC. ... T.A ACA ... A.. TTC G.. .TC ..T ... ..T ..T G.T ..T ... ..A ..A ..T ... .TG ..T ..A
YG101       --------------------------------------------------------------------------------------------------------------- T.. .CA

                                                                                                                      aa469
Dr. hsp70   GTG TAT GAG GGC GAA CGT GCG ATG ACG AAG GAC AAC AAT GCA TTG GGC ACC TTC GAT CTG TCC GGC ATT CCA CCT GCA CCA AGG GGT GTG
YG100       ..C .T. ..A ..T ... A.A ..C .A. ..T ... ... ... ..C TTG ... ..T .AG ... ..A T.. AGT ..T ... ... ..A ..T ... ..A ... ..C
YG101       ..C ..C C.A ..T ... ... .TT .AC TGT ..A ..A ... .C. TTG ... ..T GAA ... ..C T.. AAG AAC ..C ... ATG ATG ... GCT ... .AA

                                                                                                                      aa499
Dr. hsp70   CCC CAG ATA GAA GTA ACC TTC GAC TTG GAC GCC AAT GGA ATC CTG AAC GTC AGC GCC AAG GAG ATG AGT ACG GGC AAG GCC AAG AAC ATC
YG100       ..A ..A ..T ... ..C ..T ... ..T G.C ... T.T ..C ..T ..T T.. ..T ..T TC. ... GTC ..A .A. G.. ..T ..T ... T.T ..C ..G ...
YG101       ..A GTC T.G ... .CT .T. ... ..A G.T ..T ..T ..C ..T ... T.. ..G ..T .CT ... GTC ..A .A. TCT ..C ..T ... T.T TCT ... ...

                                                                                                                      aa529
Dr. hsp70   ACG ATC AAG AAC GAC AAG GGA CGC CTC TCG CAG GCC GAG ATT GAT CGC ATG GTG AAC GAG GCT GAG AAG TAC GCC GAC GAG GAC GAA AAG
YG100       ..T ..T .CC ... ... ... ..T A.A T.G ..C A.. .AA ..T ..C ..A AAG ... ..T GCT ..A ..C ..A ..A .T. AAG ..A ..A ..T ... ...
YG101       ..T ... TCT ... .CT GTT ..T A.A T.G ..T TCT .AA ..A ... ..A AAG ... ..T ... C.A ... ..A G.. .T. AAG .CT .CC ..T ... GCT

Dr. hsp70   CAT CGC CAG CGC ATA ACC TCT AGA AAT GCT CTG GAG
YG100       G.A TCT ..A A.A ..T G.T ..C .AG ..C CAA T.. ..A
YG101       -----------------------------------------------
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                                                                                                        aa24
Dr. hsp70                      met pro ala ile gly ile asp leu gly thr thr tyr ser cys val gly val tyr gln his gly lys val glu
YG100                      met ser lys  *  val  *   *   *   *   *   *   *   *   *   *   *  ala his phe ala asn asp arg  *  asp
YG101      met ala glu gly val phe gln gly  *   *   *   *   *   *   *   *   *   *   *   *  ala thr  *  glu ser ser  Δ   *   *

                                                                                                                        aa54
Dr. hsp70  ile asn ala tyr asp gln gly asn arg thr thr pro ser tyr val ala phe thr asp ser glu arg leu asn gly glu pro ala lys asn
YG100       *  ile  *  asn  *   *   *   *   *   *   *   *   *  phe  *   *   *   *   *  thr  *   *   *  ile  *  asp ala  *   *   *
YG101       *  ile  *  asn glu  *   *   *   *  val  *   *   *  phe  *   *   *   *  pro glu  *   *   *  ile  *  asp ala  *   *   *

                                                                                                                        aa84
Dr. hsp70  gln val ala met asn pro arg asn thr val phe asp ala lys arg leu ile gly arg lys tyr asp asp pro lys ile ala glu asp met
YG100       *  ala  *   *   *   *  ser  *   *   *   *   *   *   *   *   *   *   *   *  asn phe asn  *   *  glu val gln ala  *   *
YG101       *  ala  *  leu  *   *   *   *   *   *   *   *   *   *   *   *   *   *   *  ———————————————————————————————————————————

                                                                                                                        aa114
Dr. hsp70  lys his trp pro phe lys val val ser asp gly gly lys pro lys ile gly val glu tyr lys gly glu ser lys arg phe ala pro glu
YG100       *   *  phe  *   *   *  leu ile asp val asp  *   *   *  gln  *  gln  *   *  phe  *   *   *  thr  *  asn  *  thr  *   *
YG101      ———————————————————————————————————————————————————————————————————————————————————————————————————————————————————

                                                                                                                        aa144
Dr. hsp70  glu ile ser ser met val leu thr lys met lys glu thr ala glu ala tyr leu gly glu ser ile thr asp ala val ile thr val pro
YG100      gln  *   *   *   *   *   *  gly  *   *   *   *   *   *   *  ser  *   *   *  ala lys val asn  *   *   *  val  *   *   *
YG101      ———————————————————————————————————————————————————————————————————————————————————————————————————————————————————

                                                                                                                        aa174
Dr. hsp70  ala tyr phe asn asp ser gln arg gln ala thr lys asp ala gly his ile ala gly leu asn val leu arg ile ile asn glu pro thr
YG100       *   *   *   *   *   *   *   *   *   *   *   *   *   *   *  thr  *   *   *   *   *   *   *   *   *   *   *   *   *   *
YG101      ———————————————————————————————————————————————————————————————————————————————————————————————————————————————————

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Dr. hsp70  ala ala ala leu ala tyr gly leu asp  Δ   Δ  lys asn leu lys gly glu arg asn val leu ile phe asp leu gly gly gly thr phe
YG100       *   *   *  ile  *   *   *   *   *   Δ   Δ   *  lys gly  *  glu  *  his  Δ   *   *   *   *   *   *   *   *   *   *   *
YG101      val val val ile val  *   *   *  gly val gly  *  phe glu  *   Δ   *   *  his  *   *   *   *   *   *   *   *   *   *   *

                                                                                                                        aa232
Dr. hsp70  asp val ser ile leu thr ile asp glu gly ser leu phe glu val arg ser thr ala gly asp thr his leu gly gly glu asp phe asp
YG100       *   *   *  leu  *  phe  *  glu asp  *   Δ  ile  *   *   *  lys ala  *   *   *   *   *   *   *   *   *   *   *   *   *
YG101       *   *   *  leu  *  his  *  ala gly  *   Δ  val tyr thr  *  lys  *   *  ser  *  asn  *   *   *   *   *   *  gln  *   *   *

                                                                                                                        aa261
Dr. hsp70   Δ  asn arg leu val thr his leu ala glu glu phe lys arg lys tyr lys lys asp leu arg ser asn pro arg ala leu arg arg leu
YG100       Δ   *   *   *   *  asn  *  phe ile gln  *   *   *   *   *  asn  *   *   *   *  ser thr  *  gln  *   *   *   *   *   *
YG101      thr  *  leu  *  glu his phe lys  *   *  phe ———————————————————————————————————————————————   *   *   *   *   *   *

                                                                                                                        aa291
Dr. hsp70  arg thr ala ala glu arg ala lys arg thr leu ser ser ser thr glu ala thr ile glu ile asp ala leu phe glu gly gln asp phe
YG100       *   *   *  cys  *  ser gln glu asn phe val  *   *   Δ  ala gln thr ser val  *   *   *  ser lys asn  *   *  ile  *   *
YG101       *   *   *   *   *   *   *   *   *   *   *   *   *  val  *  gln thr  *  val  *  val  *  ser  *   *  asp  *  glu  *   *

                                                                                                                        aa321
Dr. hsp70  tyr thr lys val ser arg ala arg phe glu glu leu cys ala asn leu phe arg asn thr leu gln pro val glu lys ala leu asn asp
YG100       *   *  ser ile thr  *   *   *   *   *   *   *   *   *  asp  *   *   *  ser  *   *  asp  *   *   *   *  val  *  arg  *
YG101      glu ser ser leu thr  *   *   *   *   *  asp  *  asn  *  ala  *   *  lys ser  *   *  glu  *   *   *  gln val  *  lys  *

                                                                                                                        aa351
Dr. hsp70  ala lys met asp lys gly gln ile his asp ile val leu val gly gly ser thr arg ile pro lys val gln ser leu leu gln glu phe
YG100       *   *  leu  *   *  ser  *  val asp glu  *   *   *   *   *   *   *   *   *   *  ———————————————————————————————————————
YG101       *   *  ile ser  *  ser  *   *  asp glu val  *   *   *   *   *   *   *   *   *   *   *   *   *   *  lys  *   *  ser asp  *

                                                                                                                        aa381
Dr. hsp70  phe his gly lys asn leu asn leu ser ile asn pro asp glu ala val ala tyr gly ala ala val gln ala ala ile leu ser gly asp
YG100      ———————————————————————————————————————————————————————————————————————————————————————————————————————————————————
YG101       *  asp  *   *  gln  *  glu lys  *   *   *   *   *   *   *   *   *   *   *   *   *   *  gly  *   *   *  thr  *   Δ

                                                                                                                        aa409
Dr. hsp70  gln ser  Δ   Δ  gly lys ile gln asp val leu leu val asp val ala pro leu ser leu gly ile glu thr ala gly gly val met thr
YG100      ——————————————————————————————————————————————— gly  *   *   *   *   *   *   *   *   *   *   *   Δ   *   *   *   *
YG101       *   *  thr ser asp glu thr lys  *  leu  *   *  leu  *   *   *   *   *  ———————————————————————————————————————————————

                                                                                                                        aa439
Dr. hsp70  lys leu ile glu arg asn cys arg ile pro cys lys gln thr lys thr phe ser thr tyr ser asp asn gln pro gly val ser ile gln
YG100       *   *   *  ser  *   *  ser thr  *  ser thr  *  lys phe glu ile  *   *   *   *  ala  *   *   *   *   *   *  leu  *   *
YG101      ——————————————————————————————————————————————————————————————————————————————————————————————————————————— phe pro

                                                                                                                        aa469
Dr. hsp70  val tyr glu gly glu arg ala met thr lys asp asn asn ala leu gly thr phe asp leu ser gly ile pro pro ala pro arg gly val
YG100       *  phe  *   *   *   *   *  lys  *   *   *   *  leu  *   *  lys  *  glu  *   *   *   *   *   *   *   *   *   *   *   *
YG101       *   *  gln  *   *   *  val asn cys  *  glu  *  thr leu  *   *  glu  *   *   *  lys asn  *   *  met met  *  ala  *  glu

                                                                                                                        aa499
Dr. hsp70  pro gln ile glu val thr phe asp leu asp ala asn gly ile leu asn val ser ala lys glu met ser thr gly lys ala lys asn ile
YG100       *   *   *   *   *   *   *   *  val  *  ser  *   *   *   *   *   *   *   *  val  *  lys gly  *   *   *  ser asn lys  *
YG101       *  val leu  *  ala ile  *  glu val  *   *   *   *   *   *  lys  *  thr  *  val  *  lys  *   *   *   *  ser ser  *   *

                                                                                                                        aa529
Dr. hsp70  thr ile lys asn asp lys gly arg leu ser gln ala glu ile asp arg met val asn glu ala glu lys tyr ala asp glu asp glu lys
YG100       *   *  thr  *   *   *   *   *   *   *  lys glu asp  *  glu lys  *   *  ala  *   *   *   *  phe lys glu  *   *   *   *
YG101       *   *  ser  *  ala val  *   *   *   *  ser glu  *   *  glu lys  *   *   *  gln  *   *  glu phe lys ala ala  *   *  ala

Dr. hsp70  his arg gln arg ile thr ser arg asn ala leu glu
YG100      glu phe  *   *   *  ala  *  lys  *  gln  *   *
YG101      ———————————————————————————————————————————————
```

S. cerevisiae genes compared with the *Drosophila* hsp 70 gene were found to be of the same class (i.e., uncharged, aromatic, nonpolar, acidic, or basic). If amino acid substitutions of the same class were counted as homologies, the homologies to the *Drosophila* 70,000-dalton protein rose to 83 and 79% for YG100 and YG101, respectively. The amino termini were more homologous than the other portions of these genes analyzed. YG100 and YG101 were 75 and 73% homologous, respectively, to *Drosophila* hsp 70 in the amino-terminal segment (71 amino acids), compared with 72 and 64% if all segments analyzed were considered. In this same region YG100 and YG101 were 79% homologous, compared with an overall homology of 65%.

The *S. cerevisiae* genes were also compared with another member of the *Drosophila* multigene family. One of the *Drosophila* genes, which is located at cytological locus 70C, has been isolated and partially characterized (14). This gene, which is related to the hsp 70 gene, is transcribed at normal temperatures and is not induced by heat shock. The predicted sequence of the first 201 amino acids of this gene has been determined. The sequence data available allowed a comparison of 122 amino acids among the four genes (amino acids 1 through 73, 201 through 242, and 256 through 311). In these regions both the *Drosophila* hsp 70 gene and the gene contained within YG100 encode the same amino acid as the 70C *Drosophila* gene in 95 of 122 positions (78%). YG101 and the 70C *Drosophila* gene encode the same amino acid at 70 (65%) of the positions.

As expected, the two *S. cerevisiae* genes were also homologous with the hsp 70 gene at the nucleotide level. The genes contained within YG100 and YG101 were 63 and 59% homologous, respectively, to the *Drosophila* hsp 70 gene. The genes of YG100 and YG101 were 69% homologous at the nucleotide level. Some of this divergence in nucleotide sequence may be explained by a different codon preference of the two organisms; for example, *Drosophila* hsp 70 arginine residues are often encoded by CGC, whereas the *S. cerevisiae* genes tend to use the AGA codon.

Expression of RNAs homologous to YG100 and YG101. The transcription of YG100 and YG101 homologous sequences was analyzed under normal growth conditions and after heat shock. RNA was isolated from cultures growing at 23°C and from cultures rapidly shifted to 37°C and incubated for 20 min. Polyadenylic acid-containing RNA from equal numbers of cells was labeled in vitro with ^{32}P by using polynucleotide kinase and hybridized to YG100, YG101, or vector pBR322 DNA dotted onto nitrocellulose filters (31). The resulting autoradiograph is shown in Fig. 4. The intensity of the spot containing YG100 was greater when the DNA was hybridized to heat-shocked RNA than when it was hybridized to control RNA. This difference in intensity indicates that the heat-shocked cells contained more RNA homologous to YG100 than the normally growing cells did. This result suggests that transcription of RNA homologous to YG100 is increased after heat shock. Alternatively, the increased intensity could have been due to a preferential stability of YG100 homologous RNA in heat-shocked cells compared with control cells.

The heat shock-inducible sequences were localized to a portion of YG100. An analogous hybridization experiment used as a source of DNA a plasmid containing a 1.2-kb *Pst*I-*Eco*RI fragment of YG100. This fragment encompassed the amino terminus, extending from the codon of amino acid 242 to approximately 600 bases up-

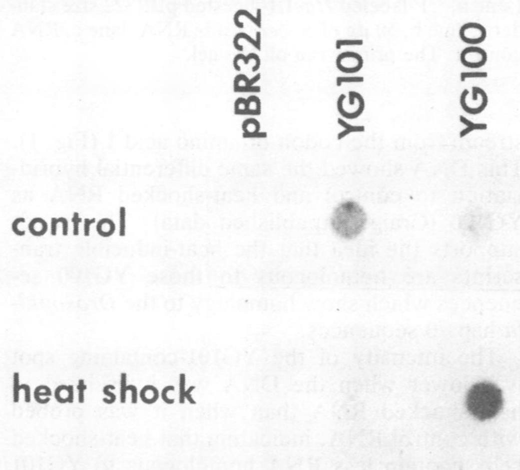

FIG. 4. Hybridization of control and heat-shocked RNAs to YG100 and YG101. A 0.5-μg amount of pBR322, YG100, or YG101 DNA was linearized by cleavage with *Hin*dIII, denatured, and spotted onto a nitrocellulose filter. Identical filters were hybridized as described in the text with polyadenylic acid-containing selected RNAs from untreated cells (control) and heat-shocked cells which had been labeled with ^{32}P in vitro. A total of 7.5×10^6 cpm of each RNA (5×10^7 cpm/μg) was hybridized in each case.

FIG. 3. Comparison of the proposed amino acid (aa) sequences of a *Drosophila* hsp 70 gene, YG100, and YG101. The amino acid sequences were deduced from the primary sequences shown in Fig. 2. The amino acids encoded by YG100 and YG101 are shown only at positions where differences occur relative to the *Drosophila* protein.

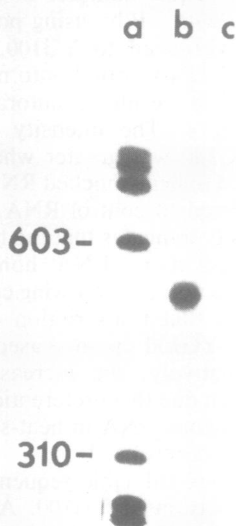

FIG. 5. Detection of RNA homologous to YG100 by cDNA synthesis. A *Hin*cII-*Kpn*I fragment that was labeled with ^{32}P at the *Hin*cII site (see Fig. 1) was used as a primer for cDNA synthesis. The cDNA was electrophoresed in a 6% acrylamide gel in 8 M urea. Lane a, ^{32}P-labeled *Hae*III-digested pBR322 size standard; lane b, 50 μg of *S. cerevisiae* RNA; lane c, RNA control. The primer ran off the gel.

stream from the codon of amino acid 1 (Fig. 1). This DNA showed the same differential hybridization to control and heat-shocked RNA as YG100 (Craig, unpublished data). This result supports the idea that the heat-inducible transcripts are homologous to those YG100 sequences which show homology to the *Drosophila* hsp 70 sequences.

The intensity of the YG101-containing spot was lower when the DNA was hybridized to heat-shocked RNA than when it was probed with control RNA, indicating that heat-shocked cells contain less RNA homologous to YG101 than control cells do. This difference is consistent with either a decrease in the transcription level or a preferential degradation of sequences homologous to YG101 upon heat shock.

The experiments described above demonstrated that RNAs homologous to recombinant plasmids YG100 and YG101 show regulation associated with heat shock. S1 nuclease and cDNA extension experiments were carried out to demonstrate more directly that sequences homologous to the putative protein-encoding regions are transcribed. A *Hin*cII-*Kpn*I fragment of YG100 which spans the protein-encoding region from the codon of amino acid 94 to the codon of amino acid 160 was 5′ end labeled with ^{32}P. The fragment was then denatured, hybridized under stringent conditions to *S. cerevisiae* RNA, and used as a primer for the synthesis of cDNA by reverse transcriptase. The size of the cDNA synthesized was determined by electrophoresis on acrylamide gels in 8 M urea. The 200-base primer was extended to approximately 510 bases (Fig. 5); in the absence of yeast RNA, no extension occurred. This result indicates that sequences homologous to the protein-encoding region are transcribed and that the size of the cDNA is consistent with an extension of about 25 bases beyond the codon for amino acid 1.

An S1 nuclease digestion experiment was performed with a *Cla*I fragment of YG101 (Fig. 6). This fragment extends from the codon for amino acid 12 to about 700 base pairs upstream from the bases that encode the amino terminus of the protein. After hybridization of the labeled *Cla*I fragment with RNA and subsequent cleavage with S1 nuclease, a 68-base fragment of DNA, which included 29 bases 5′ proximal to the codon for amino acid 1, remained. We conclude that YG101 or a very similar gene is transcribed in *S. cerevisiae* cells. Another strong band, 39 bases long, was also observed; 39 bases is the distance from the *Cla*I site to the ATG of codon

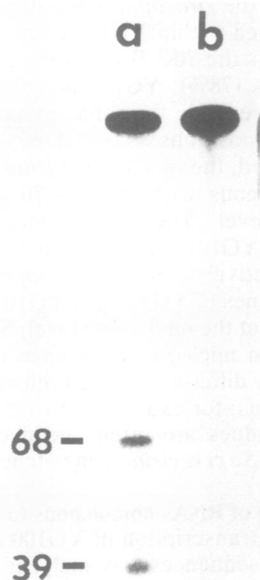

FIG. 6. Detection of RNA homologous to YG101 by S1 nuclease digestion. A *Cla*I fragment was labeled with ^{32}P, hybridized with RNA, and treated with Sl nuclease. Lane a, 50 μg of *S. cerevisiae* RNA; lane b, RNA control. The intense band in both lanes is the intact *Cla*I fragment. The sizes indicated on the left were determined from the sizes of fragments generated from a G-specific sequencing reaction carried out on the same *Cla*I fragment and run in an adjacent lane.

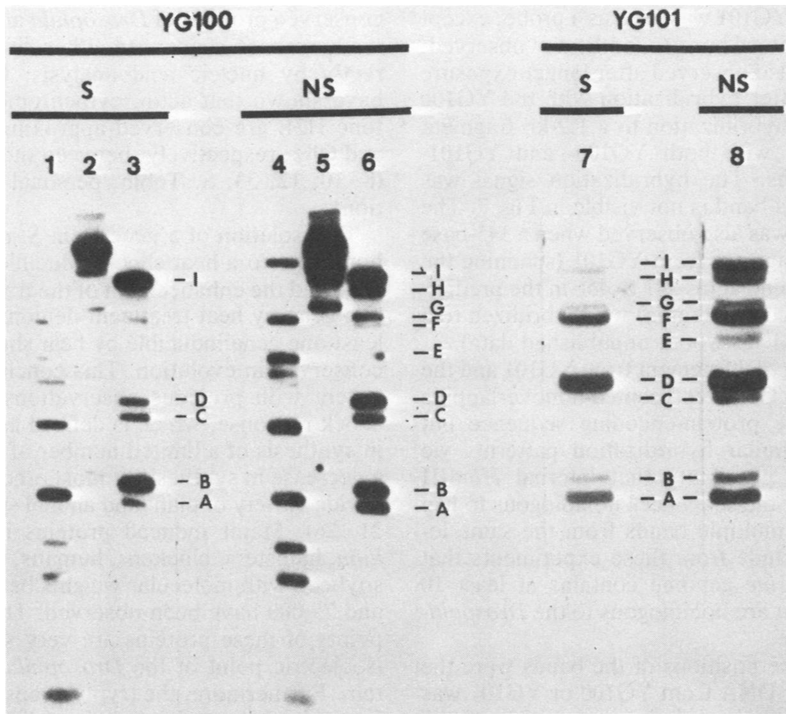

FIG. 7. Hybridizaton of YG100 and YG101 to *S. cerevisiae* genome DNA. A 3-μg portion of *S. cerevisiae* DNA was digested with *Eco*RI (lanes 1 and 4), *Bam*HI (lanes 2 and 5), or *Hin*dIII (lanes 3 and 6 through 8), electrophoresed on a 1% agarose gel, and transferred to filters. YG101 (lanes 7 and 8) and fragments of YG100 encompassing the codons of amino acids 160 through 305 (lanes 1 through 6) were labeled with ^{32}P by nick translation and end labeling, respectively, and hybridized to the filters in either 50% formamide (lanes 1 through 3 and 7) or 30% formamide (lanes 4 through 6 and 8) at 37°C. Hybridization in 50% formamide was referred to as stringent (S) conditions, whereas hybridization in 30% formamide was referred to as nonstringent (NS) conditions. The sizes of the *Hin*dIII fragments were determined by comparison with *Hin*dIII-digested adenovirus type 2 DNA run in parallel in these and other experiments. The sizes of these fragments were as follows (in kilobases): band A, 2.0; band B, 2.3; band C, 3.5; band D, 4.0; band E, 5.3; band F, 6.3; band G, 6.6; band H, 9; and band I, 10.5.

1, and this distance probably resulted from hybridization of an RNA transcribed from another member of the multigene family which was not homologous in the 5′ noncoding region. These cDNA extension and S1 nuclease digestion results indicate that RNAs are transcribed from these portions of YG100 and YG101 or from very similar genes which are also related to the *Drosophila* hsp 70 gene.

hsp 70-related sequences in the *S. cerevisiae* genome. To estimate the number of hsp 70-related sequences in the *S. cerevisiae* genome, *S. cerevisiae* DNA was digested with several restriction enzymes, subjected to electrophoresis in an agarose gel, transferred to nitrocellulose by the method of Southern (30), and hybridized with all or portions of the protein-encoding region of YG100 or YG101 (Fig. 7). If sequences homologous to hsp 70 reside in different flanking sequences, restriction fragments homologous to hsp 70 which have at least one end in non-hsp 70 DNA are likely to be unique in size. Therefore, the number of bands detected allowed an estimate to be made of the reiteration of hsp 70-related sequences in the *S. cerevisiae* genome.

End-labeled fragments of YG100 (spanning the segment of DNA which encoded amino acids 160 to 304) were hybridized to *S. cerevisiae* DNA cleaved with *Eco*RI, *Bam*HI, and *Hin*dIII. Multiple bands were observed in the digests of each of the enzymes, but the *Hin*dIII digest was the most informative. The *Bam*HI fragments which hybridized were large and therefore were not clearly resolved in this experiment. The *Eco*RI-generated restriction fragment which hybridized probably reflected an overestimate of the gene copy number because the probes from YG100 spanned an *Eco*RI site. The probe from YG100 did not contain a *Hin*dIII site; the eight bands in Fig. 7, lane 6, constitute an estimate of the gene redundancy in the *S. cerevisiae* genome. Figure 7, lane 8, shows that the pattern of hybridization of genomic *Hin*dIII-cleaved DNA was identical to the pattern obtained with

YG100 when YG101 was used as a probe, except that one additional band (band E) was observed. Band E was also observed after longer exposure of the filter after hybridization with the YG100 probe. Also, hybridization to a 1.2-kb fragment was detected with both YG100- and YG101-specific probes. The hybridization signal was weak, and this band is not visible in Fig. 7. The same pattern was also observed when a 345-base pair *Eco*RI fragment from YG101 (spanning the codons for amino acids 341 to 456 in the predicted protein-encoding region) was hybridized to a similar blot (M. Ellwood, unpublished data).

Since the *Eco*RI fragment from YG101 and the probes from YG100 represented nonoverlapping regions of the protein-encoding sequence but resulted in similar hybridization patterns, we excluded the possibility that internal *Hin*dIII sites in any of the sequences homologous to hsp 70 generated multiple bands from the same locus. We conclude from these experiments that the *S. cerevisiae* genome contains at least 10 sequences that are homologous to the *Drosophila* hsp 70 gene.

Although the positions of the bands were the same whether DNA from YG100 or YG101 was used as probe, the relative intensities of the bands were not the same. This is shown clearly in Fig. 7, lanes 3 and 7, where hybridization was performed under more stringent conditions. For instance, bands B and I were relatively intense when DNA was hybridized to YG100, whereas bands A, D, and F were more intense when YG101 was used as the probe. Clearly, the hsp 70-related sequences in *S. cerevisiae* differ in the extent of homology to YG100 and YG101.

DISCUSSION

Two members of an *S. cerevisiae* multigene family which are homologous to the gene encoding the major heat-inducible protein of *Drosophila* (hsp 70) were isolated. Our evidence suggests that transcription of one of these genes (YG100) or a closely related gene is enhanced by heat shock and that the amount of RNA homologous to the other gene (YG101) is reduced. The primary DNA sequences of YG100 and YG101 determined thus far resemble the sequences of functional genes in that distinguishing characteristics of pseudogenes, such as frameshift mutations and large deletions or insertions, have not been found. Although no mutations which would disrupt the reading frame have been found, the several small insertions or deletions found cause the addition or loss of one or two amino acids compared with the *Drosophila* hsp 70 protein. A comparison of three-fourths of the predicted amino acid sequences of YG100 and *Drosophila* hsp 70 showed 72% homology, thus demonstrating remarkable sequence conservation. Other conserved proteins of *Drosophila* and *S. cerevisiae* have been sequenced either directly or indirectly by nucleic acid analysis. Comparisons have shown that actin, cytochrome *c*, and histone H2B are conserved approximately 88, 58, and 69%, respectively, between the two species (8, 10, 12, 33; S. Tobin, personal communication).

The isolation of a gene from *S. cerevisiae* by homology to a heat shock-inducible *Drosophila* gene and the enhancement of the transcription of this gene by heat treatment demonstrate that at least one gene inducible by heat shock has been conserved in evolution. This conclusion is consistent with previous observations that a heat shock response, which is defined as an increase in synthesis of a limited number of proteins and a decrease in synthesis of most others, occurs in a wide variety of plant and animal species (3, 16, 21, 26). Major induced proteins in *Dictyostelium*, hamsters, chickens, humans, tobacco, and soybean with molecular weights between 70,000 and 75,000 have been observed. The isoelectric points of these proteins are very similar to the isoelectric point of the *Drosophila* hsp 70 protein. Furthermore, the tryptic maps of the major heat shock proteins of two diverse species (chickens and hamsters) are very similar (34). Also, Kelley and Schlessinger (17) recently demonstrated that antibodies to the chicken 70,000-dalton heat shock protein cross-react with proteins of *Drosophila*, *Xenopus*, mice, humans, and yeasts.

The function of heat shock proteins has not been determined. However, it has been shown in yeasts, *Dictyostelium*, and *Drosophila* that a pretreatment at nonlethal temperatures which results in induction of heat shock proteins dramatically improves the ability of cells to withstand a normally lethal heat shock (21, 23, 27). This increase in the ability to survive a stress suggests a physiological function for the heat shock-inducible proteins. The maintenance of a gene for a heat shock protein in evolution also indicates a probable biological importance for the heat shock response.

The results reported here indicate that *S. cerevisiae* contains a multigene family of sequences, about 10 in number, which are related to the hsp 70 gene of *Drosophila*. This is an unusually large multigene family for a yeast; the previously identified multigene families which encode proteins contain only a few members. For instance, *S. cerevisiae* contains only one actin gene (12, 28), whereas *Drosophila* contains six (11, 32). *S. cerevisiae* contains two copies of the histone H2B gene (33), whereas *Drosophila* contains approximately 100 copies (19). At least one member of the *S. cerevisiae* hsp 70-related multigene family, YG100 or a closely related

gene, is heat inducible; transcription of another member, YG101 or a closely related gene, is not enhanced by heat shock and is perhaps repressed. Recently, in *Drosophila* we have found a multigene family that is related to the hsp 70 gene (14; unpublished data). This multigene family contains both heat-inducible members (hsp 70, hsp 68) and at least three members which are transcribed under normal growth conditions and not induced by heat shock. We conclude that an analogous multigene family exists in *S. cerevisiae*. YG101 has been identified as a member which is transcribed at normal growth temperatures and is not induced by heat shock. The protein products of the genes transcribed at normal growth temperatures may perform the same or analogous functions under normal growth conditions as hsp 70 performs under conditions of stress. These results suggest that the members that are not inducible by heat shock are also conserved in evolution. Such multigene families of hsp 70-related sequences under complex transcriptional control may well be found in many plant and animal species.

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