The Caenorhabditis elegans hsp70 gene family: a molecular genetic characterization

(Recombinant DNA; nematode; heat shock; polypeptide; gene mapping; mRNA levels; promoter; introns; phage λ vectors; exons; probes)

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SUMMARY

We have isolated genomic clones representing six distinct members of the Caenorhabditis elegans 70-kDa heat-shock protein gene (hsp70) family. Each member exists as a single copy element in the C. elegans genome. Transcripts of four of the hsp70 genes have been detected by Northern-blot analysis. One member, hsp70C, appears to be a heat-shock-cognate hsp70 gene (hsc70) since its transcription is developmentally regulated and is not increased in response to heat shock. Transcripts of another gene, hsp70A, are abundant in control worms and are also increased (two- to six-fold) upon heat shock. Nucleotide sequencing of genomic and cDNA clones of hsp70A reveals that it is highly homologous to Drosophila and yeast heat-shock-inducible and heat-shock-cognate hsp70 genes. Three DNA elements homologous to the heat-shock promoter, 5′-C-GAA-TTC-G-G-3′, are located upstream from the Hsp70A-coding region. We find that hsp70A contains three introns, one of which is in a similar position with an intron in the Drosophila hsc1 and hsc2 genes. Finally, utilizing strain-specific restriction fragment length differences, we have mapped the chromosomal position of hsp70A to the far right of chromosome IV.

INTRODUCTION

All organisms tested respond to a sudden elevation of temperature by inhibiting the expression of most proteins normal to development while simultaneously synthesizing a small set of proteins, called heat-shock proteins (Hsps; reviewed by Ashburner and Bonner, 1979; Craig, 1985; Schlesinger et al., 1982). In Drosophila, genes encoding the major Hsps have been cloned and sequenced (Craig and McCarthy, 1980; Hackett and Lis, 1983; Holmgren et al., 1979; Ingolia and Craig, base pair(s); hsc70, heat-shock 70 cognate gene; HSE, heat-shock element; Hsp, heat-shock protein(s); hsp, gene coding for Hsp; HSTF, heat-shock transcription factor; kb, 1000 bp; N2, Bristol (strain); NGM, nematode growth media; RFLD, restriction fragment length difference; SDS, sodium dodecyl sulphate; SSPE, 180 mM NaCl, 10 mM NaH2PO4, 1 mM EDTA, pH 7.4.)
1982; Ingolia et al., 1980; Karch et al., 1981; Moran et al., 1979). In Drosophila the hsp70 have been grouped into three classes: a single hsp83 gene; the hsp68 and hsp70 genes; and four low-M, hsp genes, hsp22, hsp23, hsp26 and hsp28. Members within each class are structurally related and there has been a general conservation throughout evolution of the three classes of hsp genes. The Hsp70 protein is the most highly conserved of the Hsps (reviewed by Craig, 1985).

Transcription studies using cloned hsp70 genes have identified three upstream promoter elements. One of these, the TATA box, is found about 30 bp 5' to the mRNA start point of most RNA polymerase II genes (Benoist et al., 1980). The second element, the CCAAT box, is also found upstream from many polymerase II genes and confers constitutive hsp70 expression in Xenopus oocytes (Bienz, 1986). The third promoter element is found in multiple copies located upstream from the TATA box and is required for the positive control of hsp70 transcription (Pelham, 1982). A consensus element has been derived for the HSE, 5'-C--GAA--TTC-G--3'. Conservation of the mechanisms governing transcription of heat-shock genes is suggested by the fact that the Drosophila HSE functions in mammalian cells (Pelham and Bienz, 1982; Burke and Ish-Horowtiz, 1982) and that similar elements are found upstream from hsp genes of other organisms (Bienz, 1985; Pelham, 1985). A positive-acting HSTF identified in Drosophila and yeast, binds to this promoter element and is required for heat-inducible transcription (Parker and Topol, 1984; Topol et al., 1985).

The conservation throughout evolution of both the hsp70 gene family and the mechanism of induction of these genes has attracted much attention towards their study as model system for inducible gene transcription. Relatively little, however, is yet known concerning the function of Hsp70 either during heat-shock or during normal growth when some members of the hsp70 gene family (called hsc70 genes: Craig et al., 1983; Ingolia and Craig, 1982) are expressed. In addition, except for the case of Drosophila (reviewed by Craig, 1985), the molecular organization of the hsp70 gene families of other organisms has not been reported.

We report here the cloning and analysis of six members of the hsp70 gene family of C. elegans. We find that C. elegans contains both heat-shock inducible and non-heat-shock inducible hsp70 family members, none of which are linked within the approx. 120 kb of noncontiguous genomic DNA analysed here. We have completely sequenced one heat-inducible member, the hsp70A gene, and find that while it is highly homologous to both Drosophila and yeast hsp70 and hsc70 genes it contains three introns. Using strain-specific RFLPs we have mapped hsp70A to the far right of chromosome IV quantitated by one of the private bands were cut after hybridization and scintillation fluid or (ii) were quantitated using a laser scanner and detected peaks.

(c) Isolation of Caenornomonic and cDNA clone

The Drosophila hsp70 (1979) was digested with that only contains hsp7 was purified and us Χ-Chon 4 genomic DN a C. elegans λ607 Ibb Purified phase containences were subseqeublins into the plas pUC13 (Vieira and Me cDNAs, a C. elegans the vector Agt10 from grown at 20°C (a gift under high-stringency genomic hsp70 clones.

(d) Nucleotide sequences

Restriction fragment subecloned into the (Norrander et a l., 1984) constructed using exact Plasmid DNA was detached (Maniatis et al., was performed (Chat et al., 1980). Each plasmid twice. In addition, an additional sequence was obtained

(e) Genetic mapping

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quantitated by one of two methods: (i) the appropriate bands were cut from the nitrocellulose filter after hybridization and exposure and counted in scintillation fluid or (ii) the autoradiographic exposures were quantitated by scanning with an LKB laser scanner and determining the area under the peaks.

(e) Isolation of Caenorhabditis elegans hsp70 genomic and cDNA clones

The Drosophila hsp70 clone 132E3 (Moran et al., 1979) was digested with SalI and a 3.0-kb fragment that only contains hsp70 mRNA coding sequences was purified and used to probe a C. elegans λCharon 4 genomic DNA library (Snutch, 1984) and a C. elegans λ607 library (a gift of S. Bektash). Purified phage containing hsp70-homologous sequences were subsequently digested with EcoRI and subcloned into the plasmids pUR2 (Ruther, 1980) or pUC13 (Vieira and Messing, 1982). To isolate hsp70 cDNAs, a C. elegans cDNA library constructed in the vector λgt10 from RNA isolated from worms grown at 20°C (a gift of B. Meyer) was screened under high-stringency conditions with the C. elegans genomic hsp70 clones.

(d) Nucleotide sequencing

Restriction fragments from the hsp70A region were subcloned into the plasmid vector pUC19 (Norramer et al., 1983) and deletions were constructed using exonuclease III (Henikoff, 1984). Plasmid DNA was derived from mini alkali preparations (Maniatis et al., 1982) and dideoxynucleotide sequencing was performed (Chen and Seeberg, 1985; Sanger et al., 1980). Each plasmid was sequenced at least twice. In addition, approx. 40% of the hsp70A gene sequence was obtained from both strands.

(e) Genetic mapping of the hsp70A gene

We had previously identified a 3.0-kb Bristol EcoRI fragment that is located approx. 4 kb 3' to the hsp70A gene and which exhibits RFLDs between the N2 and BO strains of C. elegans (Snutch and Baillie, 1984). To map this RFLD and the tightly linked hsp70A gene to a specific chromosome, this 3.0-kb EcoRI fragment was radiolabelled and hybridized to Southern blots of DNAs isolated from strains homozygous for different regions of the Bristol genome. Each DNA was isolated from nematodes homozygous for a specific Bristol chromosome in a region of a selectable visible mutation and is randomly homozygous or heterozygous N2 and BO for all other regions of the genome (Rose et al., 1982).

Hybridization of the 3.0-kb probe to these DNAs indicated that the hsp70A gene was linked to chromosome IV in a region that carries the visible recessive mutation unc-22 (see RESULTS AND DISCUSSION, section e).

To more precisely map the hsp70A gene on chromosome IV, three factor crosses were carried out between the BO wild-type strain and N2 strains of the genotypes: unc-22 unc-31; unc-31 dpy-4; unc-26 dpy-4 (see Fig. 7 for reference). Briefly, to position the hsp70A gene RFLD relative to unc-22 (the marker used to produce DNA homozygous for this region of chromosome IV) and unc-31, a cross was constructed using unc-22(s7) unc-31(e169)/ + N2 males and wild-type BO hermaphrodites. In the F1 generation, unc-22 unc-31/BO(+) BO(+) individuals were selected, allowed to self and BO(+) unc-31/unc-22 unc-31 F2 recombinants were recovered. From the progeny of these, six individual F3 recombinants BO(+) unc-31/BO(+) unc-31 were isolated and DNA from their progeny was prepared. Hybridization with the 3.0-kb probe positioned the hsp70A gene relative to unc-22.

To position the hsp70A gene relative to unc-31 and dpy-4, BO(+) BO(+) hermaphrodites were crossed to unc-31(e169) dpy-4 (e1166)/ + N2 males and virgin F1 hermaphrodites were selected. From the F3 generation, 21 unc-31 BO(+) unc-31 BO(+) recombinants were selected and DNA from their progeny was prepared. Finally, to position the hsp70A probe relative to unc-26 and dpy-4, 24 recombinant unc-26 BO(+) unc-26 BO(+) and 30 recombinant BO(+) dpy-4/BO(+) dpy-4 strains were isolated in the F3 generation from a cross between BO hermaphrodites and N2 unc-26(e205) dpy-4(e1166)/ + males. DNA was prepared from all 54 recombinants and probed with the 3.0-kb hsp70A probe.
RESULTS AND DISCUSSION

(a) Cloning and genomic organization of the *Caenorhabditis elegans* hsp70-related sequences

To isolate genomic clones from *C. elegans* containing hsp70 homologous sequences, an EcoRI partial digest library was constructed with λCharon 4 and screened with a nick-translated *Drosophila* heat-shock-inducible hsp70 gene. Screening of five genome equivalents under moderate stringency conditions resulted in the final purification of nine recombinant phages. Hybridization with the *Drosophila* hsp70 gene probe showed that the nine phages fell into three distinct classes of hsp70-related sequences (Fig. 1). These were designated as the hsp70A, hsp70B and hsp70C classes. Comparison of the hybridization patterns of the *Drosophila* hsp70 gene probe to *C. elegans* genomic DNA (Snutch and Baillie, 1984) and to the cloned EcoRI fragments accounted for four of five *C. elegans* EcoRI genomic restriction fragments, which cross-hybridized to the *Drosophila* hsp70 gene under moderate stringency hybridization conditions.

At lower hybridization stringencies, seven to eight additional *C. elegans* genomic EcoRI fragments cross-hybridized to the *Drosophila* hsp70 gene probe (Snutch, 1984). We have further isolated three of these *C. elegans* EcoRI fragments from a total digest EcoRI library constructed into λgt11 (clones hsp70, hsp70D, and hsp70F, previously referred to as B52) and hsp70E, hsp70F hybridized to b (not shown). In addition, hsp70E and hsp70F digested with restriction enzymes within the cloned EcoRI probe hybridized to different fragments. This suggests that hsp70E and hsp70F EcoRI fragments in the *C. elegans* genome are not closely related. We interpret hsp70D and hsp70E as separate genes and that hsp70F is the sixth hsp70 supported by the North 4B section, below.

Restriction mapping of *Drosophila* hsp70A, hsp70B, and hsp70C genomic DNA clones. Classes A, B and C are represented by the individual sets of overlapping phages isolated for the hsp70A, hsp70B, and hsp70C genes, respectively. The top line for each class is the map representing the entire cloned region. Individual clones are shown beneath. R (right) and L (left) indicate the orientation of the inserts relative to the λCharon 4 vector arms. The heavy line represents homology to the *Drosophila* hsp70 gene as determined by blot hybridization. H, HinfIII; E, EcoRI; X, XbaI; O, XhoI; S, SalI; K, KpnI; B, BamHI. 5' and 3' indicate the direction of transcription of the hsp70 genes as determined by hybridization to specific regions of the *Drosophila* hsp70 gene.

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**Fig. 1.** Restriction maps of the *C. elegans* hsp70A, hsp70B and hsp70C genomic DNA clones. Classes A, B and C represent the individual sets of overlapping phages isolated for the hsp70A, hsp70B and hsp70C genes, respectively. The top line for each class is the restriction map representing the entire cloned region. Individual clones are shown underneath. R (right) and L (left) indicate the orientation of the inserts relative to the λCharon 4 vector arms. The heavy line represents homology to the *Drosophila* hsp70 gene as determined by blot hybridization. H, HinfIII; E, EcoRI; X, XbaI; O, XhoI; S, SalI; K, KpnI; B, BamHI. 5' and 3' indicate the direction of transcription of the hsp70 genes as determined by hybridization to specific regions of the *Drosophila* hsp70 gene.
hsp70C clones, patterns of the C. elegans genomic and to the cloned revealed that the probe for four of the restriction fragments of Drosophila hsp70 gene isolation conditions. For example, the hsp70D and hsp70E clones were isolated three of from a total digest (clones hsp70E, referred to as B5 2.6, hsp70F hybridized to both 5' and 3' coding regions (not shown). In addition, hybridization of hsp70D, hsp70E and hsp70F probes to genomic DNA digested with restriction enzymes that did not cut within the cloned EcoRI fragments showed that each probe hybridized to differently sized genomic DNA fragments. This suggested that the hsp70D, hsp70E and hsp70F EcoRI fragments were not adjacent fragments in the C. elegans genome. Since hsp70D and hsp70E are not closely linked within the C. elegans genome, we interpret these results to mean that hsp70D and hsp70E are 3' portions of distinct hsp70 genes and that hsp70F represents the major coding portion of a sixth hsp70 gene. This interpretation is supported by the Northern-blot analysis described in section b, below.

Restriction mapping and subsequent blot hybridizations with the Drosophila probe showed that the three C. elegans hsp70 clones, hsp70A, hsp70B and hsp70C, contained sufficient homology to the Drosophila hsp70 gene to each encode at least a 70-kDa polypeptide (Fig. 1). Based on their respective restriction maps, these three genes showed distinctive differences and thus form distinct members of the hsp70 family. Comparison of genomic DNA blots probed with hsp70A, hsp70B and hsp70C flanking regions showed that no detectable rearrangements had occurred during cloning (not shown). In addition, hybridization of individual cloned fragments from all six of the hsp70 homologous regions (including hsp70D, hsp70E and hsp70F) to C. elegans genomic DNA digested with various restriction enzymes showed that each cloned hsp70 gene was a single-copy element (not shown). None of the six cloned hsp70 gene regions overlap in the approximately 120 kb of DNA studied.

(b) Expression of the hsp70 genes

The expression of the C. elegans hsp70 homologous sequences was analysed by hybridization to total RNA isolated from heat-shocked and control worms. Fig. 2 shows a Northern-blot analysis under high-stringency hybridization conditions to control and heat-shock RNA. At this stringency, the hsp70A and hsp70B genes cross-hybridized to about 10% of the level of self-hybridization, while no other cross-hybridization was detected among any other hsp70 gene. Fig. 2 shows that transcripts from the hsp70A gene were found in control worms and were increased in response to heat shock. In both heat-shocked and control RNA samples the hsp70A mRNA was 2.4 kb in size. Densitometer scanning of this autoradiogram showed that the hsp70A gene transcripts were increased three-fold in response to heat shock. In contrast to the hsp70A gene, no transcripts from the hsp70B gene were detected in either control or heat-shock RNA samples at normal autoradiographic exposure times (Fig. 2). With longer exposure times, transcripts in the 2.4-kb size range homologous to the hsp70B gene were detected, but these were not present at levels greater than the level expected for cross-hybridization to the hsp70A gene transcripts. Fig. 2 also shows that hsp70C gene transcripts were detected in control and heat-shock RNA samples, but that no heat-inducible increase was observed. The major hsp70C message was 2.6 kb while a minor component at 2.8 kb was also observed. The amount of the 2.8-kb mRNA was slightly increased in RNA preparations from heat-shocked worms.

Fig. 2 also shows Northern blots of the remaining three hsp70 clones hybridized to RNA from heat-shocked and control worms. The hsp70F gene was transcribed in control worms as a 2.7-kb mRNA and which was slightly enhanced (approx. two-fold) in response to heat shock. The hsp70D gene was also transcribed in control worms, as a 2.4-kb mRNA, and its expression was enhanced approx. eight-fold in heat-shocked worms. We have observed heat-shock-inducible increases of up to 50-fold over control RNA levels for the hsp70D gene. Experimental differences appear to reflect variations in the level of hsp70D transcripts in control RNA preparations (not shown). We have not detected transcripts of the hsp70E gene, either in control or heat-shock RNA samples.
Since both the hsp70 to be expressed in continuous level of expression development. Fig. 3 shows, the relative abundance of gene transcripts at various developmental stages. Transcripts of hsp70 throughout C. elegans are abundant in L1 larvae at this level by the L1 larval stage. The hsp70A gene was identified in L1 larvae and decreased with development. While these N origin, in order to eliminate hybridization between further characterization or primer-extension a.

Fig. 2. Hybridization of C. elegans hsp70-homologous regions to control and heat-shock RNA. Equal amounts of total RNA (lane 1, heat shock; lane 2, control) were denatured in 2.2 M formaldehyde, 50% formamide for 10 min at 65°C and electrophoresed at 70 V for 5 h through a 1.1% agarose gel containing 2.2 M formaldehyde as described (Maniatis et al., 1982). Marker lanes were cut off and stained in 0.5 µg/ml of ethidium bromide in 0.1 M ammonium acetate, 0.1 M β-mercaptoethanol. The RNA was transferred to nitrocellulose as described by Thomas (1980) and hybridized for 30 h to the indicated C. elegans hsp70 probes (specific activity = 1-2 x 10^6 cpm/µg) under high-stringency conditions (see MATERIALS AND METHODS, section b). The filters were exposed for three days with an intensifying screen. The numbers next to the bands indicate the size (x 10^3 nt) of the hybridizing mRNA.

Fig. 3. Post-embryonic ex and control RNAs. Total and electrophoresed at 7 Lanes: 1, L1 larval RNA Hybridization was under expression of the hsp70C as described in panel A. METHODS, section b). The bands indicate the si
Since both the hsp70A and hsp70C genes appeared to be expressed in control worms, we then asked how the level of expression of these genes changes during development. Fig. 3 shows, by Northern-blot analysis, the relative abundance of the hsp70A and hsp70C gene transcripts at various times during development. Transcripts of the hsp70C gene were detected throughout C. elegans development, being most abundant in L1 larvae and decreasing to about 15\% of this level by the adult stage. Transcripts of the hsp70A gene were also detected throughout C. elegans development, being most abundant in L1 larvae and decreasing by about 50\% by the adult stage. Fig. 3 also shows that transcription of the hsp70A gene is heat-shock-inducible at all stages tested. While these Northern-blot data are informative, in order to eliminate the possibility of cross-hybridization between hsp70 gene family members, a further characterization involving RNA protection or primer-extension assays will be required.

(c) Sequence analysis of the hsp70A gene region

The fact that the hsp70A gene was abundantly expressed in control worms, was moderately heat-shock-inducible and appeared to be the C. elegans hsp70 element most closely related to the Drosophila hsp70 gene, suggested it as an interesting candidate for further analysis. The entire sequence of the hsp70A gene and flanking regions is shown in Fig. 4. Within the 3-kb region sequenced an open reading frame corresponding to the previously deduced sequence of a Drosophila hsp70 gene (Ingolia et al., 1980) was found. The open reading frame was found to be interrupted by three short introns of 49, 194 and 55 bp. The position of the first intron in hsp70A (aa 69) is similar to that of an intron in the non-heat-shock-inducible Drosophila hsc1 (aa 66) and hsc2 (aa 58/59) genes (Craig et al., 1983). The sequence of the intron boundaries (5'--GTAAGT .... TTCAG--3') matched that found for other

Fig. 3. Post-embryonic expression of hsp70A and hsp70C genes. (A) Northern blot of the hsp70A gene to various stage-specific heat-shock and control RNAs. Total RNA (17 \mu g) from each stage was denatured with 2.2 M formaldehyde, 50\% formamide at 65\(^\circ\)C for 10 min and electrophoresed at 70 V for 5 h through a 1.1\% agarose gel containing 2.2 M formaldehyde as described (Maniatis et al., 1982). Lanes: 1, 1L larvae RNA; 2, L3 and L4 larvae RNA; 3, adult worm RNA. HS and C refer to heat-shock and control RNA, respectively. Hybridization was under high-stringency conditions for 36 h (see MATERIALS AND METHODS, section b). (B) Developmental expression of the hsp70C gene. Total RNA (17 \mu g) isolated from each stage of control worms was denatured, electrophoresed and blotted as described in panel A. Hybridization was with the hsp70C gene under high-stringency conditions for 28 h (see MATERIALS AND METHODS, section b). Lanes: 1, 1L larvae RNA; 2, L2 larvae RNA; 3, L3 and L4 larvae; 4, adult worm RNA. The numbers next to the bands indicate the size (\times 10^3 nt) of the hybridizing mRNA.
Fig. 4. Nucleotide sequence letters. Dashes represent insertions and putative TATA box as with asterisks. The sequence

C. elegans introns (K): of the introns were corresponding region: of hsp70A (pCc 6.2; ) pCc 6.2 cDNA includ polypeptide and exon codon. The mature h-642-aa polypeptide o have previously been eukaryotic genes in th existence of introns in hsp16 gene family (F and as we report her suggests a fundament between C. elegans h other organisms.

Upstream from th quences which are C HSE 5′–C –GAA– homology), HSE2 (88% homology) (F1 elctrode sequence in TATA box, TAAAT
Fig. 4. Nucleotide sequence of the hp70A gene region (Fig. 2). A partial sequence of hp70A cDNA (pCe 6.2) is shown in lower-case letters. Dashes represent nucleotides which are present in the genomic nucleotide sequence but not in the cDNA sequence. The HSEs and putative TATA box are indicated. The regions exhibiting dyd symmetry capable of forming a stem-and-loop structure are marked with asterisks. The sequence is numbered from the initiating ATG codon.

C. elegans introns (Karn et al., 1983). The positions of the introns were confirmed by sequencing the corresponding regions of a nearly full-length cDNA of hp70A (pCe 6.2; Fig. 4, lower-case letters). The pCe 6.2 cDNA included the first met residue of the polycistron and extended 150 bp 3' to the UAA stop codon. The mature hp70A mRNA would encode a 642-aa polypeptide of 69.8 kDa. Heat-shock genes have previously been considered unique among eukaryotic genes in their relative lack of introns. The existence of introns in four members of the C. elegans hp16 gene family (Russnak and Candido, 1985), and as we report here, in at least the hp70A gene, suggests a fundamental difference in gene structure between C. elegans heat-shock genes and those of other organisms.

Upstream from the coding region are three sequences which are homologous to the consensus HSE 5'-C-<GAA-<TTG-<G-3': HSE1 (88% homology), HSE2 (75% homology) and HSE3 (88% homology) (Fig. 4). Examination of the nucleotide sequence in this region showed a putative TATA box, TAAATT, 25 nt downstream from the genomic XbaI site (from -113 to -108). Two sequences analogous to the cap site signals in sea urchin genes (PyCATTCCu; Sures et al., 1978) were found from -80 to -75 and from -98 to -93, 28 and 10 nt downstream from the putative TATA box (Fig. 4). Flanking the putative TATA box are two repeats, one inverted with respect to the other. The first one, from -118 to -114, has 1 nt difference from the second sequence, from -103 to -99 (Fig. 4). The presence of inverted repeats is also seen around the TATA box in a Drosophila heat-shock-inducible hp70 gene (Ingolia et al., 1980). Encompassing the TATA box and HSE1 was a palindromic sequence stretching from -150 to -138 and from -118 to -106 with eleven out of 13 matches (Fig. 4). These two stretches are capable of pairing with each other to form a stem-and-loop structure. The resulting loop is 19 nt long. HSE2 and HSE3 do not have any sequences surrounding them that are capable of forming stem-and-loop structures. The final sequences of note, flanking HSE1 and HSE2, are three CCAAT boxes, CCAAT1 from -125 to -121 and CCAAT2 from -169 to -165 and CCAAT3 from...
(d) Conserved features

Overall, hsp70A is homologous in hsp70 and hsc70 gen (Fig. 5). For example 75% homologous vs 77% homologous witn YG102 (not show to the Drosophila hsc70 also shows that a s between hsp70A and YG102 is extreme C terminal recently speculated a of the high degree of related genes and rel

(e) Genetic mapping

Our previous resul the hsp70A gene con variation between C. elegans (Snutch a RFLDs in the hsp7 fragmen 3' to the con as a mapping probe. copy DNA but, a mutations, shifts to strain (3.7, 2.3 and C of hybridization of f for regions flanking N2 chromosomes. T and BO patterns in r mosomes I, III, V an (III, IV), indicating chromosomes. In co chromosome IV DN much greater intensity small amount of BO: DNA suggested th chromsome IV but

Fig. 5. Comparison of deduced amino acid sequences for hsp70A to a heat-inducible hsp70 gene from Drosophila (Ingoldia et al., 1980). The hsc70 cognate gene from Drosophila, mg34 (Craig et al., 1983; M. Slater and E.A. Craig, pers. commun.), and the yeast hsp70 gene YG100 (M. Slater and E.A. Craig, pers. commun.). Amino acid matches to the C. elegans sequence hsp70A are indicated by a dot and amino acids in hsp70A that are not represented in the other sequences are indicated by a dash.
-205 to -201 (Fig. 4). CCAAT1 is within the loop of the potential stem-and-loop structure.

(d) Conserved features of hsp70A

Overall, hsp70A is highly homologous to both hsp70 and hsc70 genes from Drosophila and yeast (Fig. 5). For example, at the amino acid level hsp70A is 75% homologous with a Drosophila hsp70 gene and 71% homologous with the yeast hsp70 genes YG100 and YG102 (not shown). It is also 81% homologous to the Drosophila hsc4 gene (mg34 in Fig. 5). Fig. 5 also shows that a similar homology profile exists between hsp70A and these other hsp70 family members, the N terminus and the initial approximate 500 aa are most highly conserved, while there is marginal conservation at the C-terminal portion of hsp70 related genes. An exception to this, as noted by Hunt and Morimoto (1985), is the conservation between hsp70-related genes of the peptide, EEVD, at the extreme C terminal. Pelham (1986) has recently speculated as to the functional significance of the high degree of conservation amongst hsp70-related genes and related stress proteins.

(e) Genetic mapping of the hsp70A gene

Our previous results showed that regions flanking the hsp70A gene contain large amounts of sequence variation between the BO and N2 strains of C. elegans (Schnell and Baillie, 1984). Of the many RFLPs in the hsp70A region, a 3.0-kb N2 EcoRI fragment 3’ to the coding element was chosen for use as a mapping probe. This fragment represents single-copy DNA but, as a result of apparent point mutations, shifts to three EcoRI bands in the BO strain (3.7, 2.3 and 0.5 kb). Fig. 6 shows the results of hybridization of this probe to DNA homozygous for regions flanking visible mutations on several N2 chromosomes. The pattern showed both the N2 and BO patterns in roughly equal amounts for chromosomes I, III, V and the reciprocal translation, eTi (III, IV), indicating random assortment in these chromosomes. In contrast, the lane homozygous for chromosome IV DNA showed the N2 pattern in a much greater intensity than the BO pattern. The small amount of BO signal in the N2 chromosome IV DNA suggested that the hsp70A probe is linked to chromosome IV but not tightly linked to the marker used to select the chromosome IV DNA (unc-22).

The hsp70A gene was next positioned relative to unc-22 and unc-31, loci about 1.3 map units apart on the right arm of chromosome IV (Fig. 7). From a cross between wild-type BO hermaphrodites and unc-22 unc-31/+/+ N2 males, six recombinant worms with the genotype BO(+) unc-31/BO(+).unc-31 were isolated in the F3 generation and DNA was prepared. Each of these strains contains BO chromosome IV DNA to the left of the crossover point and N2 chromosome IV DNA to the right. The worms should be random N2 and BO for the remaining chromosomes. Fig. 6 (lane unc-31) shows the results of the hybridization of the 3.0-kb probe to the pooled DNA. Only the N2 pattern occurred indicating that the hsp70A gene was to the right of unc-22.
The hsp70A gene was further positioned on chromosome IV by probing recombinants in the unc-31 to dpy-4 region. Twenty-one recombinants with the genotype unc-31 BO(+) unc-31 BO(+) were isolated, cultured individually on plates and DNA prepared from twelve individual recombinants and from a pool of all 21 recombinants. Fig. 8 shows hybridization of the 3.0-kb DNA probe to eight individual DNA preparations and to the pool of 21 recombinants. While the eight individual recombinants only showed the BO pattern, hybridization to the pooled sample showed a small amount of the N2 pattern, indicating that the hsp70A gene is to the far right of unc-31 but to the left of dpy-4.

This result was confirmed by a final mapping experiment in which 54 recombinants were isolated in the region between unc-26 and dpy-4. In this analysis, 30 BO(+)/dpy-4/BO(+) dpy-4 and 24 of the reciprocal recombinants unc-26 BO(+)/unc-26 BO(+) were isolated and their DNA probed. All 30 dpy-4 DNAs showed the N2 pattern, and 22 of 24 of the unc-26 DNAs showed the BO pattern with the remaining two having the N2 pattern (not shown). These results suggested that the hsp70A gene is 1/21 of the unc-26 to dpy-4 distance, nearest to dpy-4 (Fig. 7).

(f) Conclusions

(1) We have isolated six distinct members of the C. elegans hsp70 gene family from genomic DNA libraries. We estimate from the hybridization of cloned hsp70 probes to genomic DNA blots that C. elegans possesses nine to twelve hsp70-related genes. The unique expression pattern of the various C. elegans hsp70 family members appears similar to the complex patterns found for yeast and Drosophila hsp70 gene families (Craig, 1985), and suggests a general conservation throughout evolution of a family of related proteins whose expression is essential both during normal development and in response to environmental stress.

(2) At the present level of analysis we are able to divide the six cloned hsp70 family members into four distinct groups. One group, consisting of hsp70A and hsp70F, is abundantly expressed in control worms and is increased slightly (two- to six-fold) in response to heat shock. Hsp70-related genes with similar properties have been described in yeast and Drosophila (Craig, 1985). Transcripts of a second group, consisting only of hsp70C at this time, are present at moderate levels throughout C. elegans development and are not increased at all in response to heat shock. Transcripts of a third group, consisting of hsp70B and hsp70E, have not been detected. These two genes are clearly not heat-shock-inducible hsp70 genes, nor are their transcripts required in moderate to large amounts in growing worms. In addition, hybridization of pro hsp70 genes to D. melanogaster species, that while sequenced related to the hsp70 genes, no sequences conserved between (Snatch, 1984). Thus a recent addition to been recently lost and either of these sequenced genes may be pseudogenes of hsp70D, detectable in contrast are increased eightfold. These Drosophila hsp70 ge C. elegans hsp70 sequencing of the systematic analysis of several various groups mo (3) While most nearly identical hsp70 loci (ish-H addition to several six cloned C. elegans copy elements within genome. Analysis hsp70B and hsp70D elements similar to shock-inducible n (Lis et al., 1981) hsp70 locus (not s

(4) Nucleotide hsp70A gene was and hsc70 genes hsp70A appears to number both hsp70 unc transcripts are a hsp70A cDNAs w the cDNAs isolated from RN hsp70A transcript non-heat-shock tested and in adult hsc70 genes hsp70A gene is is in contrast to hsc70.
hybridization of probes representing the six cloned hsp70 genes to DNA isolated from a related nematode species, Caenorhabditis briggsae, showed that while sequences can be detected which are related to the hsp70A, hsp70C, hsp70D and hsp70F genes, no sequences related to hsp70B or hsp70E are conserved between these two closely related species (Snutch, 1984). Thus, these two genes may either be a recent addition to the C. elegans genome or have been recently lost from the C. briggsae genome. In either of these scenarios, the hsp70B and hsp70E genes may be pseudogenes. A fourth hsp70 group consists of hsp70D. Transcripts of hsp70D are barely detectable in control worm RNA preparations and are increased eight- to 50-fold after heat shock. In these respects, this gene more closely resembles the Drosophila hsp70 genes. Further detailed analysis of the C. elegans hsp70 family, including nucleotide sequencing of the remaining genes and a more qualitative analysis of mRNA expression, may define the various groups more precisely.

(3) While most Drosophila strains contain five nearly identical hsp70 genes clustered at two cytological loci (Ish-Horowicz and Pinchikos, 1980), in addition to several divergent hsc70 genes, we find the six cloned C. elegans hsp70 sequences to be single-copy elements which are dispersed in the C. elegans genome. Analysis of DNA flanking the hsp70A, hsp70B and hsp70C genes showed no evidence for elements similar to the tandem copies of the heat-shock-inducible middle repetitive DNA elements (Lis et al., 1981) which flank the Drosophila 87C hsp70 locus (not shown).

(4) Nucleotide sequencing showed that the hsp70A gene was highly homologous to both hsp70 and hsc70 genes of Drosophila and yeast. In fact, hsp70A appears to possess some properties common to both hsp70 and hsc70 genes. First, hsp70A transcripts are abundant in growing worms, as hsp70A cDNAs were found to represent 0.25% of the cDNAs isolated from a cDNA library constructed from RNA isolated from a mixed population of worms grown at 20°C (not shown). Second, hsp70A transcripts were found to be present under heat-shock growth conditions in all larval stages and in adult worm RNA preparations. Third, like the hsc70 genes of Drosophila (Craig, 1985), the hsp70A gene is interrupted by introns. Finally, in contrast to hsc70 genes, we find that transcription of hsp70A is moderately enhanced by heat shock and that upstream from the hsp70A coding element three sequences homologous to the HSE are found. It will be interesting to determine the molecular basis for the relatively low level of heat-shock inducibility of the hsp70A gene with its three flanking HSEs, as compared to the much greater induction found with the C. elegans hsp16 genes (Russnak and Candido, 1985).

(5) It has been suggested for Drosophila and other organisms, that heat-shock genes are relatively devoid of introns to circumvent a block in RNA splicing which occurs during a severe heat-shock (> 37°C; Yost and Lindquist, 1986). All five C. elegans heat-shock genes sequenced to date (four hsp16 genes (Russnak and Candido, 1985) and hsp70A reported here) contain at least one intron. Thus, if the theory proposed by Yost and Lindquist (1986) also applies to C. elegans, we would expect that transcripts from these genes would be correctly processed and translated only under conditions of mild or moderate heat shock.

(6) The localization of the chromosomal position of hsp70A to the far right arm of chromosome IV provides both a cloned marker for this region of the C. elegans genome and a defined region in which a mutational analysis could be directed in an effort to identify the nature of the hsp70A gene product.

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