Gene, 64 (1988) 241–255
Elsevier

GEN 02352

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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Received 1 September 1987
Accepted 22 December 1987
Received by publisher 25 January 1988

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–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,

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Abbreviations: aa, amino acid(s); BO, Bergerac (strain); bp, 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 NaH_2PO_4, 1 mM EDTA, pH 7.4.

1982; Ingolia et al., 1980; Karch et al., 1981; Moran et al., 1979). In *Drosophila* the *hsp* have been grouped into three classes: a single *hsp83* gene; the *hsp68* and *hsp70* genes; and four low-M_r *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-Horowitz, 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 RFLDs we have mapped *hsp70A* to the far right of chromosome IV.

MATERIALS AND METHODS

(a) Heat shock and culturing of nematodes

C. elegans, var. Bristol, wild-type strain N2 was maintained on NGM plates supplemented with *Escherichia coli* strain OP50 as a food source (Brenner, 1974). For large-scale RNA and DNA preparations, worms were cultured on high peptone plates and seeded with a lawn of *E. coli* strain B (Rose et al., 1982). Nematodes were heat-shocked at 35°C for 4 h on NGM plates and subsequently allowed to recover at 20°C for 90 min. The nematodes were collected with 0.04 M NaCl and RNA was isolated using guanidine hydrochloride as previously described (Snutch and Baillie, 1983).

(b) Isolation of nucleic acids and hybridizations

C. elegans genomic DNA and total RNA were isolated as previously described (Snutch and Baillie, 1984). Plasmid and phage λ DNA isolation were by standard techniques (Davis et al., 1980). DNA probes were nick-translated to specific activities between 5×10^7 and 2×10^8 cpm/μg. DNA and RNA filters were prehybridized at the hybridization temperature in 5 × SSPE, 0.2% SDS, and 2.5 × Denhardts (0.05% each of Ficoll, polyvinylpyrrolidine, and bovine serum albumin) for 30 min. Hybridizations were carried out in a fresh aliquot of the above solution that included the denatured, nick-translated probe. The following two sets of hybridization criteria were employed: (1) moderate stringency — hybridization at 65°C, washing at 65°C in 2 × SSPE; (2) high stringency — hybridization at 68°C, washing at 70°C in 0.2 × SSPE. Blots were

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(c) Isolation of *Caenor* nomic and cDNA clone

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(d) Nucleotide sequen

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(e) Genetic mapping

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*Eco*RI fragment was

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.

(c) Isolation of *Caenorhabditis elegans hsp70* genomic and cDNA clones

The *Drosophila hsp70* clone 132E3 (Moran et al., 1979) was digested with *Sal*I 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. Bektesh). Purified phage containing *hsp70*-homologous sequences were subsequently digested with *Eco*RI 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 (Norrander 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 dideoxy sequencing was performed (Chan 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 *Eco*RI 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 *Eco*RI 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 *Eco*RI 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 phage. Hybridization with the *Drosophila hsp70* gene probe showed that the nine phage fell into three distinct classes of *hsp70*-related sequences (Fig. 1). These were designated as the *hsp70A*, *hsp70B* and *hsp70C* clo
Comparison of the hybridization patterns o
Drosophila hsp70 gene probe to *C. elegans* geno
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hsp70A, *hsp70B*, and *hsp70C* genes revealed tha
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At lower hybridization stringencies, seven to n
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cross-hybridized to the *Drosophila hsp70* gene pro
(Snutch, 1984). We have further isolated three
these *C. elegans Eco*RI fragments from a total dig
*Eco*RI library constructed into λ607 (clones *hsp70*
hsp70D, and *hsp70F*, previously referred to as B5 2.6

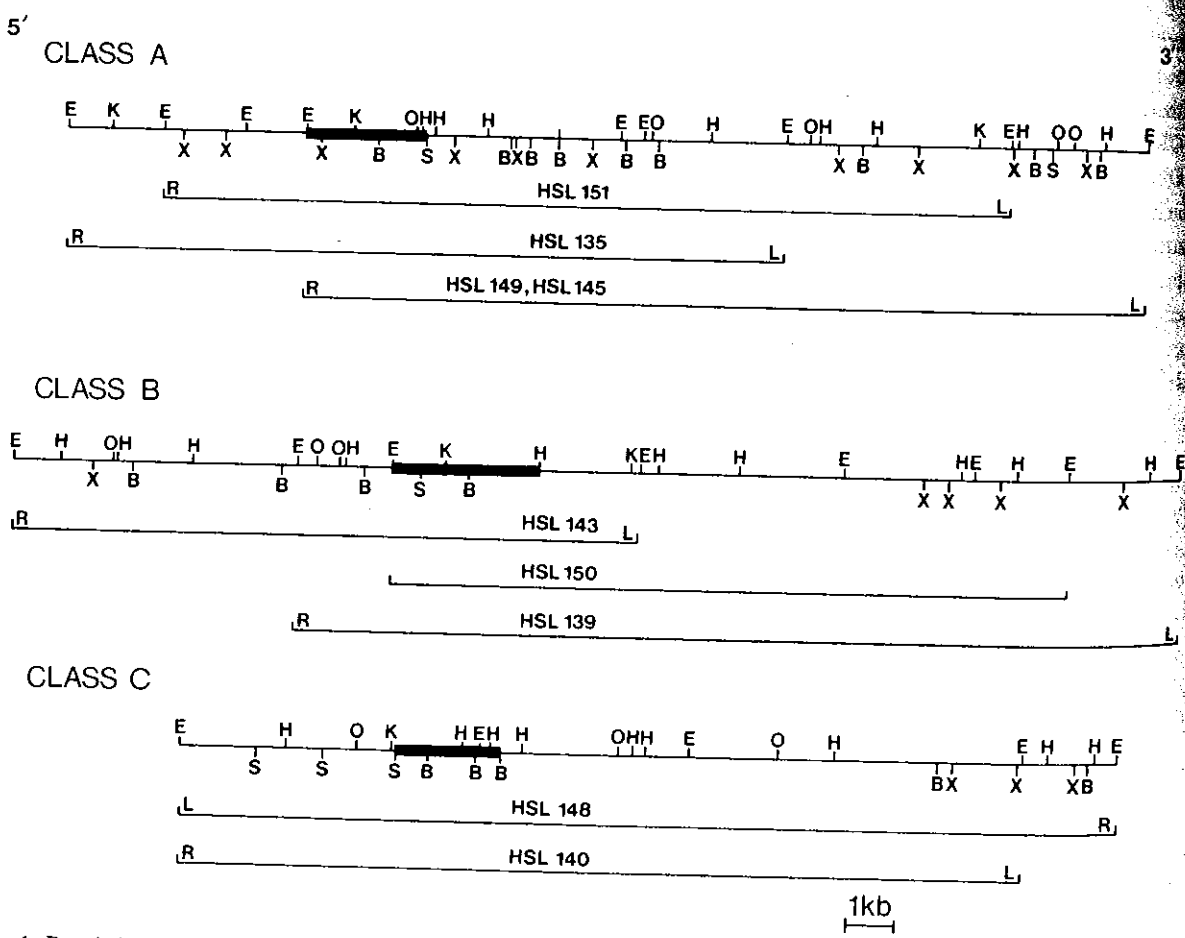

Fig. 1. Restriction maps of the *C. elegans hsp70A*, *hsp70B* and *hsp70C* genomic DNA clones. Classes A, B and C represent the individual sets of overlapping phage isolated for the *hsp70A*, *hsp70B* and *hsp70C* genes, respectively. The top line for each class is the restriction map representing the 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, *Hin*dIII; E, *Eco*RI; X, *Xba*I; O, *Xho*I; S, *Sal*I; K, *Kpn*I; B, *Bam*HI. 5′ and 3′ indicate the direction of transcription of the *hsp70* genes as determined by hybridization to specific regions of the *Drosophila hsp70* gene.

B9 1.7, and B4 2.9, respectively; Snutch, 1984). Since the *hsp70D*, *hsp70E* and *hsp70F* clones were isolated as single *Eco*RI fragments from the λ607 library, no flanking nucleotide sequences were available for analysis. However, we could determine whether each of these fragments represented intact genes or portions thereof by hybridization to the *Drosophila hsp70* gene cut into 5′- and 3′-coding regions. For example, the *hsp70D* and *hsp70E* clones only hybridized to a probe representing the 3′-coding portion of the *Drosophila hsp70* gene. In contrast, *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 *Eco*RI fragments showed that each probe hybridized to differently sized genomic DNA fragments. This suggested that the *hsp70D*, *hsp70E* and *hsp70F Eco*RI 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% 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.

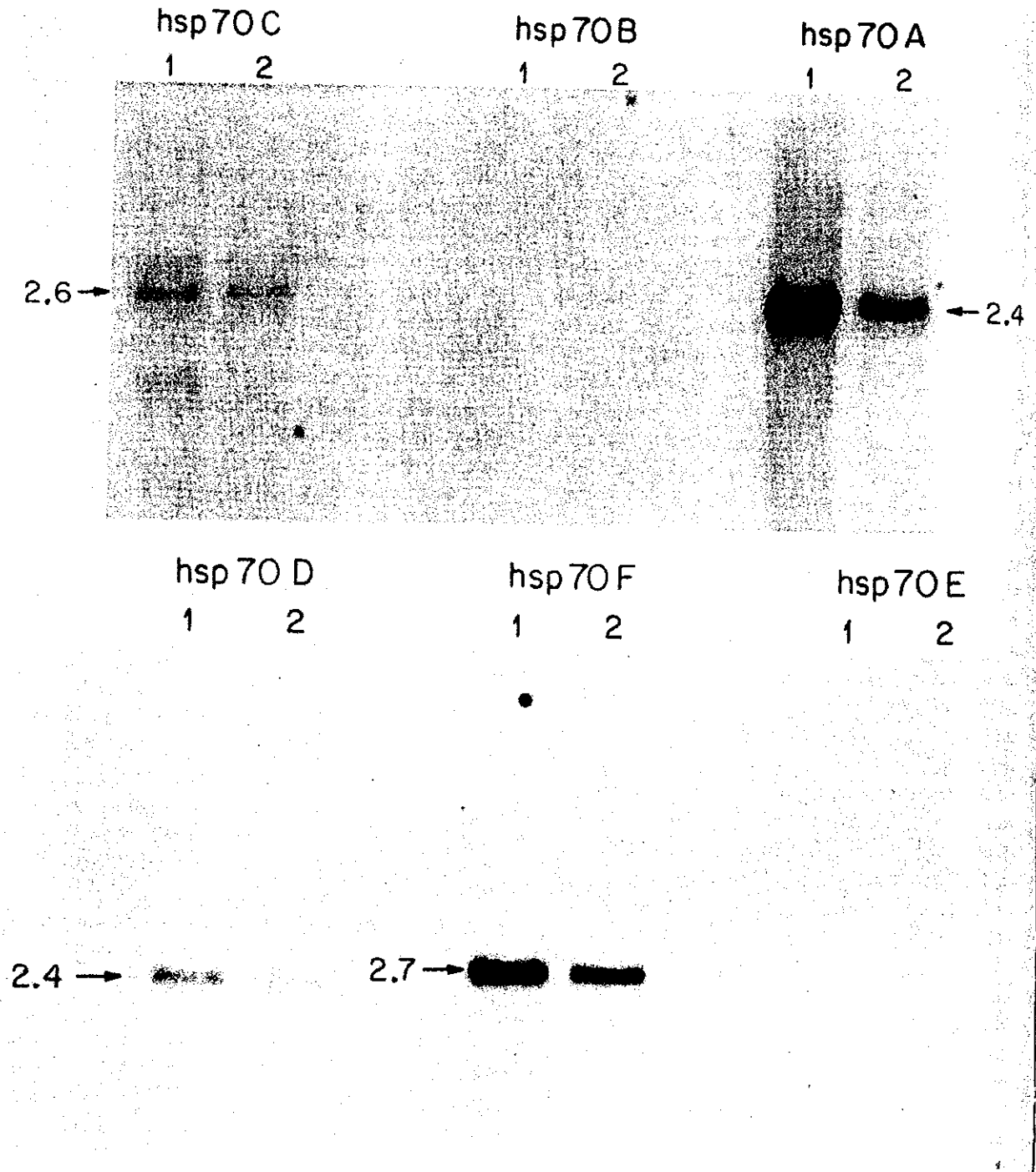

Fig. 2. Hybridization of *C. elegans hsp70*-homologous regions to control and heat-shock RNA. Equal amounts of total RNA (lane 1, heat shock; lane 2, control) were denatured in 2.2 M formaldehyde, 50% formamide for 10 min at 65 °C and electrophoresed at 70 V for 5 h through a 1.1% agarose gel containing 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 (sp. act. = 1–2 × 10^8 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 (× 10^3 nt) of the hybridizing mRNA.

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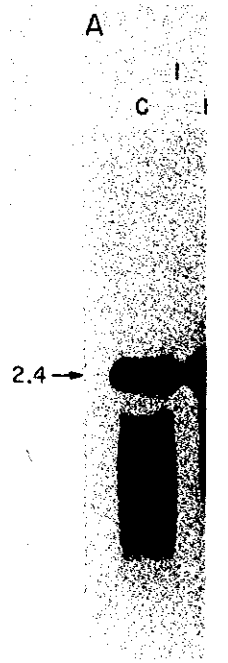

Fig. 3. Post-embryonic ex
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Lanes: 1, L1 larvae RNA
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METHODS, section b).
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Since both the *hsp70A* and *hsp70C* genes appeared to be expressed in 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 ..... TTTCAG–3′) matched that found for other

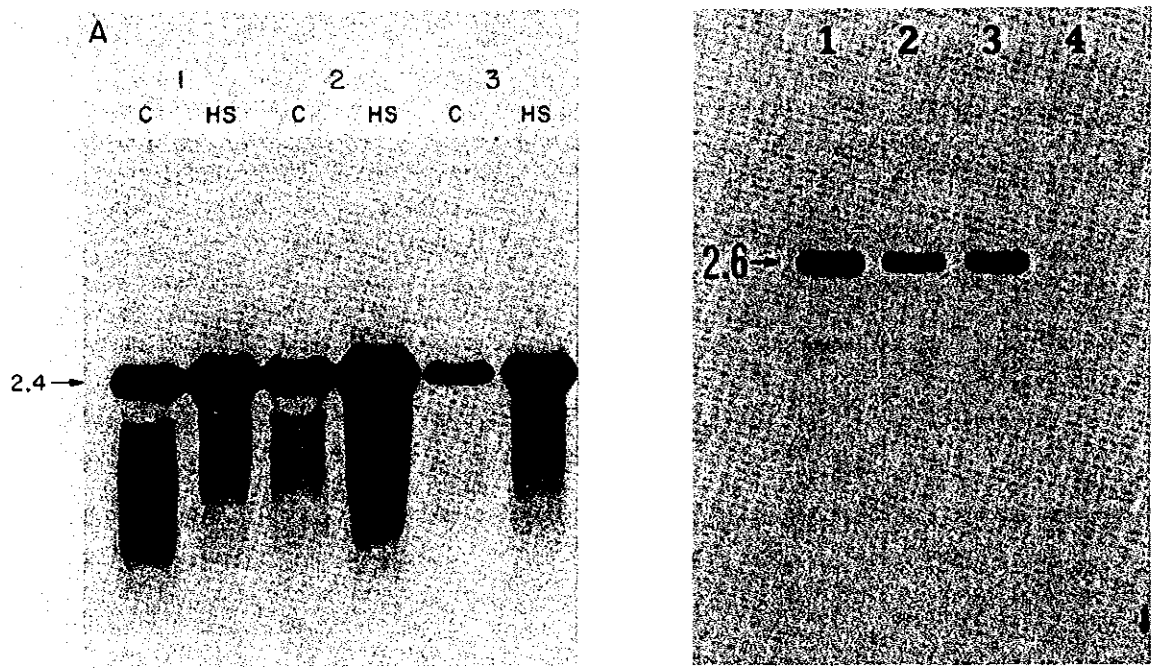

Fig. 3. Post-embryonic expression of *hsp70A* and *hsp70C* genes. (**A**) Northern blot of the *hsp70A* gene to various stage-specific heat-shock and control RNAs. Total RNA (17 μg) from each stage was denatured with 2.2 M formaldehyde, 50% formamide at 65°C for 10 min and electrophoresed at 70 V for 5 h through a 1.1% agarose gel containing 2.2 M formaldehyde as described (Maniatis et al., 1982). Lanes: 1, L1 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 μg) 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, L1 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.

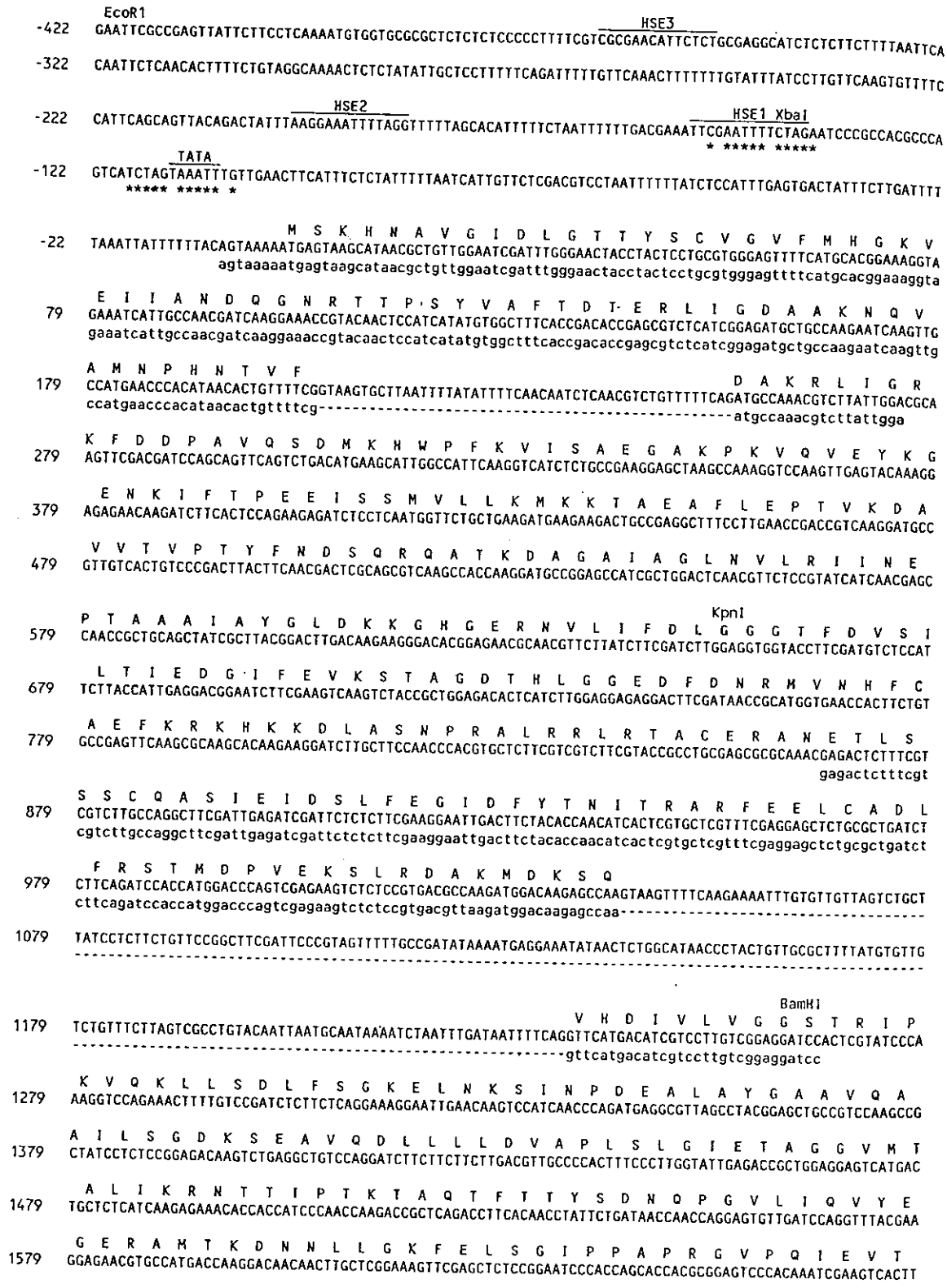

Fig. 4. Nucleotide sequen
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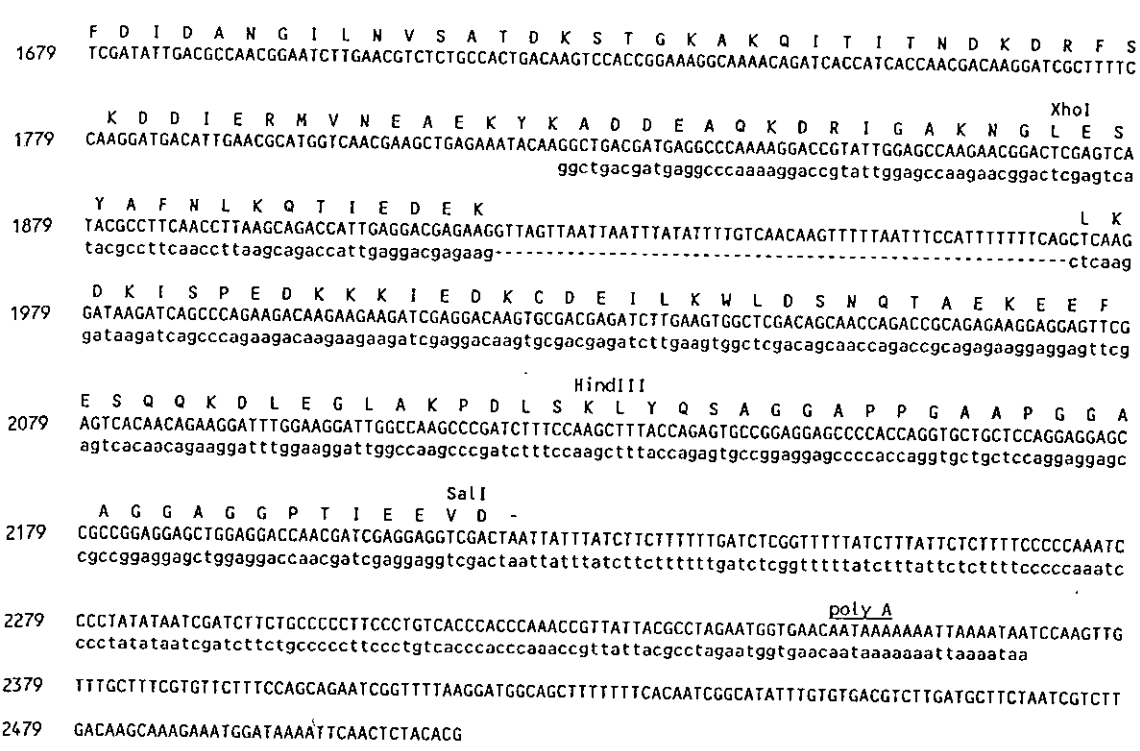

Fig. 4. Nucleotide sequence of the *hsp70A* gene region (Fig. 2). A partial sequence of *hsp70A* cDNA (pCe 6.2) is shown in lower-case letters. Dashes represent nucleotides which are present in the genomic nucleotide sequence but not in the cDNA sequence. The HSEs and putative TATA box are indicated. The regions exhibiting dyad 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 *hsp70A* (pCe 6.2; Fig. 4, lower-case letters). The pCe 6.2 cDNA included the first met residue of the polypeptide and extended 150 bp 3′ to the UAA stop codon. The mature *hsp70A* 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 hsp16* gene family (Russnak and Candido, 1985), and as we report here, in at least the *hsp70A* 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– –TTC– –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 *Xba*I site (from −113 to −108). Two sequences analogous to the cap site signals in sea urchin genes (PyCATTCPu; 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 *hsp70* gene (Ingolia et al., 1980). Encompassing the TATA box and HSE1 was a palidromic 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

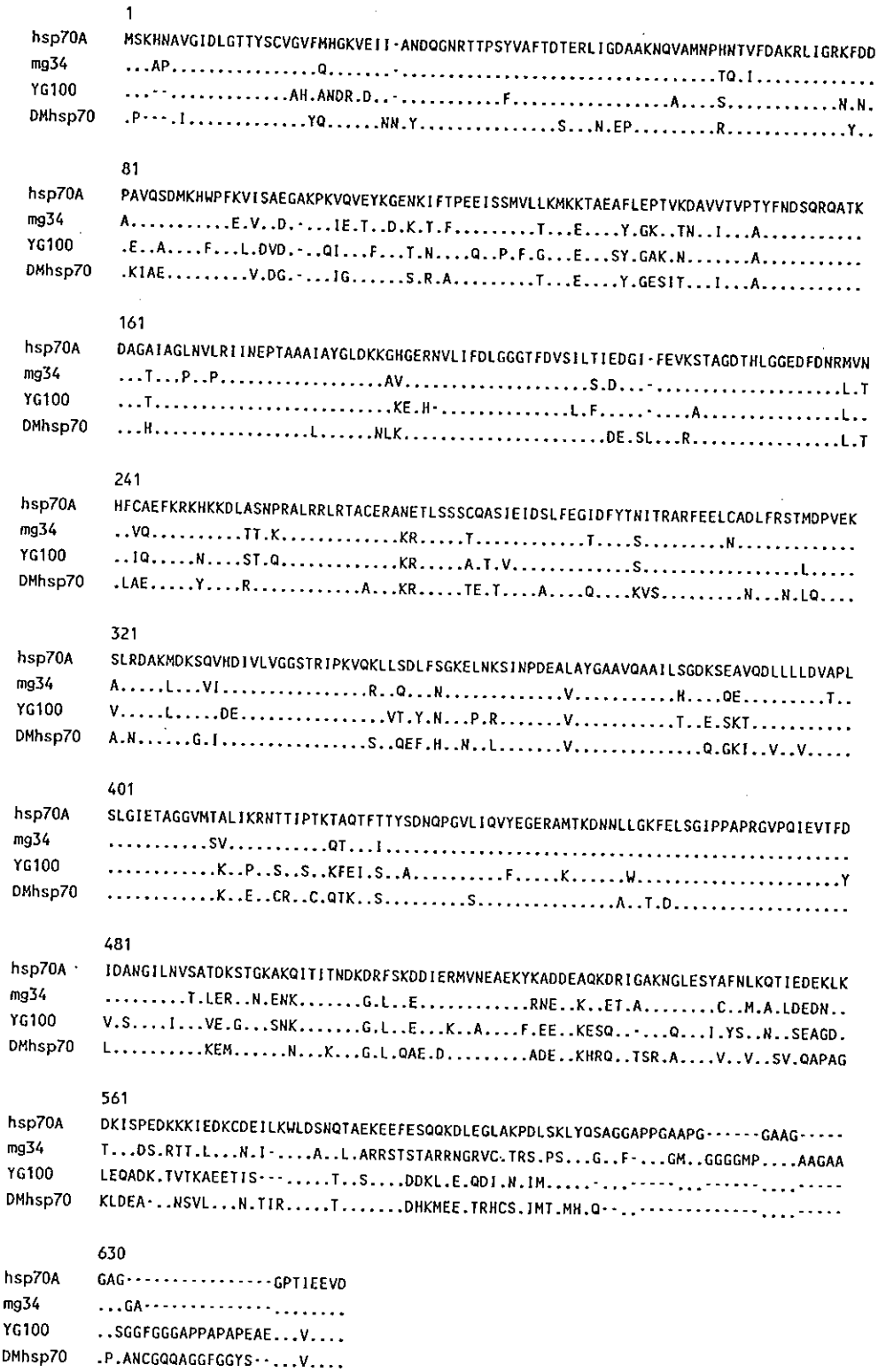

Fig. 5. Comparison of deduced amino acid sequences for *hsp70A* to a heat-inducible *hsp70* gene from *Drosophila* (Ingolia et al., 1980), the *hsc4* cognate gene from *Drosophila*, *mg34* (Craig et al., 1983; M. Slater and E.A. Craig, pers. commun.), and the yeast *hsp70* gene YG100 (M. Slater and E.A. 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
of the potential stem-

(d) Conserved feature

Overall, *hsp70A* is
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(e) Genetic mapping

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−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 77% 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 550 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 terminus. 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* (Snutch and Baillie, 1984). Of the many RFLDs in the *hsp70A* region, a 3.0-kb N2 *Eco*RI 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 *Eco*RI 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, *eT1 (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

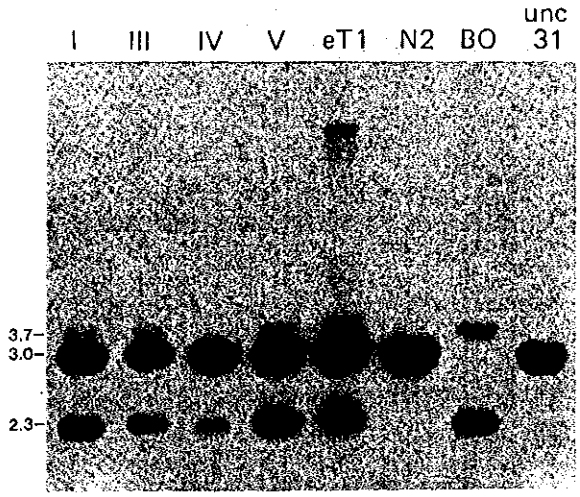

Fig. 6. Hybridization of ^{32}P-labeled N2 3.0-kb *hsp70A* DNA probe to *Eco*RI-digested genomic DNA homozygous for portions of individual N2 chromosomes. Four μg of each DNA (see MATERIALS AND METHODS, section e) was digested with *Eco*RI and electrophoresed through a 0.8% agarose gel containing 89 mM Tris, 89 mM boric acid and 1 mM EDTA. DNA was transferred to nitrocellulose by the bidirectional method of Smith and Summers (1980). Hybridization was at moderate stringency (see MATERIALS AND METHODS, section b) for 30 h. The filter was exposed for two days with an intensifying screen. I, chromosome I DNA; III, chromosome III DNA; IV, chromosome IV DNA; V, chromosome V DNA; *eT1*, translocation between chromosome V and chromosome III; *unc*-31, pooled DNA from six BO(+) *unc*-31/BO(+) *unc*-31 recombinants. The numbers on the left margin are the sizes of genomic fragments (in kb) homologous to the N2 3.0-kb probe. The 0.5-kb BO fragment (see RESULTS AND DISCUSSION, section e) has run off the end of the gel.

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.

Fig. 7. The region flanking *dpy*-4 on the right arm of chromosome *IV*. The relative positions of nonessential genes is shown. The position of the *hsp70A* gene with 95% confidence limits is marked (see RESULTS AND DISCUSSION, section e).

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 1/27 of the *unc*-26 to *dpy*-4 distance, nearest to *dpy*-4 (Fig. 7).

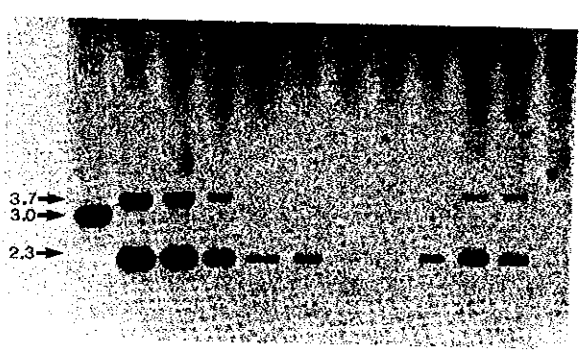

Fig. 8. Hybridization of the N2 *hsp70A* 3.0-kb DNA probe to *unc*-31 BO(+)/*unc*-31 BO(+) recombinants. The DNA samples and agarose gel were treated as described in the legend to Fig. 6. The filter was exposed for five days with an intensifying screen. See MATERIALS AND METHODS, section e for explanation of the recombinant DNA samples. Lanes: 1, pooled DNA from 21 recombinants; 2–9, individual recombinants. Note the small amount of N2 pattern in the pooled sample. The numbers on the left margin are the sizes of genomic fragments (in kb) homologous to the N2 3.0-kb probe. The 0.5-kb BO fragment (see RESULTS AND DISCUSSION, section e) has run off the end of the gel.

(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 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 Pinchios, 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* gene 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 non-heat-shock growth conditions in all larval stages tested 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.

ACKNOWLEDGEMENTS

We thank K. Beckenbach, M. Rogalski, L. Hale and H. Hemani for their technical assistance throughout various portions of this work. We also thank Dr. I. Kovesdi for the λCharon 4 arms, Dr. L. Moran for the *Drosophila hsp70* probe and M. Slater and E.A. Craig for communicating their unpublished sequence data. T.P.S. thanks R. Rosenbluth and Dr. T. Rogalski for teaching him the ways of the worms. We would also like to thank K. Beckenbach, R. Rosenbluth and Drs. M. Smith, R. Hackett and A. Rose for their comments on this manuscript. This work was supported by predoctoral fellowships from Simon Fraser University and from the Natural Sciences and Engineering Council of Canada to T.P.S., a predoctoral fellowship from Simon Fraser University and a studentship from the

Medical Research Council of Canada to M.F.P.H. and by research grants from NSERC of Canada to D.L.B.

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Communicated by D.T. Denhardt.