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Alterations in the pattern of gene expression following heat shock in the nematode *Caenorhabditis elegans*

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Exposure of the nematode *Caenorhabditis elegans* to elevated temperatures induces the preferential synthesis of eight major polypeptides of approximate molecular weights 81 000, 70 000, 41 000, 38 000, 29 000, 19 000, 18 000, and 16 000. In pulse-labelled worms these peptides first appear at 29°C and continue to be synthesized up to lethal temperatures. They are heat inducible at every stage of development. While temperature elevation induces the synthesis of the heat-shock polypeptides, the *in vivo* synthesis of most other proteins present before heat shock is suppressed. In contrast, *in vitro* translation of mRNA from heat-shocked worms shows no alteration from the pattern of normal 20°C mRNAs except for the appearance of the heat-shock mRNAs. An *in vitro* study of RNA from control and heat-shocked dauer larvae shows that this developmental variant possesses little translatable mRNA but, upon heat shock, synthesizes a set of messages corresponding to the heat-shock polypeptides. The low background of this system will be especially useful in the analysis and purification of heat-shock mRNA for molecular cloning experiments. Extensive similarities between the *Drosophila* and *C. elegans* heat-shock responses are shown, including homology between the 70-kdalton heat-shock genes of the two organisms.

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L'exposition du nématode *Caenorhabditis elegans* à des températures élevées induit la synthèse préférentielle de huit polypeptides majeurs dont les poids moléculaires approximatifs sont de 81 000, 70 000, 41 000, 38 000, 29 000, 19 000, 18 000 et 16 000. Dans les vers soumis à un marquage court, ces peptides apparaissent d'abord à 29°C et leur synthèse continue jusqu'aux températures létales. Ils sont inductibles par la chaleur à chacun des stades du développement. Tandis que l'élévation de température induit la synthèse des polypeptides de choc thermique, la synthèse *in vivo* de la plupart des autres protéines présentes avant le choc thermique est supprimée. En revanche, la traduction *in vitro* des mRNA provenant des vers soumis au choc thermique suit le même profil que celle des mRNA normaux à 20°C, sauf pour ce qui est de l'apparition des mRNA induits par le choc thermique. Une étude *in vitro* des RNA provenant des larves "dauer" témoins et des larves soumises au choc thermique montre que cette variante développementale possède peu de mRNA traduisibles, mais qu'après un choc thermique, elle synthétise un groupe de messages correspondant aux polypeptides de choc thermique. La faible production de ce système sera spécialement utile dans l'analyse et la purification des mRNA induits par choc thermique dans les expériences de clonage moléculaire. *Drosophila* et *C. elegans* montrent beaucoup de ressemblances dans leur réponse au choc thermique, dont l'homologie entre les gènes de 70 kdaltons induits par choc thermique dans ces deux organismes.

[Traduit par la revue]

Introduction

A wide range of organisms, including insects (1), yeast (2), and several vertebrates (3), have been shown to respond to elevated temperatures by coordinately expressing a set of peptides called HSPs. While the biological significance of HSPs is unknown, their ubiquity implies that they confer a distinct selective advantage.

The response has been extensively studied in the fruit fly *Drosophila melanogaster* (reviewed in Ref. 4). In *Drosophila*, heat shock results in very rapid and extensive changes to the normal developmental pattern of gene activity. At the transcriptional level, Ritossa (5, 6) has demonstrated that a unique set of puffs could be induced by subjecting larvae to elevated temperatures. Temperature shock results in the regression of puffs normal to development while inducing puffs at nine loci. Further studies have shown that alterations in the patterns of protein and mRNA synthesis parallel the changes in chromosomal puffing patterns and that seven to nine polypeptides are made from newly synthesized mRNAs (1, 7, 8).

While heat-shocked *Drosophila* cells show a reduced synthesis of normal cellular proteins, they still retain their normal complement of 25°C mRNAs (7). A translational control mechanism exists whereby heat-shocked mRNA is selectively translated in heat-shocked cells, while nonshocked mRNA translation is suppressed (9, 10).

Recently, genes coding for most of the *Drosophila* HSPs have been cloned and sequenced. The major 70-kdalton-HSP gene is present in five copies located at two

ABBREVIATIONS: HSP, heat-shock peptide; NGM, nematode growth media; TCA, trichloroacetate; SDS, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetate.

cytological loci, while the lower molecular weight HSPs (22, 23, 26, and 27 kdaltons) are encoded by single copy genes grouped at cytological locus 67B (11–15). None of these genes contain intervening sequences.

The ubiquitous nature of the heat-shock response suggests a need to characterize it at the level of DNA sequence in several species. Such a comparison would provide valuable insight into the conservation and identification of regulatory sequences responsible for the expression of these coordinately expressed genes. The nematode *Caenorhabditis elegans* is an excellent model organism for the biochemical and genetical analysis of eukaryotic regulation (16).

We report here the demonstration of a heat-shock response in *C. elegans*. We show that heat shock of *C. elegans* produces dramatic alterations in the pattern of peptide synthesis normal to development, including the induction of a major 70-kdalton HSP. Homology between the *Drosophila* and *C. elegans* 70-kdalton HSP gene is also shown. The heat-shock response appears to be under both transcriptional and translational control, as demonstrated by *in vivo* and *in vitro* peptide synthesis patterns. Finally, using a *C. elegans* developmental variant, we demonstrate how a system highly enriched for the heat-shocked mRNAs may be used for molecular cloning experiments.

Materials and methods

Culturing and labelling

Experiments were performed with the nematode *C. elegans* N2 strain which was originally obtained from Brenner (Medical Research Council, Cambridge, England). Nematodes were cultured on NGM plates at 20°C and handled as described by Brenner (16). Radioactive *Escherichia coli* was prepared by growing strain B in a low sulphate medium supplemented with 10 mCi of [^{35}S]sulphate (25 Ci/mg, Amersham; 1 Ci = 37 GBq) as described (17). Labelled eggs were isolated by dissolving gravid adults, which had grown from the L_3 stage on labelled *E. coli*, in 2% sodium hypochlorite – 0.05 *M* sodium hydroxide for 10 min (18).

RNA isolation

Worms were washed off plates with 0.04 *M* NaCl and pelleted lightly. Ten volumes of cold 6 *M* guanidine hydrochloride – 0.2 *M* sodium acetate – 0.1 *M* β-mercaptoethanol (pH 5.0) was added and the suspension was immediately passed twice through a French Press at 12 000 psi (pounds per square inch; 1 psi = 6.894 757 kPa) onto ice. After spinning out the carcasses, half a volume of 95% ethanol was added and the RNA was precipitated overnight at −20°C. Nematode RNA was subsequently purified as described by Chirgwin et al. (19). The final RNA pellet was washed twice with 95% ethanol, dried under air, and dissolved to a concentration between 1 and 2 mg/mL in sterile water.

Heat shock

For RNA preparations, the resuspended worm pellets were immersed in water baths at the desired temperatures (20, 29, 31, 33, and 35°C). After 15 min, the worms were spotted onto prewarmed NGM plates and transferred to an incubator for various times. RNA was then purified as described in RNA isolation.

For *in vivo* heat-shock polypeptide synthesis, worms were collected and spotted onto plates containing radioactively labelled *E. coli* and allowed to feed at 20°C for 45 min. At time 0, they were collected, immersed into a water bath for 15 min, and then spotted onto prewarmed plates containing labelled *E. coli* for the duration of the heat shock. Following heat shock, the worms were spotted onto nonradioactive *E. coli* at 20°C for 30 min. Finally, worms were washed off, pelleted, and boiled in 50 μL of loading buffer (20). A portion of each sample was then separated on 10 or 12% acrylamide slab gels as described by Laemmli (20). After electrophoresis, gels were stained in 0.25% Coomassie blue, destained, dried, and autoradiographed.

Cell-free protein synthesis

Total RNA (2 μg) was translated in a cell-free rabbit reticulocyte lysate (21) for 60 min as described by the supplier New England Nuclear. Translation was monitored by the incorporation of [^{35}S]methionine into TCA-precipitable material. The reaction was terminated by the addition of 1 μg RNase and incubation at room temperature for 10 min. Translation samples were mixed 1:1 with loading buffer and approximately equal numbers of counts were electrophoresed on SDS gels as described by Laemmli (20).

Transfer of DNA to nitrocellulose

Nematode DNA was isolated from N2 worms as described by Emmons et al. (18), except that it was further purified through a 0.89 g $CsCl_2$ – 0.25 mg ethidium bromide per millilitre equilibrium gradient. DNA samples were completely digested with either *Eco*RI or *Hind*III restriction endonucleases and 4 μg of DNA per lane was loaded onto 0.7% agarose gels. Electrophoresis was performed in 89 m*M* Tris – 89 m*M* borate – 2.5 m*M* EDTA (pH 8.3) buffer overnight at a 15 mA current. DNA fragments were transferred from the gel to two pieces of nitrocellulose (Schleicher and Schuell, No. BA 85, 0.45-μm pore diameter) by the bidirectional transfer method of Smith and Summers (22).

Transfer of RNA to nitrocellulose

Poly(A)$^+$ heat-shock and control RNA was isolated from total RNA by passage through oligo(dT) (Collaborative Research) as described by Lee et al. (23). Both poly(A)$^+$ and total RNA were denatured in deionized 1 *M* glyoxal – 50% dimethyl sulphoxide – 10 m*M* sodium phosphate (pH 7.0) at 50°C for 60 min and then electrophoresed through a 1.1% agarose gel and transferred to nitrocellulose using the method of Thomas (24).

Nick translation

We screened *C. elegans* DNA and RNA samples for sequences homologous to a *D. melanogaster* 70-kdalton-HSP genomic clone. Plasmid 132 E3 (11) was digested with *Sal*I and the fragments were separated out on a 0.5% low melting point agarose gel (Bethesda Research Laboratories). A 3-kilobase fragment containing a single copy of the *Drosophila* 70-kdalton HSP gene was purified from the gel by the method of Langridge et al. (25). This DNA was then nick translated to a

specific activity of approximately 10^7–10^8 cpm/μg using [α-^{32}P]dCTP as described by Rigby et al. (26).

Hybridization

DNA and RNA filters were prehybridized in 0.2% SDS – 2.5 times Denharts (1 times is 0.02% bovine serum albumin, 0.02% Ficoll, 0.02% polyvinyl pyrolidone) – 5 times SSPE (1 times is 0.18 *M* NaCl, 10 m*M* sodium phosphate, 1 m*M* EDTA, pH 7.0) for 2 h at 57°C. Hybridization was carried out under these conditions except that the solution was made 10% dextran sulphate. After 72 h at 57°C, hybridization was stopped by washing filters in 0.2% SDS – 2 times SSPE at 45°C four times. Filters were then exposed to Kodak NS-2T film at −80°C for 48–96 h.

Results

Heat-shock peptides of C. elegans

Short exposure of *C. elegans* to temperatures 10 to 15° above normal 20°C culture conditions causes dramatic changes in the pattern of peptide synthesis. Figure 1 illustrates the *in vivo* peptide synthesis of worms cultured at various temperatures. There are several striking aspects of the temperature response. First, as compared with control worms, 29°C results in the immediate appearance of a 70 kdalton HSP which is not present at 20°C and which appears to increase in relative abundance as the temperature increases from 29 to 35°C (Fig. 1) In all heat-shock experiments conducted, the 70-kdalton HSP is always the major HSP. Also to be noted at 29°C is the appearance of two smaller peptides of 16 and 18 kdaltons, which sometimes appear in controls and are enhanced by heat shock.

Second, as the culture temperature is increased from 29 to 35°C, different peptide expression patterns develop. A comparison of lanes 2–5 (Fig. 1) shows that, while the 16-kdalton HSP is initially induced at 29°C, it subsequently disappears at temperatures of 33°C or greater. Also, as the temperature is increased to 35°C, peptides of 81, 29, 19, and two around 40 kdaltons appear. Two of these peptides (19 and 29 kdaltons) do not appear in control worms while the remainder (81, 41, and 38 kdaltons) are visible prior to heat shock and appear to be enhanced with increasing temperature.

In general, the nematode heat-shock response varies, depending upon the severity of shock, but always includes the induction of a major 70-kdalton HSP. The apparent molecular weights of the nematode HSPs are shown compared with protein standards and to the *Drosophila* HSPs (Fig. 2). As the molecular weights of some of the nematode HSPs were found to vary slightly between experiments, the values should be considered nominal.

Finally, the synthesis of many peptides normal to development decrease with elevated temperatures. These changes in labelling are not due to protein catabolism, as worms which are prelabelled at 20°C and then shifted to 35°C on unlabelled *E. coli* show no differences from the control *in vivo* peptide pattern (data not shown).

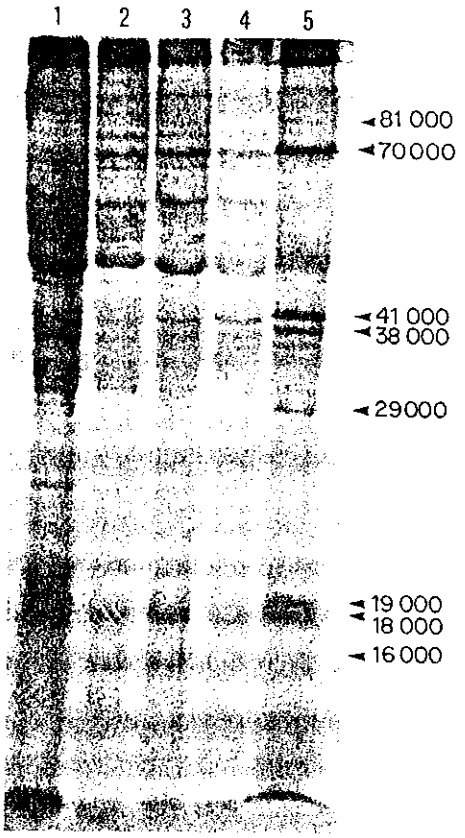


FIG. 1. *In vivo* polypeptide synthesis in worms exposed to various temperatures. A population of worms mixed with respect to age was allowed to feed on ^{35}S-labelled *E. coli* at 20°C for 45 min. At time 0, the worms were suspended in 0.04 *M* NaCl at the indicated temperature for 15 min, then spotted onto prewarmed plates and transfered to an incubator at that temperature for 4 h. After heat shock, the worms were allowed to feed on nonradioactive *E. coli* for 30 min, boiled, and then loaded onto a 12.5% SDS–acrylamide slab gel, electrophoresed, and autoradiographed. Lane 1 shows control worms at 20°C, while lanes 2–5 show worms incubated at 29, 31, 33, and 35°C, respectively.

Heat shock during development

The life cycle of *C. elegans* involves development from the egg, through four larval stages (L_1–L_4) to the adult, over a 3.5-day period at 20°C. At temperatures of 28°C and higher, the worms do not reproduce but stop growing and die slowly (with a half-life of about 7 h at 35°C). To test whether the heat-shock response was unique to any particular developmental stage, we pulse labelled synchronous populations of worms at each stage. Figure 3 shows that HSP induction at 33°C is not dependent upon a given developmental stage; that is they exhibit no stage-specific banding pattern differences.

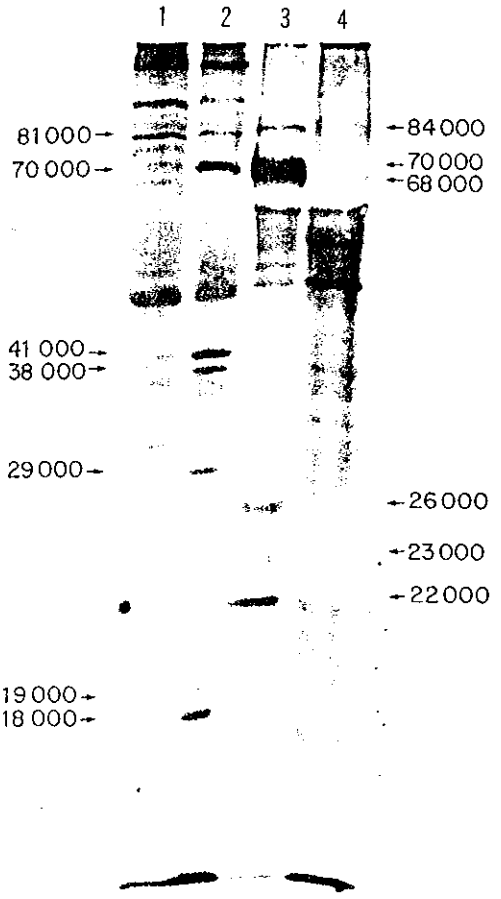


FIG. 2. Comparison of *C. elegans* and *D. melanogaster* heat-shock patterns. Lane 1 shows worm control peptide pattern (20°C) while lane 4 shows *Drosophila* control (25°C). Lanes 2 and 3 are worm and *Drosophila* heat shock, respectively. Worms were heat shocked at 35°C while the *Drosophila* shock was at 37°C. *Drosophila* samples were obtained from L. Moran.

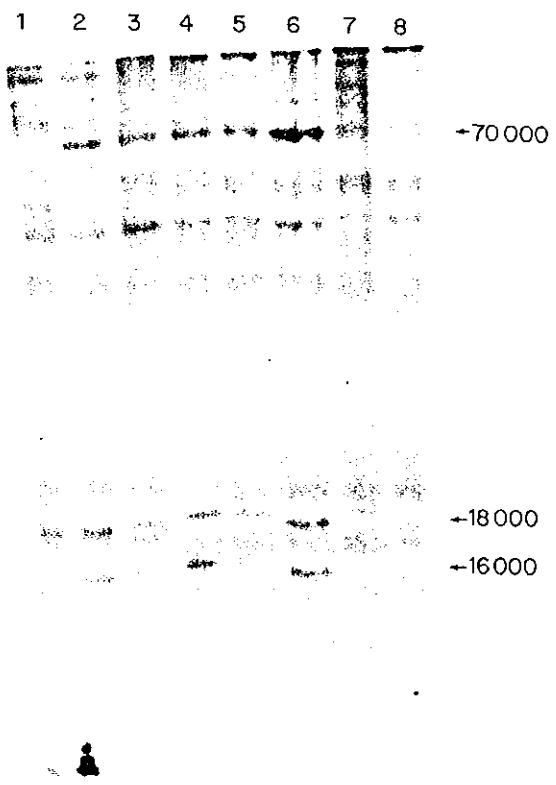
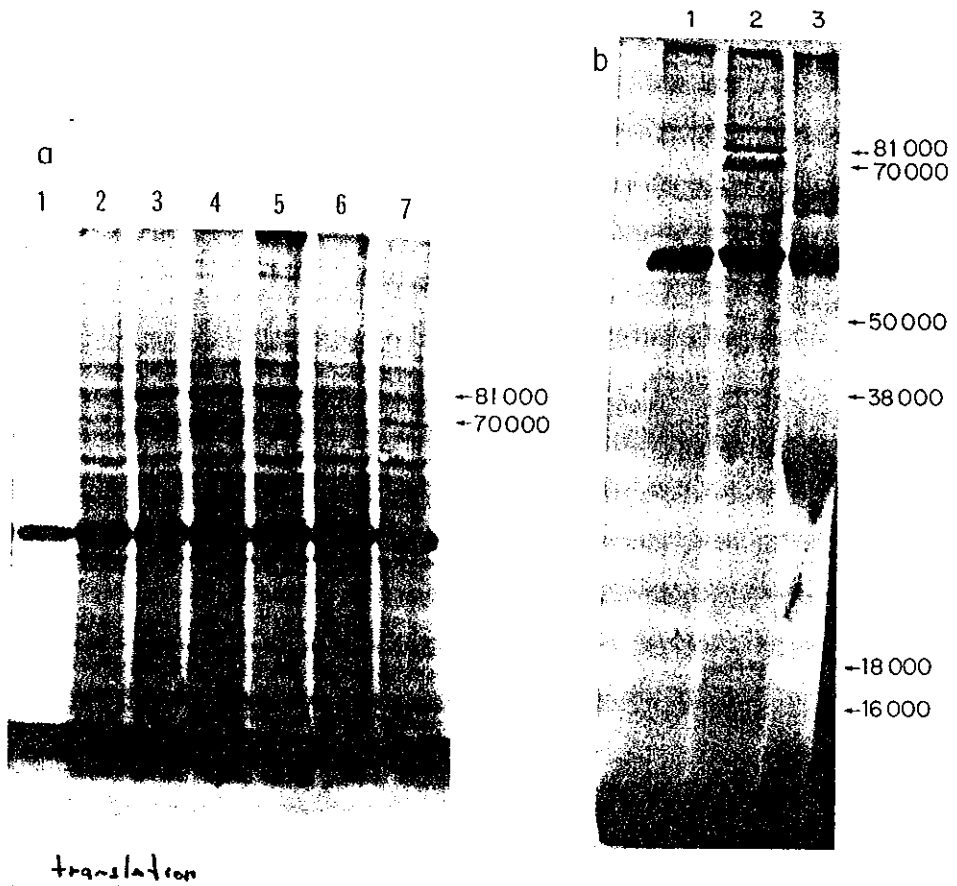


FIG. 3. Heat-shock induction at each developmental stage. Synchronous populations of worms were grown from eggs isolated by dissolving gravid adults in 2% sodium hypochlorite – 0.05 *M* sodium hydroxide for 10 min, and then filtering through a 50-μm Nitex filter. Heat shock was at 33°C as described in Fig. 1 legend. Labelled eggs were obtained by growing worms from the L_3 stage to adult on ^{35}S-labelled *E. coli* and isolating the eggs as described. Lanes 1 and 8 show control worms, while lanes 2 through 7 are stages egg, L_1, L_2, L_3, L_4, and adult, respectively.

Comparison with Drosophila *heat-shock response*

The well-documented heat-shock response of *Drosophila* indicates that changes in gene expression following heat shock provide a good model system for studying the regulation of eukaryotic gene expression (4). Figure 2 compares the heat-shock response of *D. melanogaster* to that of *C. elegans*. Lanes 1 and 2 show the nematode *in vivo* labelling patterns at 20 and 35°C, while lanes 3 and 4 show those for *Drosophila* at 37 and 25°C, respectively. The heat-shock responses show a number of similarities. Both *Drosophila* and *C. elegans* produce a unique set of polypeptides, which account for the majority of protein synthesis during heat shock. While heat-shocked *Drosophila* essentially synthesizes only HSPs, the nematode has a slightly higher background of other proteins. This difference is probably the result of the prelabelling with radioactively labelled *E. coli* required in the worms. As the heat-shock severity is increased in the worms, pharyngeal pumping decreases and eventually feeding stops altogether; consequently, they must be labelled prior to heat shock.

Similarities exist between the electrophoretic mobilities of the induced HSPs of *Drosophila* and *C. elegans*. The nematode 81-kdalton HSP and the *Drosophila* 83-kdalton HSP appear quite similar on one-dimensional electrophoretic gels. More striking are the similarities between the 70-kdalton HSP of the two species. In both cases the 70-kdalton HSP accounts for the majority of heat-induced polypeptide synthesis. Homology between the *Drosophila* and nematode 70-kdalton HSP is indicated by comigration on one-dimensional denaturing gels (data not shown). In contrast, the lower molecular weight HSPs of the two species do not appear to co-migrate.



translation

FIG. 4. *In vitro* ~~transcription~~ of total RNA from heat-shocked and control worms. Heat-shock and RNA purification were as described in Materials and methods. Purified RNA (2 μg) was translated in a cell-free rabbit reticulocyte lysate for 60 min. Translated samples were mixed 1:1 with loading buffer and approximately equal numbers of TCA-precipitable counts were electrophoresed. (*a*) Heat-shock and control RNA from growing worms. Lane 1 shows the endogenous RNA synthesis in the reticulocyte lysate, and lanes 2 and 7 show control RNA at 20°C, while lanes 3–6 show heat shock at 35°C for 3, 2, 1, and 0.5 h, respectively. (*b*) Translation of control dauer larvae and heat-shocked dauer larvae RNA. Lane 1, control; lane 2, heat shocked; lane 3, no message.

Translation of heat-shock messages in vitro

To study the mechanisms that control the reduction of nonheat-shock polypeptide synthesis and the induction of HSP synthesis during temperature shock, we analysed translatable mRNAs extracted from control and heat-shocked worms. Figure 4*a* shows the products of mRNA translated in a rabbit reticulocyte lysate. Lane 1 shows a single band around 50 kdaltons when no message is added and corresponds to the reticulocyte endogenous protein synthesis. Lanes 2 and 7 show the *in vitro* translation products of total mRNA isolated from worms grown at 20°C, while lanes 3–6 show the products of total mRNA isolated from worms heat shocked at 35°C. It is evident that there is no detectable difference in the nonheat-shock mRNA products, between heat-shocked and control worms. Thus, the *in vivo* reduction of nonheat-shock polypeptide synthesis during heat shock is not the result of degradation of the nonheat-shock mRNA, but rather due to a suppression of its translation.

The presence of nonheat-shock mRNA makes the analysis of mRNA coding for the low molecular weight HSPs difficult. Figure 4*a* shows, however, that there is little translatable mRNA coding for the 81- and 70-kdalton peptides at 20°C, but that they do appear at 35°C. The time course shows the appearance of translatable amounts of these mRNAs within 60 min of heat shock and their maximum accumulation after 4 h at the elevated temperature. While the 70-kdalton HSP is the major *in vivo* labelled HSP, the *in vitro* analysis here shows that both the 70- and 81-kdalton HSPs are translated in equal amounts.

Heat shock of C. elegans *dauer larva*

Having studied heat shock in normally growing

worms we decided to study its effect on the *C. elegans* dauer larva. This facultative larval stage is formed when environmental conditions are unfavorable. Both the behaviour (cessation of pharyngeal pumping) and morphology (formation of an impermeable cuticle) indicate that this juvenile variant is specialized for survival in harsh environmental conditions (27). Since the dauer larvae cannot feed they probably have a limited energy supply and, therefore, might not respond to heat shock as would a growing worm. To test this we subjected 7- to 10-day dauer larvae to a 4-h heat-shock and isolated total RNA. Figure 4*b* shows the *in vitro* translation of control and heat-shocked dauer larvae mRNA.

Several aspects of the results are interesting. First, compared with control dauer larvae it is apparent that a new set of mRNAs appear in the heat-shocked worms. The fact that worms in this stage will spend some of their limited energy on the production of the heat-shock mRNAs is surprising and possibly indicates the importance of the heat-shock response to the survival of the organism. Second, relative to growing worms, the dauer larvae do not appear to be actively synthesizing or accumulating translatable amounts of mRNA. Third, the low content of nonheat-shock mRNA in this system allows detection of heat-shock mRNA coding for the low molecular weight HSPs. The results indicate that the heat-shocked dauer larvae produce mRNAs coding for the same size polypeptides as the HSPs produced in growing worms. In addition, however, the heat-shocked dauers show at least one other inducible polypeptide (approximately 50 kdaltons) which is not detectable in growing worms.

Conservation of the 70-kdalton-HSP gene

A major stress-inducible polypeptide of 70 kdaltons has been reported in a variety of organisms (1, 3, 30–34). Homology between the 70-kdalton HSP of distant organisms is shown by the fact that trout, *Drosophila*, and nematode 70 kdalton HSP comigrate on one-dimensional denaturing gels (T. Snutch and R. Kothary, unpublished results). To test if the genomic sequences coding for this ubiquitous HSP are conserved throughout evolution, we hybridized a ^{32}P-labelled *Drosophila* 70-kdalton-HSP genomic clone to nematode DNA and RNA.

Figure 5*a* shows *Eco*RI and *Hind*III digested *C. elegans* DNA which has been transfered to nitrocellulose and hybridized to a *Drosophila* 70-kdalton-HSP clone. The result indicates that the *Drosophila* 70-kdalton-HSP clone is homologous to *C. elegans* genomic sequences. While several fragments hybridize, one fragment in particular (6.6 kilobase *Eco*RI, 9.5 kilobase *Hind*III) shows the majority of conserved sequences.

Further evidence that the 70-kdalton HSP is conserved between *Drosophila* and *C. elegans* is shown in Fig. 5*b*. Control and heat-shock poly(A)$^+$ RNAs were hybridized to the *Drosophila* probe using a "Northern" blot. This result indicates that the probe is specific for a heat-inducible mRNA of approximately 2300 nucleotides.

Discussion

Heat shock of *C. elegans* induces the synthesis of at least eight polypeptides of 81, 70, 41, 38, 29, 19, 18, and 16 kdaltons. The patterns of control and heat-shock peptides were determined by allowing worms to feed on ^{35}S-labelled *E. coli* for 45 min prior to and then throughout the duration of the heat shock. Since worms decrease feeding during heat shock, prelabelling was required to provide sufficient radioactivity to detect the HSPs. This prelabelling may have contributed to the background of nonheat-shock peptide synthesis.

The evidence from *in vivo* and *in vitro* polypeptide expression patterns suggests that the *C. elegans* heat-shock response is under both transcriptional and translational control. The appearance of *in vitro* translatable amounts of the 81- and 70-kdalton-HSP mRNAs within 60 min of temperature elevation suggests transcriptional control. This is further supported by Northern blot analysis. Hybridization of a ^{32}P-labelled *Drosophila* 70-kdalton-HSP gene to nematode control and heat-shock RNA shows the induction of a specific mRNA upon heat shock. The size of this message is that expected for the mRNA coding for a 70-kdalton peptide. The presence of a lightly hybridizing band in the control mRNA may indicate the presence of heat-shock cognate genes (28) in the nematode. Alternatively, it may be simply that the act of collecting worms off plates is sufficiently stressful to induce the 70-kdalton HSP.

The result also suggests that the nematode genomic DNA which hybridizes to the *Drosophila* probe corresponds to the nematode 70-kdalton-HSP gene(s). From the number and intensity of bands we estimate that *C. elegans* has two to three 70-kdalton-HSP genes. This conclusion has been verified by the isolation of a set of phage from a "Charon 4 library" which accounts for all the bands present on the genomic "Southern" (T. P. Snutch and D. L. Baillie, unpublished results).

A translational control of heat shock, similar to that described in *Drosophila* (9, 10), may also exist in *C. elegans*. While nonheat-shock polypeptide synthesis is reduced *in vivo* during heat shock, analysis of *in vitro* translation products shows that the RNA coding for these polypeptides is still present and functional. The selective translation of heat-shock mRNA has been shown not to involve any of the newly synthesized HSPs, but probably involves modification of protein-synthesizing factors and a special stability of the heat-shock mRNA (9, 10).

Dauer larvae have been shown to be resistant to a

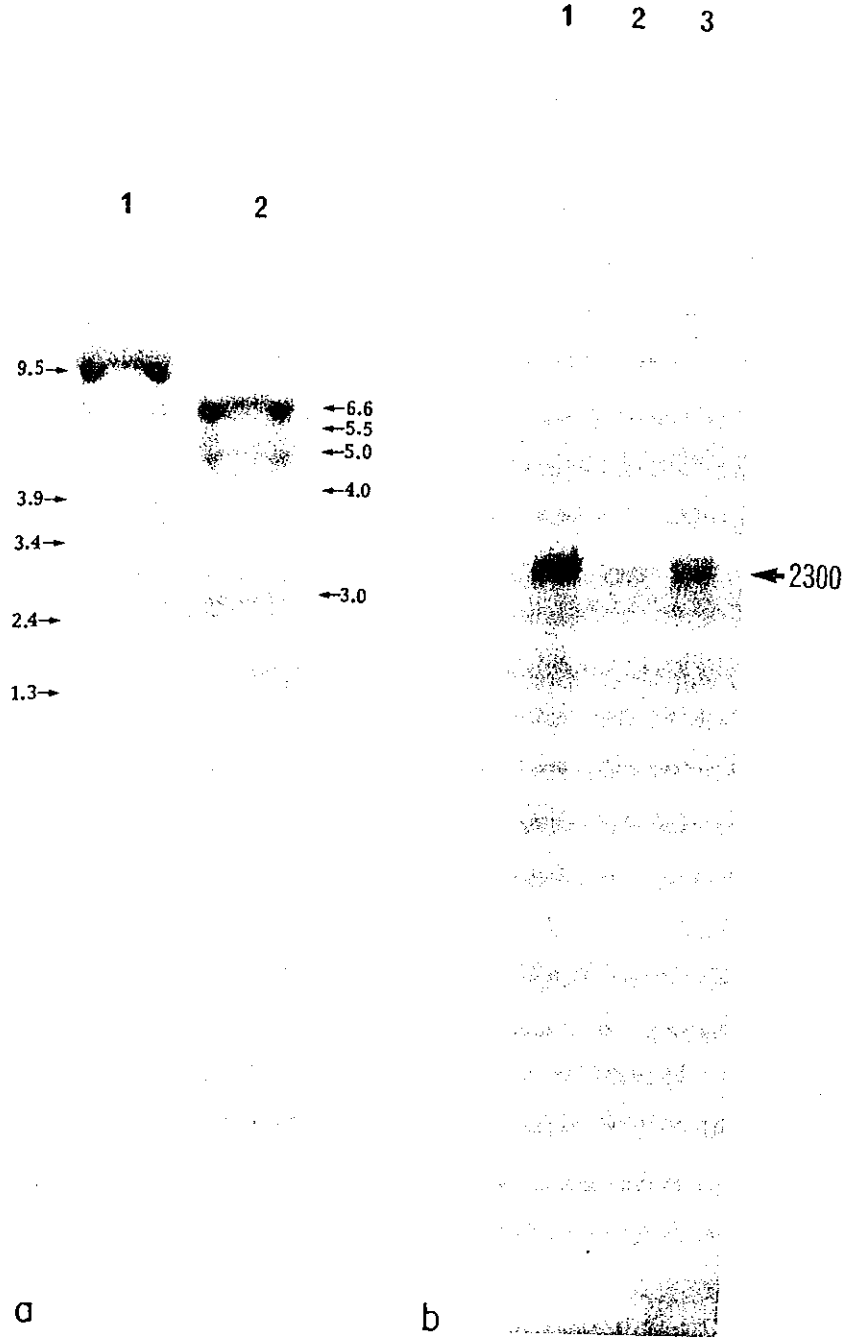


FIG. 5. (*a*) Hybridization of ^{32}P-labelled *Drosophila* 70-kdalton-HSP gene to *Hind*III (lane 1) and *Eco*RI (lane 2) digested *C. elegans* DNA. Sizes are in kilobases. (*b*) Hybridization of the same probe to heat-shock and control RNA. Lane 1, total RNA (40 μg) from heat-shocked dauer larvae; lane 2, poly(A)$^+$ RNA (10 μg) from control growing worms; lane 3, poly(A)$^+$ RNA (10 μg) from heat-shocked growing worms. Sizes are in nucleotides.

variety of chemical insults, osmotic shock, anoxia, and thermal stress (27, 29). We feel that our results are particularly novel in that these normally quiescent dauer larvae of *C. elegans* will react to temperature elevation by expending energy to actively synthesize the heat-shock mRNAs. The level of nonheat-shock mRNA in the dauer larvae is extremely low and the newly synthesized heat-shock mRNA accounts for a large percentage

of the total. Compared with growing worms, the dauer larvae are greatly enriched for heat-shock messages. For the purposes of obtaining molecular probes for the heat-shock genes, the enriched dauer preparation will eliminate the requirement for specific mRNA purifications, allowing direct construction of a cDNA library enriched for all HSP probes.

The nematode 70-kdalton HSP is the most prevalent HSP. A stress-induced peptide of similar molecular weight has also been shown in a variety of organisms (1, 3, 30–34). While the function of this HSP is unknown, its ubiquitous nature suggests a fundamental role in the heat-shock response. We report here that in addition to similar molecular weights, the *Drosophila* and *C. elegans* 70-kdalton-HSP genes share considerable homology. We are now attempting to isolate the nematode 70-kdalton-HSP gene(s) from a genomic library using the *Drosophila* probe. Sequence analysis of regions 5′ to the gene should provide valuable information into the conservation of sequences responsible for the regulation of these genes.

This is the first report of a heat-shock response in the phylum Nematoda and emphasizes the widespread nature of this response. This is further emphasized by the ability of dauer larvae to make heat-shock messages in the apparent absence of other message production. It is noteworthy that, although the heat-shock induction patterns vary between organisms, the 70 000 dalton peptide is apparently highly conserved and produced immediately upon heat shock. The nematode *C. elegans* with its well documented genetics is also well suited for biochemical analysis and is, therefore, an ideal organism in which to study the regulation of a set of heat-inducible genes.

Acknowledgements

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