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MINI-REVIEW

THE HSP70 MULTIGENE FAMILY OF *CAENORHABDITIS ELEGANS*

MARK F. P. HESCHL* and DAVID L. BAILLIE†

*Department of Physiological Chemistry, University of Wisconsin-Madison, 1300 University Avenue, Madison, Wisconsin, 53706 USA (Tel: 608 262 1347); and †Institute of Molecular Biology and Biochemistry, Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, V5A 1S6 Canada

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Abstract—1. The heat shock response of the nematode *Caenorhabditis elegans* has been characterized.

2. There are at least nine genes in the hsp70 multigene family of *C. elegans*.

3. Five of the hsp70 genes have been characterized and assigned to one of at least three hsp70 gene subfamilies. One of the subfamilies consists of an hsp70 protein that has the potential to be translocated into the endoplasmic reticulum and another subfamily consists of a protein that has the potential to be translocated into the mitochondria.

4. The *C. elegans* hsp70 multigene family has several unique characteristics including introns in the heat inducible hsp70 genes, at least one *trans*-spliced hsp70 mRNA and two grp78 related genes, one of which is highly heat inducible.

5. The identification and characterization of *C. elegans* hsp70 multigene family is the basis for a genetic characterization of the regulation and function of a gene family during the development of a multicellular eukaryote.

INTRODUCTION

All organisms respond to an increase in temperature by inducing the synthesis of a number of proteins referred to as heat shock proteins or hsps. The heat shock response is a universal response and can be induced by other forms of stress including anoxia, exposure to ethanol and heavy metal ions. In addition, either the hsps themselves or closely related hsp-like proteins (heat shock cognates or hsc) are normally present in cells and organisms and have an important role in normal cellular functioning. During heat shock, protein synthesis normal to development is repressed while synthesis of the hsps is enhanced or initiated. Repression of protein synthesis occurs before translation but after transcription (Lindquist, 1986; Lindquist and Craig, 1988). Heat shock expression of the hsp genes is mediated, in part, by heat shock elements (HSE) at the start of the gene (Bienz and Pelham, 1987).

The hsp70s (mol. wt 70,000) are evolutionarily highly conserved proteins. The hsp70 proteins are encoded by individual genes belonging to a multigene family, each gene differentially expressed under a number of different physiological conditions. Hsp70 multigene families have been identified in many organisms including *Drosophila melanogaster* (Lindquist and Craig, 1988), humans (Mues *et al.*, 1986), *Saccharomyces cerevisiae* (Craig, 1989) and, the subject of this review, *Caenorhabditis elegans* (Snutch *et al.*, 1988; Heschl and Baillie, 1989a, 1989b). The hsp70 and hsc70 proteins have been localized to the cytoplasm, the nucleus (Lindquist and Craig, 1988), the endoplasmic reticulum (ER) (Munro and Pelham, 1986; Rose *et al.* 1989; Normington *et al.*, 1989) and the mitochondria (Craig *et al.*, 1989; Engman *et al.*, 1989; Leustek *et al.*, 1989). Recent evidence suggests that the hsp70-related proteins are associated with other proteins and appear to be involved in: (1) the translocation of proteins across intracellular membranes into the ER and the mitochondria; (2) the secretion of proteins; (3) the binding of exposed hydrophobic sites on unfolded or malformed proteins and incompletely assembled protein complexes; and (4) the disassembly of folded protein complexes (Deshaies *et al.*, 1988; Ellis and Hemmingson, 1989; Rothman, 1989).

The heat shock response of Caenorhabditis elegans

Caenorhabditis elegans is a small, free-living soil nematode found commonly throughout many parts of the world and is well suited for combined biochemical and genetical analyses (Kenyon, 1988; Wood *et al.*, 1988). Feeding primarily on bacteria, this nematode reproduces with a life cycle of approximately 3 days under ideal conditions. After hatching the nematode undergoes four larval moults culminating in the mature, adult form. Each larval stage is designated L1 through L4. There are two adult forms, the self-fertilizing hermaphrodite and the male, each comprised of approximately 1000 somatic nuclei; the cell lineages of both are completely known (Sulston, 1988). Under conditions of limited food supplies, the L2 larva can enter an alternative developmental pathway to produce the dauer larva. This specialized L3 larva does not feed, is resistant to desiccation and stress, is altered in energy metabolism, is

arrested in development (Riddle, 1988) and may be transcriptionally silent (Snutch and Baillie, 1983).

The nematode, when shifted from 20°C to temperatures above 28°C, stops growing, fails to reproduce and slowly dies. Induction of the hsps first becomes apparent after exposure to temperatures greater than 29°C and up to at least 35°C. The synthesis of proteins normal to development is repressed post-transcriptionally upon heat shock. Eight sets of proteins ranging in mol. wts from 81,000 to 16,000 are induced upon heat shock. Hsp29, hsp19 and hsp16 are induced at 29°C, with the synthesis of hsp16 gradually decreasing as the severity of the stress increases. Synthesis of hsp81, hsp70, hsp41 and hsp38 are enhanced during heat stress. Hsp70, the major heat inducible protein, is synthesized immediately upon heat shock. Dauer larvae display a heat shock response and synthesize a set of the hsp mRNAs inducible during normal development. The only apparent difference is that the dauer larvae synthesize at least one extra protein of mol. wt approximately 50,000 when compared to nematodes growing normally (Snutch and Baillie, 1983).

There are at least nine members of the hsp70 multigene family in *C. elegans*. Six of these genes have been cloned and analyzed (Snutch *et al.*, 1988). Five of these cloned genes have been characterized and assigned to subfamilies based on nucleotide identity between each other and homology to other known hsp70-like genes (Snutch *et al.*, 1988; Heschl and Baillie, 1989a, 1989b). There are at least three hsp70 subfamilies with one or more gene members that have been named according to the first hsp70 gene defined for each subfamily. The HSP-1 subfamily is comprised of the *hsp-1* and *hsp-2*ps genes, the HSP-3 subfamily is comprised of the *hsp-3* and *hsp-4* genes and the HSP-6 subfamily is comprised of the *hsp-6* gene. The structural relationships of each of the members of the hsp70 multigene family of *C. elegans* is summarized in Fig. 1. A comparison of the *C. elegans* hsp70 multigene family to the *S. cerevisiae* hsp70 multigene family (Craig, 1989) indicates that several gene members and subfamilies remain to be identified in *C. elegans*. The *hsp-1* gene has been mapped genetically to the right end of linkage group (LG) IV (Snutch *et al.*, 1988) while the *hsp-2*ps and *hsp-3* genes have been assigned to positions on the cloned *C. elegans* genome and both have been mapped by *in situ* hybridizations to LGX (left) (as described in Coulson *et al.*, 1986, 1988; D. Albertson, pers. commn).

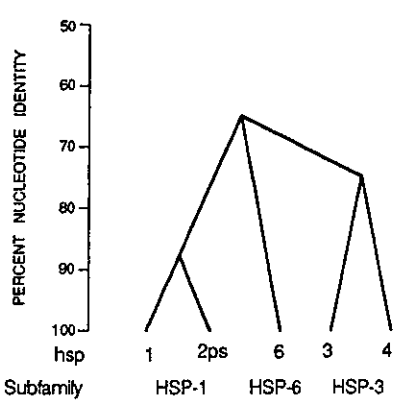

Fig. 1. Structural relationships of members of the *C. elegans* hsp70 multigene family. Approximate nucleotide identities are based on complete (*hsp-1*, *hsp-2*ps and *hsp-3*) and partial (*hsp-4* and *hsp-6*) DNA sequence data. The sequence data is compiled from Snutch *et al.*, 1988; Heschl and Baillie, 1989a,b; and our unpublished data.

The HSP-1 subfamily

The *hsp-1* gene is expressed throughout nematode development. Upon temperature upshift, *hsp-1* mRNA synthesis is enhanced 2–6-fold. The 5′ regulatory region of the *hsp-1* gene contains several copies of the HSE, consistent with the heat inducibility of the gene (Snutch *et al.*, 1988). In the unstressed dauer larva, it appears that the *hsp-1* gene is transcribed at levels comparable to those observed at other larval stages (B. Dalley and M. Golomb, pers. commn). Characterization of the *hsp-1* gene and its predicted protein product, hsp70A, suggests that hsp70A is closely related to the *Drosophila* heat inducible hsp70s and the constitutively expressed, heat inducible hsc70 and the *S. cerevisiae* SSA hsp70 subfamily (Snutch *et al.*, 1988).

During our analysis of the *hsp-1* gene and two other heat inducible hsp70 genes, *hsp-4* and *hsp-6* (discussed below), we detected the presence of three introns or non-coding, intervening sequences. The heat inducible hsp16 genes of *C. elegans* also have introns (Russnack and Candido, 1985; Jones *et al.*, 1986). At first, this does not seem unusual as *C. elegans* genes characterized to date contain introns (Blumenthal and Thomas, 1988). However, it is unusual for heat inducible hsp genes to be interrupted by introns. Yost and Lindquist (1986) demonstrated in *Drosophila* that intron excision (or *cis*-splicing) from pre-mRNAs is transiently inhibited during a severe heat stress, including excision of the sole intron from the *Drosophila* hsp83 pre-mRNA. If intron excision is inhibited upon severe heat shock in the nematode then the introns in the heat inducible hsp70 and hsp16 genes should not be excised. It has also been demonstrated that the *hsp-1* mRNA is *trans*-spliced (Bektesh *et al.*, 1988; our unpublished results). *Trans*-splicing involves the attachment of an exon encoded elsewhere in the genome to the pre-mRNAs presumably by mechanisms similar to those used in *cis*-splicing (Blumenthal and Thomas, 1988). Therefore, *trans*-splicing should also be inhibited upon heat shock. It follows that if the pre-mRNAs are not processed, heat shock proteins (or other proteins synthesized from a *cis*- or *trans*-spliced mRNA) would not be synthesized. Inconsistent with this idea is the observation that heat shock protein synthesis occurs after prolonged heat stresses (Snutch and Baillie, 1983). There are three possibilities to explain these observations. First, some of the uncharacterized hsp genes may not have introns or be *trans*-spliced thereby accounting for the protein synthesis observed. Second, the range of heat stresses tested by Snutch and Baillie (1983) was not severe enough to inhibit intron splicing in the nematode. Third, the nematode may have developed a splicing mechanism that is resistant to heat shock and is highly selective for the heat shock gene pre-mRNAs upon heat stress. Such a system has evolved in trypanosomes where all

pre-mRNAs are *trans*-spliced normally during development but only the hsp pre-mRNAs are *trans*-spliced upon heat shock (Muhich and Boothroyd, 1989). In all three cases it should be noted that the observed heat stress induced lethality is probably the result of the other debilitating effects of heat stress such as the observed lack of feeding or, possibly, dehydration.

The DNA immediately surrounding *hsp-1* (i.e. 20–30 kilobases on either side) has accumulated a greater number of nucleotide changes than regions further upstream or downstream of *hsp-1* as detected in a comparison of the *hsp-1* gene region between two strains of *C. elegans*. The accumulation of these nucleotide changes could result if the region surrounding *hsp-1* was always in an open configuration (i.e. transcriptionally active) in the germline and, therefore, susceptible to DNA damage (Snutch and Baillie, 1984). This proposal would imply that the *hsp-1* mRNA is stored in the oocyte as a maternal mRNA or is necessary for the production of the gametes.

The *hsp-2*ps gene is closely related to the *hsp-1* gene. No transcripts have been detected for the *hsp-2*ps gene. Cross-hybridization of the *C. elegans* *hsp-2*ps gene to the genome of the closely related nematode *C. briggsae* did not reveal the presence of a *hsp-2*ps homolog in *C. briggsae* (Snutch *et al.*, 1988). This raised the possibility that the *hsp-2*ps gene, gained after the divergence of *C. elegans* and *C. briggsae*, was either a pseudogene or a recent gene duplication not expressed under the conditions tested. A comparison of the DNA sequences of the *hsp-1* and *hsp-2*ps genes revealed that they shared 88% identity at the nucleotide level. Further analysis demonstrated that the *hsp-2*ps gene, with respect to *hsp-1*, was truncated missing the last third of the *hsp-1* gene, yet contained the first two introns and part of the transcribed, untranslated sequence (but not the *trans*-spliced leader sequence) and had several mutations which disrupted the coding region. These observations confirmed the identification of *hsp-2*ps as a pseudogene of *hsp-1* (Heschl and Baillie, 1989a). Based on the greater mutability of the *hsp-1* gene region and the structure of *hsp-2*ps, we proposed that the *hsp-2*ps gene was probably generated by transposition of a copy of the normal *hsp-1* gene on chromosome IV to the X chromosome and not by reverse transcription of a mRNA (Heschl and Baillie, 1989a).

The HSP-3 subfamily

The *hsp-3* gene is constitutively expressed and not heat inducible. Transcripts from the *hsp-3* gene have been detected throughout development being most abundant at the L1 larval stage (Snutch *et al.*, 1988). The *hsp-3* coding region is interrupted by three introns. Unexpectedly, we detected an identity to the HSE in the 5′ region of the *hsp-3* gene (Heschl and Baillie, 1989b). This would suggest that *hsp-3* might be heat inducible but heat inducibility was not detected under the conditions tested (Snutch *et al.*, 1988). Characterization of hsp70C, the predicted *hsp-3* gene product, revealed that hsp70C, with respect to hsp70A, had a long hydrophobic amino terminus which is characteristic of proteins imported into the ER (Colman and Robinson, 1986; Verner and Schatz, 1988). Hsp70C also had the carboxyl terminal sequence KDEL (Lys-Asp-Glu-Leu) which is characteristic of proteins retained in the ER (Pelham, 1989). We concluded (Heschl and Baillie, 1989b) that hsp70C was closely related to the ER-localized mammalian grp78 (glucose regulated protein) also known as BiP (immunoglobulin heavy chain binding protein). Expression of the mammalian grp78 is enhanced when glucose levels are low or if calcium ionophores are present but not upon heat shock (Lee, 1987). It will be interesting to determine if expression of *hsp-3* is enhanced upon starvation as L2 larvae enter the dauer larvae developmental pathway, a situation potentially analogous to glucose starvation.

The *hsp-4* mRNA is barely detectable under normal growth conditions. Upon heat stress, synthesis of the *hsp-4* mRNA is enhanced as much as 50 times that of the control level (Snutch *et al.*, 1988). Characterization of the last half of *hsp-4* revealed the presence of at least two introns in the *hsp-4* gene. The *hsp-4* gene product, hsp70D, is closely related to both the hsp70C and grp78 proteins (our unpublished results). However, the carboxyl terminus is HDEL (His-Asp-Glu-Leu) instead of KDEL similar to the *S. cerevisiae* grp78 equivalent, KAR2 (Rose *et al.*, 1989; Normington *et al.*, 1989). Like the polypeptide KDEL, HDEL is important for the retention of KAR2 in the ER of yeast (Pelham, 1989). *KAR2* is normally expressed at high levels during growth and expression is further enhanced upon heat stress (Rose *et al.*, 1989; Normington *et al.*, 1989). Assuming that hsp70D is translocated into the ER as are grp78 and KAR2, then the situation in *C. elegans* appears to be unique in that the nematode contains two grp78-like genes, one that is constitutively expressed and one that is highly heat inducible. If we consider *S. cerevisiae* to more closely represent the ancestral situation with the constitutively expressed, heat inducible grp78-like gene, it would be interesting to explore the apparent division of expression and probable division of function of the grp78s as seen in the nematode system and the apparent loss of a highly heat inducible grp78 gene as seen in the mammalian system.

During the course of our characterization of the *hsp-3* gene, we compared the 5′ regulatory region of *hsp-3* to the rat grp78 gene. If these two genes are functionally similar as proposed then elements used to mediate the expression of the grp78 homologs should be conserved. In fact, such a conserved element was detected (Heschl and Baillie, 1989b). The corresponding element from the rat grp78 gene has been shown to direct expression of the rat grp78 gene as well as to bind a putative regulatory protein (Resendez *et al.*,1988). A comparison of the *C. elegans* 5′ regulatory region was extended to the regulatory region of the *hsp-3* homolog from *C. briggsae*. Similarly, if any elements are important for the regulation of the *hsp-3* homologs in *Caenorhabditis*, these too should be conserved between these sister species. Several conserved elements were detected including, but not limited to, the HSE, the element identified in the rat/*C. elegans* comparison and several identities to SV40 and adenovirus enhancers (Heschl and Baillie, 1990). The presence of

identities to mammalian viral enhancers in *C. elegans* suggests that these gene regulatory elements are relatively ancient and have either been recruited by the mammalian viruses or there exists an unidentified virus or viruses distantly related to the mammalian viruses which can infect *Caenorhabditis* sp. With the development of integrative transformation techniques which allow the correct expression of the transformed genes (Fire, 1986; Fire and Waterston, 1989), the ability of the conserved elements to direct expression of the *hsp-3* genes can now be tested.

The HSP-6 subfamily

The *hsp-6* gene is constitutively expressed and heat inducible (Snutch *et al.*, 1988). Several copies of the HSE in the 5′ regulatory region were detected, consistent with the heat inducibility of *hsp-6*. The first two-thirds of the *hsp-6* gene contains two introns. Analysis of the predicted partial *hsp-6* protein product, hsp70F, with respect to hsp70A, revealed the presence of an amphiphilic leader sequence rich in serine and threonine (Heschl and Baillie, 1989b). This is characteristic of leader sequences on proteins imported into the mitochondria (Colman and Robinson, 1986; Roise and Schatz, 1988; Verner and Schatz, 1988). A comparison of hsp70F to known hsp70-like proteins suggested that hsp70F was more closely related to the prokaryotic hsp70 homolog from *Escherichia coli*, dnaK, than known eukaryotic hsp70s (Heschl and Baillie, 1989b). Subsequently, a number of hsp70 proteins have been demonstrated to be translocated into the mitochondria (Craig *et al.*, 1989; Engman *et al.*, 1989; Leustek *et al.*, 1989). Like hsp70F, these proteins are more closely related to the bacterial hsp70 homolog than eukaryotic hsp70s. The close identity of these mitochondrial imported proteins with the bacterial hsp70 homolog is not too surprising since it is widely believed that mitochondria arose through a symbiotic relationship between bacteria and the primitive eukaryotic cell.

Perspectives

The isolation of mutant eukaryotic hsp genes has been done primarily in the unicellular organism *S. cerevisiae*. In such a system, the effects of some mutant genes may not be readily detectable. For example, mutations in individual members of the yeast hsp70 SSA subfamily have no apparent effect. However, when two or more SSA mutations are combined, there are visible effects on the growth or viability of the yeast (Craig, 1989). The lack of mutant heat shock protein genes in higher multicellular eukaryotes, such as *Drosophila melanogaster*, may reflect the redundancy of the hsp70 genes in these sytems. The relative simplicity of *C. elegans* offers an alternative to *S. cerevisiae* and the more complex eukaryotic systems to combine both biochemistry and genetics to study multigene families. The identification of the hsp70 multigene family from *C. elegans* represents the first step towards a genetic dissection of the heat inducible and developmentally regulated hsp70 genes in a multicellular eukaryote. There are many questions concerning the regulation and the roles of the hsp70s during development that can be answered using the nematode. These include determining the potential maternal expression of the *hsp-1* gene, the role and regulation of the two grp78-like proteins in the formation of the dauer larva, the developmental regulation of the hsp70 genes, the effects of hsp70 mutants on the development of the nematode and the effects of severe heat stresses on the processes of *cis*- and *trans*-splicing. The answers to these questions will not only give us insights into the regulation and function of the hsp70 genes during development and heat stress but will also provide us with valuable clues into the regulation of other gene families during the development of multicellular eukaryotes.

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REFERENCES

Bektesh S., Doren K. van and Hirsh D. (1988) Presence of the *Caenorhabditis elegans* spliced leader on different mRNAs and in different genera of nematodes. *Genes Dev.* **2**, 1277–1283.

Bienz M. and Pelham H. R. B. (1987) Mechanisms of heat-shock gene activation in higher eukaryotes. *Adv. Genet.* **24**, 31–72.

Blumenthal T. and Thomas J. (1988) *Cis* and *trans* mRNA splicing in *C. elegans*. *TIG* **4**, 305–308.

Colman A. and Robinson C. (1986) Protein import into organelles: hierarchial targeting signals. *Cell* **46**, 321–322.

Coulson A., Sulston J., Brenner S. and Karn J. (1986) Toward a physical map of the genome of the nematode *Caenorhabditis elegans*. *Proc. natn. Acad. Sci. USA* **83**, 7821–7825.

Coulson A., Waterston R., Kiff J., Sulston J. and Kohara Y. (1988) Genome linking with yeast artificial chromosomes. *Nature* **335**, 184–186.

Craig E. A. (1989) Essential roles of 70 kDa heat inducible proteins. *BioEssays* **11**, 48–52.

Craig E. A., Kramer J., Schilling J., Werner-Washburne M., Holmes S., Kosic-Smithers J. and Nicolet C. M. (1989) *SSC1*, an essential member of the yeast HSP70 multigene family, encodes a mitochondrial protein. *Mol. Cell. Biol.* **9**, 3000–3008.

Deshaies R. J., Koch B. D. and Schekman R. (1988) The role of stress proteins in membrane biogenesis. *TIBS* **13**, 384–388.

Ellis R. J. and Hemmingson S. M. (1989) Molecular chaperones: proteins essential for the biogenesis of some macromolecular structures. *TIBS* **14**, 339–342.

Engman D. M., Kirchhoff L. V. and Donelson J. E. (1989) Molecular cloning of mtp70, a mitochondrial member of the hsp70 family. *Mol. Cell. Biol.* **9**, 5163–5168.

Fire A. (1986) Integrative transformation of *Caenorhabditis elegans*. *EMBO J.* **5**, 2673–2680.

Fire A. and Waterston R. H. (1989) Proper expression of myosin genes in transgenic nematodes. *EMBO J.* **8**, 3419–3428.

Heschl M. F. P. and Baillie D. L. (1989a) Identification of a heat-shock pseudogene from *Caenorhabditis elegans*. *Genome* **32**, 190–195.

Heschl M. F. P. and Baillie D. L. (1989b) Characterization of the hsp70 multigene family of *Caenorhabditis elegans*. *DNA* **8**, 233–243.

Heschl M. F. P. and Baillie D. L. (1990) Functional elements and domains inferred from sequence comparisons of a heat shock gene in two nematodes. *J. molec. Evol.* (in press).

Jones D., Russnack R. H., Kay R. J. and Candido E. P. M. (1986) Structure, expression, and evolution of a heat shock gene locus in *Caenorhabditis elegans* that is flanked by repetitive elements. *J. biol. Chem.* **261,** 12,006–12,015.

Kenyon C. (1988) The nematode *Caenorhabditis elegans*. *Science* **240,** 1448–1453.

Lee A. S. (1987) Coordinated regulation of a set of genes by glucose and calcium ionophores in mammalian cells. *TIBS* **12,** 20–23.

Leustek T., Dalie, B., Amir-Shapira D., Brot N. and Weissbach H. (1989) A member of the Hsp70 family is localized in mitochondria and resembles *Escherichia coli* DnaK. *Proc. natn. Acad. Sci. USA* **86,** 7805–7808.

Lindquist S. (1986) The heat-shock response. *A. Rev. Biochem.* **55,** 1151–1191.

Lindquist S. and Craig E. A. (1988) The heat-shock proteins. *A. Rev. Genet.* **22,** 631–677.

Mues G. I., Munn T. Z. and Raese J. D. (1986) A human gene family with sequence homology to *Drosophila melanogaster* Hsp70 heat shock gene. *J. biol. Chem.* **261,** 874–877.

Muhich M. L. and Boothroyd J. C. (1989) Synthesis of trypanosome hsp70 mRNA is resistant to disruption of *trans*-splicing by heat shock. *J. biol. Chem.* **264,** 7107–7110.

Munro S. and Pelham H. R. B. (1986) An hsp70-like protein in the endoplasmic reticulum identity with the 78 kD glucose-regulated protein and immunoglobulin heavy chain binding protein. *Cell* **46,** 291–300.

Normington K., Kohno K., Kozutsumi Y., Gething M.-J. and Sambrook J. (1989) *S. cerevisiae* encodes an essential protein homologous in sequence and function to mammalian BiP. *Cell* **57,** 1223–1236.

Pelham H. R. B. (1989) Control of protein exit from the endoplasmic reticulum. *A. Rev. Cell Biol.* **5,** 1–23.

Resendez E. Jr., Wooden S. K. and Lee A. S. (1988) Identification of highly conserved regulatory domains and protein-binding sites in the promoters of the rat and human genes encoding the stress-inducible 78-kilodalton glucose-regulated protein. *Mol. Cell. Biol.* **8,** 4579–4584.

Riddle D. L. (1988) The dauer larva. In *The nematode Caenorhabditis elegans* (Edited by Wood W. B. *et al.*), pp. 393–412. Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, USA.

Roise D. and Schatz G. (1988) Mitochondrial presequences. *J. biol. Chem.* **263,** 4509–4511.

Rose M. D., Misra L. M. and Vogel J. P. (1989) *KAR2*, a karyogamy gene, is the yeast homolog of the mammalian BiP/GRP78 gene. *Cell* **57,** 1211–1221.

Rothman J. E. (1989) Polypeptide chain binding proteins: catalysts of protein folding and related processes in cells. *Cell* **59,** 591–601.

Russnack R. H. and Candido E. P. M. (1985) Locus encoding a family of small heat shock genes in *Caenorhabditis elegans*: Two genes duplicated to form a 3.8-kilobase inverted repeat. *Mol. Cell. Biol.* **5,** 1268–1278.

Snutch T. P. and Baillie D. L. (1983) Alterations in the pattern of gene expression following heat shock in the nematode *Caenorhabditis elegans*. *Can. J. Biochem. Cell Biol.* **61,** 480–487.

Snutch T. P. and Baillie D. L. (1984) A high degree of DNA strain polymorphism associated with the major heat shock gene in *Caenorhabditis elegans*. *Mol. Gen. Genet.* **195,** 329–335.

Snutch T. P., Heschl M. F. P. and Baillie D. L. (1988) The *Caenorhabditis elegans* hsp70 gene family, a molecular genetic characterization. *Gene* **64,** 241–255.

Sulston J. (1988) Cell Lineage. In *The nematode* Caenorhabditis elegans (Edited by Wood W. B. *et al.*), pp. 123–155. Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, USA.

Verner K. and Schatz G. (1988) Protein translocation across membranes. *Science* **241,** 1307–1313.

Wood W. B. *et al.* (eds) (1988) *The nematode* Caenorhabditis elegans. Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, USA.

Yost, H. J. and Lindquist S. (1986) RNA splicing is interrupted by heat shock and is rescued by heat shock protein synthesis. *Cell* **45,** 185–193.