Molecular Characterization of a Novel, Developmentally Regulated Small Embryonic Chaperone from *Caenorhabditis elegans*

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Low molecular weight chaperones inhibit protein aggregation and facilitate refolding of partially denatured polypeptides in cells subjected to physical and chemical stresses. The nematode *Caenorhabditis elegans* provides a system amenable for investigations on roles for chaperone proteins in normal homeostasis and development. We characterized a *C. elegans* gene and cDNAs that encode a novel, small embryonic chaperone-like protein (SEC-1) that is composed of 159 amino acids. The central core of SEC-1 (residues 45–126) is ~40% identical with a corresponding segment of mammalian Hsp27 and αB crystallin. Expression of SEC-1 in *Escherichia coli* confers thermostolerance on the bacterium. SEC-1 mRNA is evident only in *C. elegans* oocytes and developing embryos. Translation and accumulation of SEC-1 protein is temporally coupled with a prolonged burst of intense protein synthesis and rapid mitogenesis during early embryogenesis. As the rate of protein synthesis decreases during late embryogenesis, levels of SEC-1 and its cognate mRNA detectably decline. Induction/deinduction of SEC-1 is precisely regulated by intrinsic developmental factors rather than extrinsic stresses. In vivo injection of *C. elegans* oocytes with antisense oligonucleotides that complement the 5′-end of SEC-1 mRNA arrests nematode development at an early stage after fertilization. Thus, SEC-1 appears to be adapted to perform essential functions in early embryogenesis.

Small heat shock proteins (sHsps) are ubiquitously expressed in organisms ranging from *Escherichia coli* to humans (1–5). The principal sHsp prototypes in mammals are Hsp27 (~200–210 amino acid residues) and the α crystallins (~175 residues) (6, 7). Human Hsp27 and α crystallin are 35% identical in their central and COOH-terminal regions, and both proteins oligomerize to generate high molecular weight (>600,000) complexes (6–8). α-crystallins and Hsp27 are often expressed at low levels in unstressed cells and tissues. However, the biosynthesis and accumulation of sHsps are induced by heat shock, interleukin-1, toxic chemicals (arsenite, oxidative stress), and hydrolysis of ATP by the chaperones; in contrast, sHsps promote folding in an ATP-independent manner (8, 18, 19). Investigations on sHsps have focused principally on the ability of these proteins to protect cells against physical, chemical, and biological stresses. However, the properties of small molecular chaperones are also well suited for subserving physiological roles in unstressed cells. For example, the rate and level of protein synthesis are markedly elevated at various stages of embryogenesis in many organisms. As a consequence, cellular machinery that ensures efficient, high fidelity folding of polypeptides may be required for embryonic homeostasis. Hsp70 chaperones are absent during early developmental stages in mammals, and the forced expression of these proteins in *Drosophila* embryos results in lethality (1, 4). Thus, embryonic expression of small molecular chaperone proteins, which inhibit nascent polypeptide aggregation and promote accurate folding of newly synthesized proteins, could potentially support and facilitate the rapid progression of early development. Small molecular chaperones that reversibly associate with actin filaments may also be intimately involved in the multiple cycles of cytoskeleton reorganization that accompany serial cell divisions and extensive cell differentiation in embryos.

At present, little is known about expression, structure, regulation, and possible functions of sHsp-related proteins during early embryogenesis in mammals and most other organisms. The nonparasitic nematode *Caenorhabditis elegans* is a good model system for investigations on these topics. Adult *C. elegans* are composed of 959 somatic cells, which are organized...
into highly specialized tissues that constitute reproductive, digestive, muscular, hypodermal, and nervous systems (23–25). The cellular and developmental biology of the nematode have been characterized in exceptional detail, and the lineage of each cell in the animal has been established (23–25). Moreover, the molecules and mechanisms that govern signal transduction and development in C. elegans are universal and control similar processes in higher organisms, including mammals (26, 27). Embryogenesis in C. elegans is an invariant, stereotypical process that is completed over a period of 14 h (28). Approximately 60% of the adult’s ultimate constellation of cells is generated from the zygote during the first 6 h of embryogenesis. Morphogenesis of the embryo into an L1 larva ensues in the subsequent 8 h. Compression of intensive embryonic protein synthesis and multiple cytoskeletal rearrangements into a relatively brief time frame creates an environment in which the demand for efficient and accurate protein folding is extremely high.

Previous investigations on C. elegans failed to identify small molecular chaperones that are expressed in normally developing, unstressed embryos. Candido and co-workers (29, 30) characterized four homologous C. elegans genes (hsp16–41, hsp16–48, hsp16–1, and hsp16–2) that encode putative α-helices composed of 143 or 145 amino acid residues. Portions of the central and COOH-terminal regions of these predicted C. elegans polypeptides share substantial sequence identity/similarity with corresponding, highly conserved regions in mammalian Hsp27 and α crystallin homologs. However, C. elegans hsp16–1, hsp16–2, hsp16–41, and hsp16–48 genes are fully repressed and inducible during early embryogenesis (31). Moreover, accumulation of the various nematode HSP16 proteins at later stages of development is strictly dependent upon heat shock or other stresses. We now report the discovery of a C. elegans gene (sec-1) that encodes a novel, developmentally regulated chaperone protein (M, 18,000) that performs critical functions in early embryogenesis.

**EXPERIMENTAL PROCEDURES**

**Growth of C. elegans**—The Bristol N2 strain of C. elegans was grown at 20 °C as described previously (32). To synchronize C. elegans for developmental studies, embryos were hatched in the absence of nutrients and then transferred to plates containing E. coli as a food source. Under these conditions, the worms develop synchronously into reproductive adults (33). L1 larvae were harvested 6 h after feeding, L2 larvae at 20 h, L3 at 29 h, L4 larvae at 40 h, young adult worms at 53 h, and egg-laying adult nematodes at 75 h. A purified population of embryos was obtained by alkaline hypochlorite treatment of gravid egg-laying adults (33). L1 larvae were harvested 6 h after feeding, L2 larvae 10 min and then incubating at 42 °C for 8 h. The hybridized primer was extended using avian myeloblastosis virus reverse transcriptase, as described by Reeder et al. (42). The extended product was characterized by electrophoresis on a 6% polyacrylamide gel containing 7 m urea and subsequent autoradiography.

**Expression and Purification of Recombinant SEC-1 Fusion Protein**—A 507-bp XhoI-EcoRI restriction fragment of SEC-1 cDNA was subcloned into the pRSSET-C expression plasmid (Invitrogen). This plasmid was transformed with the expression plasmid and induced with 1 μM isopropropyl-thio-galactopyranoside (IPTG) for 1 h at 37 °C. The host bacterium contains a chromosomal copy of the phage T7 RNA polymerase gene under the control of the lac promoter. Bacteria were harvested, disrupted, and separated into soluble and particulate fractions as described for previous studies (43). The SEC-1 fusion protein was recovered in the pellet fraction, because it is insoluble in the standard 20 mM Tris-HCl buffer system (pH 8) used for bacterial lysis. Recombinant SEC-1 was dissolved in 20 mM Tris-HCl (pH 8.0), 0.5 mM NaCl supplemented with 8 m urea and purified to near homogeneity by nickel-chelate chromatography (in the presence of 8 m urea) as described previously (43). When urea was eliminated by extensive dialysis against 50 mM sodium acetate, pH 4.9, the SEC-1 fusion protein remained soluble (see “Results” for details). Approximately 5 mg of highly purified SEC-1 fusion protein was obtained from a 500-ml culture of E. coli.

**Production of Antibodies Directed against SEC-1**—Samples of the SEC-1 fusion protein were injected into rabbits (0.2 mg initial injection; 0.2 mg for each of three booster injections) at Hazelton Corning Laboratories (Vienna, VA) for the generation of antisera. Serum was collected at 3-week intervals.

**Electrophoresis of Proteins and Western Immunoblot Assays**—Samples of proteins were denatured in gel loading buffer and subjected to electrophoresis in a 12% polyacrylamide gel containing 0.1% SDS as described previously (44). Albumin (M, 67,000), ovalbumin (M, 43,000), carbonic anhydrase (M, 29,000), myoglobin (M, 17,000), and cytochrome
C. elegans homogenates were prepared as described previously (45). Western blots of C. elegans proteins and SEC-1 fusion protein were blocked, incubated with antibodies (1:2000), and washed as described previously (46). SEC-1 was visualized by an indirect chemiluminescence procedure as previously reported (46).

**Thermotolerance Studies—** E. coli BL21(DE3) was transformed with pRSET-C expression plasmids that contained no insert, a cDNA encoding a portion of the cytoplasmic domain of a C. elegans stress-inducible gene (e.g., a protein-tyrosine phosphatase). In the latter two instances, the recombinant proteins were constitutively expressed in the absence of inducer (see "Results").

**Ablation of SEC-1 Function by Antisense Oligonucleotides—** RNase protection assay using RNA isolated from a programmed oocyte (S1) was injected into a second gonadal oocyte (S2) and subsequently, the bacteriaweresubjectedtoheatstressbyshiftingthe incubation temperature to 58°C for 30 min. After rapid cooling, the cells were maintained at 28°C for 48 h. Growth was monitored by determination of the optical density at 600 nm (OD₆₀₀) values at various times.

**Abolition of SEC-1 Function by Antisense Oligonucleotides—** The derived amino acid sequence of SEC-1 is aligned with the sequences of rat HSP16–1 (29) and human Hsp 27 (7) in A and with C. elegans Hsp16–1 (29) and Hsp16–2 (29) in B. Amino acids that are identical in the three aligned proteins (*) and residues conserved in SEC-1 and one of the aligned polypeptides (+) are marked.

**RESULTS**

**Characterization of cDNAs That Encode a Novel Small Chaperone from C. elegans—** A cDNA library was prepared in the bacteriophage λZAP II, using template mRNA isolated from nematodes that were stressed with 2 mM CdCl₂ (35). Recombinant phage that contain cDNAs encoding stress-induced proteins were identified by differential screening. A 521-bp cDNA insert from a recombinant phage designated Z500 encodes a partial polypeptide (amino acid residues 4–159, Fig. 1) with a calculated Mr of 17,839. The remaining cDNA sequences of SEC-1 (Fig. 1) and proteins in standard databases have the identical amino acid residues conserved in SEC-1 and one of the aligned polypeptides (+) are marked.

**A**

| SAC 1 | NHSLC | -Ptyx- | RTYFYDFP- | KMDNYX- | QKKXQXX- |
|-------|-------|-------|---------|--------|---------|
| SEC-1 | MNT1HEW- | -SFFPNSF- | GGLSESL- | LPSLTS- | TATL|

**B**

| SAC 1 | NHSLC | -Ptyx- | RTYFYDFP- | KMDNYX- | QKKXQXX- |
|-------|-------|-------|---------|--------|---------|
| SEC-1 | MNT1HEW- | -SFFPNSF- | GGLSESL- | LPSLTS- | TATL|
| SEC-1 | VAYKPKFEMW- | -SFFPNSF- | GGLSESL- | LPSLTS- | TATL|

**Nomenclature—** The novel C. elegans polypeptide (Fig. 1) exhibits structural and functional features associated with chaperones and is expressed only during embryogenesis. Therefore, it is named SEC-1² for small embryonic chaperone-1. Data documenting the developmentally controlled expression of SEC-1 and various structural and functional properties of the protein are presented in Figs. 2–7, Table I, and text below.
Fig. 3. Analysis of the copy number and transcription initiation site for the sec-1 gene. Southern gel analysis is presented in A. C. elegans high molecular weight DNA was digested with PstI (lane 1), XbaI (lane 2), Sau3AI (lane 3), BglII (lane 4), and BamHI (lane 5). Fragments resolved on a 0.6% agarose gel were transferred to a Nytran membrane and were probed with 32P-labeled cDNA corresponding to the sec-1 gene. The gel was calibrated with a standard series of HindIII fragments of λDNA as described previously (32). The sizes determined for the hybridizing DNA fragments in lanes 1–5 were 1.5, 4.0, 5.3, 3.4, and 5.0 kb, respectively. A, primer extension analysis was performed as described under “Experimental Procedures.” An autoradiogram of the relevant portion of a 6% polyacrylamide, 7M urea gel is shown. Lanes 1–4 received standard dideoxynucleotide sequencing reactions that were used to calibrate the gel for measuring the size of the extended product (see “Experimental Procedures”) was detected (lane P). Its length indicates that the transcription initiation site lies 47 nucleotides upstream from the initiator ATG codon. No larger extension products were observed in the upper portion of the gel (data not shown). No signals were obtained when template was omitted, an unrelated primer was used, or an excess of nonradioactive primer was employed (data not shown).

were compared by using BLASTX, BLASTP, and FASTA homology search computer programs (38, 39). The searches indicated that SEC-1 is a unique, previously uncharacterized protein. However, the SEC-1 sequence has motifs that are conserved in two families of proteins: C. elegans HSP16 isoforms (32–38% overall identity with SEC-1) and vertebrate low molecular weight shock proteins (29, 30). Central core regions of SEC-1, HSP16–1, and HSP16–48 are not highly related to SEC-1 are not highly related to the SEC-1 sequence has motifs that are conserved in two families of proteins: C. elegans HSP16 isoforms (32–38% overall identity with SEC-1) and vertebrate low molecular weight shock proteins (29, 30). Central core regions of SEC-1, HSP16–1, and HSP16–48 (Fig. 2 B) are aligned in Fig. 2 A. The central core of SEC-1 (residues 45–126) shares ~40% sequence identity with its counterparts in α-crystallin and Hsp27. This domain apparently promotes proper folding of partially denatured polypeptides in stressed cells (5). The NH2 (residues 1–44) and COOH (127–159) termini of SEC-1 are not highly related to α-crystallin or Hsp27 (~17–20% identity achieved with large gaps in the alignments, Fig. 2A).

SEC-1 is maximally homologous with small C. elegans heat shock proteins (29, 30). Central core regions of SEC-1, HSP16–1, and HSP16–48 are ~50% identical and 65% similar (Fig. 2B), thereby suggesting that the three nematode proteins evolved from a common ancestor. The NH2 and COOH termini of SEC-1 differ substantially (~25% sequence identity after introducing several gaps) from corresponding regions in both HSP16–1 and HSP16–48 (Fig. 2B). SEC-1 also has a unique Ser/Thr-rich COOH-terminal extension.

The predicted pI for SEC-1 is 8.2, whereas pI values for HSP16 isoforms lie within the range of 5.0–5.9. Since the internal pH in C. elegans is ~6.2 (49), SEC-1 will exhibit a net positive charge in the physiological milieu. HSP16 proteins will be almost neutral or negatively charged. SEC-1 is enriched 2–5-fold in His residues relative to HSP16 proteins. Imidazole side chains will contribute a substantial level of positive charge to SEC-1 at the physiological pH, which may promote interactions with unfolded acidic proteins.

Characterization of the C. elegans sec-1 Gene—When Southern blots of restriction enzyme digests of C. elegans DNA were probed with 32P-labeled SEC-1 cDNA under conditions of high stringency, the pattern was not altered when low stringency conditions were employed (data not shown). It is likely that the sec-1 gene occurs as a single copy in the C. elegans genome. A segment of DNA that contains the SEC-1 coding region was isolated from a C. elegans genomic library in the bacteriophage AEML4. The sequence for 729 bp of 5′-flanking DNA, the sec-1 gene, and 251 bp of 3′-flanking DNA is presented in Fig. 4. Comparison of the cDNA and gene structures revealed that the primary transcript has one short intron (56 bp) that interrupts codon 93. The transcription start site was mapped to an A residue 47 bp upstream from a potential ATG codon by primer extension analysis (Fig. 3A). A classical TATA box at nucleotides ~31 to ~27 (asterisks). The poly(A) signal at nucleotides 624–629 is also marked with asterisks. 3 The term “small chaperone” is synonymous with “sHsp.”

Fig. 4. Sequence of the sec-1 gene and contiguous flanking DNA. Nucleotides in exons are shown in uppercase letters; nucleotides in introns and flanking DNA are indicated with lowercase letters. The transcription start site is assigned position +1. The 5′-flanking region includes a TATA box at nucleotides ~31 to ~27 (asterisks). The poly(A) signal at nucleotides 624–629 is also marked with asterisks.
480-nucleotide coding region flanked by 5’- and 3’-untranslated sequences that are 47 and 57 nucleotides long, respectively. If the transcript receives a typical poly(A) tail (0.65 kb), SEC-1 mRNA was visualized by autoradiography. 

**FIG. 5. Effects of stress and stage of development on expression of SEC-1 mRNA.** Northern blots that contain size-fractionated RNAs from *C. elegans* were prepared as described under “Experimental Procedures.” Blots were probed with 32P-labeled SEC-1 cDNA corresponding to nucleotides 56–319 in Fig. 1. Hybridization of the probe with (0.65 kb) SEC-1 mRNA was visualized by autoradiography. A, fractionated RNAs (4 μg) from control *C. elegans* (lane 2) and *C. elegans* incubated with 0.2 mM CdCl2 for 24 h (lane 1) were analyzed. B, lanes of a Northern gel received 4 μg of total RNA from either embryos (E) or larvae (L1–L4) at various stages of postembryonic development. C (upper part), Northern blot analysis was performed on samples of total RNA (20 μg) isolated from control *C. elegans* maintained at 20 °C (lane 3) or heat-stressed nematodes that were incubated for 3 h (lane 1) or 1 h (lane 2) at 31 °C. The lower part shows a portion of the same blot that was hybridized with a 32P-labeled cDNA probe for myosin light chain mRNA, as described previously (53). Levels of the two myosin light chain transcripts do not vary with stress or development (not shown). Thus, the resulting signals document the integrity of the RNA samples and show that equal amounts of RNA were loaded in each lane.

**FIG. 6. Expression and purification of recombinant SEC-1 fusion protein.** A, *E. coli* BL21 was transformed with a recombinant expression plasmid that encodes the His-tagged SEC-1 fusion protein described under “Experimental Procedures” and “Results.” A control transformation was performed with pRSET-C plasmid that lacks a cDNA insert. Transformed *E. coli* were grown to *A*<sub>oo</sub> = 0.6. Subsequently, aliquots of the cultures were incubated in the presence or absence of 1 mM IPTG. After 1 h at 37 °C, the bacteria were harvested, and *E. coli* proteins were fractionated by electrophoresis on a 0.1% SDS-12% polyacrylamide gel (see “Experimental Procedures”). Polypeptides were visualized by staining with Coomassie Blue. Lane 1 received 50 μg of protein from *E. coli* harboring the control (no cDNA insert) plasmid; lanes 2–4 contained 50, 50, and 25 μg of protein, respectively, from *E. coli* transformed with recombinant pRSET-C that encodes the SEC-1 fusion protein (apparent *M*, 25,000). Samples applied to lanes 1 and 2 were obtained from uninduced *E. coli*; samples in lanes 3 and 4 were from *E. coli* that were induced with 1 mM IPTG. B, The SEC-1 fusion protein from IPTG-induced *E. coli* was dissolved and purified, via nickel chelate affinity chromatography, in buffer containing 8 M urea (see “Experimental Procedures”) and “Results”). Urea was removed from replicate samples of purified fusion protein by dialysis against either 10 mM sodium phosphate, pH 7.4, 0.15 M NaCl or 50 mM sodium acetate, pH 4.9. Dialyzed samples were centrifuged at 30,000 × *g* for 10 min, and supernatant and pellet fractions were collected. After boiling in SDS loading buffer, aliquots (25 μl) of these fractions were analyzed by denaturing electrophoresis as described above. Lanes 1 and 2 contained the pellet and supernatant fractions, respectively, obtained by dialysis at pH 7.4; lanes 3 and 4 received the pellet and supernatant fractions, respectively, isolated after dialysis at pH 4.9.

Production and Characterization of Antibodies Directed against SEC-1—A cDNA fragment that encodes the full-length SEC-1 polypeptide was cloned into the expression plasmid pRSET-C (see “Experimental Procedures”). A “His-tagged” SEC-1 fusion protein (apparent *M*, 25,000) was synthesized constitutively in the bacterium (Fig. 6A, lanes 1 and 2). This is probably due to the binding of endogenous lac repressor protein by lac promoter/operator sequences in the high copy number pRSET plasmid. Partial depletion of the repressor results in inducer-independent synthesis of T7 RNA polymerase, which efficiently utilizes the plasmid T7 promoter to initiate transcription of SEC-1 mRNA. Nevertheless, incubation of transformed *E. coli* with inducer for 1 h doubled the level of SEC-1 fusion protein (Fig. 6A, lanes 2 and 3). Recombinant SEC-1 protein was dissolved in buffer containing 8 M urea and purified to homogeneity via nickel chelate chromatography, under denaturing conditions. Dialysis of purified SEC-1 fusion polypeptide against 10 mM sodium phosphate buffer, pH 7.4, containing 0.15 M NaCl resulted in quantitative precipitation of the protein (Fig. 6B, lanes 1 and 2). In contrast, dialysis against 50 mM sodium acetate, pH 4.9, eliminated urea and yielded a highly purified soluble preparation of SEC-1 (Fig. 6B, lanes 3 and 4).

Purified SEC-1 was used to immunize rabbits. The resulting antibodies avidly bound recombinant SEC-1, readily detecting 1 ng of antigen at high dilution (Fig. 7A). Moreover, the antibodies complexed a single 18-kDa polypeptide in cytosol derived from embryos and a mixed population of *C. elegans* (Fig. 7B, lanes 3 and 4). No signals were observed when the antisera were preincubated with a large excess of recombinant SEC-1 (Fig. 7B, lanes 1 and 2).
Distribution of SEC-1 mRNA and Protein in Situ—In situ hybridization analysis was used to monitor SEC-1 mRNA accumulation in embryos and adult nematodes. *C. elegans* engaged in early and middle phases of embryogenesis stain intensely for SEC-1 mRNA (Fig. 9A, arrowheads). SEC-1 mRNA content declines precipitously to background levels as the embryos undergo morphogenesis (elongation and folding) to generate L1 larvae (Fig. 9A, arrowheads). Immunofluorescence microscopy demonstrated that SEC-1 protein is abundant and widely distributed in ellipsoid embryos that have not yet entered the postproliferative, morphogenesis stage (Fig. 9C, arrowheads). Animals at later stages of embryogenesis (Fig. 9C, arrowheads) have markedly lower or undetectable amounts of SEC-1 protein. In adult nematodes SEC-1 mRNA is highly expressed in the gonadal arms (developing oocytes) (Fig. 9B). The transcript is not detected in any other cells of adult *C. elegans*.

**SEC-1 Confers Thermotolerance on E. coli**—The recombinant
pRSET plasmid that mediates constitutive expression of recombiant SEC-1 protein in E. coli (see Fig. 6; see above) was exploited to test a possible functional role for the C. elegans protein. A highly diluted sample of transformed E. coli was heated at 58 °C for 30 min and then incubated in standard growth medium at 28 °C for 40 h. E. coli transformed with pRSET containing a cDNA encoding an unrelated protein or pRSET lacking an insert were used as controls. Bacteria that accumulated the SEC-1 protein (Fig. 6) grew to a high density after the intense thermal stress (Table I). E. coli transformed with the control plasmids did not survive. The ability of SEC-1 to protect E. coli from heat shock demonstrates that the C. elegans protein can perform a chaperone-associated function in intact cells.

SEC-1 Is Essential for the Progression of Embryogenesis—Expression of the SEC-1 polypeptide during embryogenesis was inhibited by introducing into oocytes antisense DNA oligonucleotides, which complement codons near the 5′-end of SEC-1 mRNA. Oligonucleotides were microinjected into the gonadal syncytium at a site where multiple oocyte nuclei share a common cytoplasm. The subsequent generation of oocyte plasma membranes during a later step in oogenesis permanently sequesters the antisense oligonucleotides in numerous individual oocytes. Inspection of embryos produced (as externally deposited eggs) by injected animals revealed that inhibition of SEC-1 expression yielded aborted embryos that are composed of a disorganized mass of cells (Fig. 10, A and B). Injection of sense oligonucleotides had no effect on embryogenesis (Fig. 10C). Thus, SEC-1 appears to mediate an essential function(s) that is crucial for the normal progression of embryogenesis.

DISCUSSION

The C. elegans sec-1 gene encodes a novel 18-kDa polypeptide that is selectively expressed during embryogenesis. SEC-1 mRNA is produced in the maternal gonad and packaged into oocytes. SEC-1 protein is abundant during the time period when a fertilized oocyte rapidly develops into an embryo containing a precisely arranged constellation of ~550 cells. Since most C. elegans genes are transcriptionally silent during early embryogenesis (28), it is probable that all or most of the SEC-1 protein is derived from translation of maternally synthesized mRNA molecules. However, the possibility that early sec-1 gene transcription augments SEC-1 mRNA and protein content in embryos has not been excluded.

The size and structural properties of SEC-1 suggest that the 18-kDa protein is a member of the small chaperone/shSHP superfamily. The central core region (residues 45–126) of SEC-1 shares high levels of sequence identity with analogous segments of both mammalian chaperones and four C. elegans shSP proteins. Similarities among SEC-1, chaperones, and nematode heat shock proteins include motifs that promote correct refolding of denatured proteins in stressed cells (5). The evolutionary conservation of structural features is consistent with the idea that C. elegans SEC-1 mediates protein folding and/or inhibits polypeptide aggregation in vivo (8, 18, 19). Furthermore, SEC-1 confers thermotolerance, a chaperone-associated function (1–5), on E. coli (Table I). This parallels the ability of mammalian Hsp27 and crystallin transgenes to protect various cells and organisms against heat and chemical stresses (9–12).

Results in this paper indicate that physiological roles of SEC-1 are not redundant with functions performed by C. elegans HSPs 16–1, 16–2, 16–41 and 16–48. Several structural features suggest a functional divergence between SEC-1 and other chaperones. Sequences at the NH₂ and COOH termini of SEC-1 are not highly related to sequences in any other proteins. The four C. elegans HSP16 isoforms are acidic proteins with pI values in the range of 5–6. In contrast, SEC-1 is a basic polypeptide that is highly enriched in His residues. Thus, SEC-1 contains distinct subsets of amino acid side chains that may generate novel higher order structures and/or surfaces. Such domains could bind proteins that are not complexed by HSP16 isoforms or large chaperones.

Aspects of the organization and regulation of the C. elegans sec-1 gene are unique. Like genes encoding the C. elegans HSP16 isoforms, the sec-1 gene contains a single intron. However, the sec-1 intron is inserted within codon 93. This generates two exons that encode similarly sized segments of the conserved central core region of the SEC-1 protein (Figs. 1–3). The nematode hsp16 genes encode the entire conserved core domain in a single exon (29, 30). This leads to speculation that the C. elegans sec-1 and hsp16 genes evolved from an intronless ancestor gene and acquired intervening sequences after they diverged.

During the course of our studies, the DNA sequence corresponding to the sec-1 gene was deposited in the EMBL data base (Accession number Z35640) by the C. elegans Genome Sequencing Project (56). The sequence determined for the sec-1 structural gene and its associated 5′- and 3′-flanking DNA in this laboratory and the data provided by the sequencing project are in complete agreement. No other information on the structure, regulation, and function of the sec-1 gene or SEC-1 mRNA and protein has been previously published or deposited in data bases.

The four C. elegans hsp16 genes are organized as tandem head to head pairs (hsp16–1 linked to hsp16–48; hsp16–2
paired with hsp16–41) that share overlapping promoter regions (29, 30). Transcription of each hsp16 gene is controlled by heat-inducible promoter elements that precede TATAA boxes by ~20 bp (29, 30). Heat stress induces hsp16 gene transcription and HSP16 isomor accumulation in virtually every somatic cell in larval and adult nematodes (31). No expression is observed in the absence of stress. In contrast, sec-1 is a single copy gene that is expressed in unstimmed oocytes and embryos. The sec-1 structural gene is preceded by a TATAA box, but the 5'-flanking DNA lacks both cis heat-inducible promoter elements and sequences that govern cadmium-activated transcription. The absence of stress-activated enhancers is confirmed by the observations that SEC-1 mRNA is not induced by elevated temperature or CdCl2 (Fig. 5). Expression of the sec-1 gene is regulated by developmental factors (presumably transcription factors). SEC-1 mRNA is abundant in adult gonad (especially in oocytes) and in early to midstage embryos. Neither SEC-1 mRNA nor SEC-1 protein was detected in nongonadal tissues of adult C. elegans. Both macromolecules remain undetectable throughout larval development (L1–L4 larvae). Thus, the sec-1 gene and its protein product appear to be specifically adapted for functions associated with embryogenesis. SEC-1 cDNAs were discovered in a screen for cadmium (stress)-induced gene products. However, CdCl2 does not elicit an increase in SEC-1 mRNA content. Together the physiological adaptation of C. elegans to CdCl2 and the pattern of SEC-1 expression during development described herein provide an explanation for the paradoxical enrichment of SEC-1 transcripts in the "stress-induced" cDNA library. Nematodes adapt to CdCl2 by ceasing to expel developing embryos (as eggs) to the external milieu. Instead, the eggs hatch internally, and the macrochordates of developing large quantities of nascent polypeptides that are produced during the intense biosynthetic phase of early embryonic development. By analogy with mammalian small chaperones (10–14) SEC-1 may also play a central role in regulating the assembly and disassembly of cytoskeleton as embryonic cells rapidly proliferate and differentiate. Thus, SEC-1 may be expressed to prevent, rather than respond to, biological stress. SEC-1 may function independently or in concert with C. elegans Hsp60 and Hsp70 chaperones. C. elegans genes encoding the larger chaperones have been cloned and characterized (50, 51), but the expression and physiological roles of the Hsp60 and Hsp70 proteins during embryogenesis have not been systematically explored. Hsp70 is not normally expressed in Drosophila embryos. Moreover, forced constitutive expression of this chaperone during early embryogenesis resulted in lethality (4). Whether SEC-1 functions in concert with large chaperones independently, it is the only candidate small chaperone in C. elegans for a key role in early development. Finally, in view of the potential importance of SEC-1 and a lack of knowledge regarding roles for small chaperones in mammalian development (1–5), it might be fruitful to search for SEC-1 homologues or analogs in higher organisms.

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REFERENCES

1. Lindquist, S., and Craig, A. E. (1988) Annu. Rev. Genet. 22, 631–677
2. Nover, L., and Scharf, K.-D. in Heat Shock Response (Nover, L., ed) pp. 41–128, CRC Press, Inc., Boca Raton, FL
3. Ciocca, D. R., Oesterreich, S., Channess, G. M., McGuire, W. L., and Fuqaus, S. A. W. (1990) J. Natl. Cancer Inst. 82, 1558–1570
4. Parsell, D. A., and Lindquist, S. (1993) Annu. Rev. Genet. 27, 437–496
5. Jakob, U., and Buchner, J. (1994) Trends Biochem. Sci. 19, 205–211
6. Quax-Jeskes, Y., Quax, W., van Rens, G., Khan, P. M., and Bloemendal, H. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 5819–5823
7. Hickey, E., Brandon, S. E., Potter, R., Stein, G., Stein, J., and Weber, L. A. (1986) Nucleic Acids Res. 14, 4127–4145
8. Merck, K. B., Groenen, P. J. A., Voorter, C. E., deHaard-Hoeckeman, W. A., Horvitz, J., Bloemendal, H., and de Jong, W. (1993) J. Biol. Chem. 268, 1046–1052
9. Sax, C. M., and Piatigorsky, J. (1994) Adv. Enzymol. 69, 155–201
10. Crepiten, R., and Landry, J. (1989) J. Cell. Biol. 137, 7–15
11. Lavoie, J. N., Gingras-Breton, G., Tanguay, R. M., and Landry, J. (1993) J. Biol. Chem. 268, 3420–3429
12. Lavoie, J. N., Hickey, E., Weber, L. A., and Landry, J. (1993) J. Biol. Chem. 268, 24210–24214
13. Miron, T., Vancompernolle, K., Vandezande, J., Wilchek, M., and Geiger, B. (1991) J. Cell. Biol. 114, 255–261
14. Miron, T., Wilchek, M., and Geiger, B. (1988) Eur. J. Biochem. 178, 543–553
15. Landry, J., Lambert, H., Zhou, M., Lavoie, J. N., Hickey, E., Weber, L. A., and Anderson, C. W. (1992–93) J. Biol. Chem. 267, 794–803
16. Rouse, J., Cohen, P., Trigon, S., Morange, M., Alsenzo-Llamazaes, A., Zamanillo, D., Hunt, T., and Nebreda, A. R. (1994) Cell 78, 1027–1037
17. Freeech, N. W., Rawlinson, F., Jain, K., Cowley, S., Hausan, J., and Saklatvala, J. (1994) Cell 78, 1039–1049
18. Horwitz, J. (1992) Proc. Natl. Acad. Sci. 89, 10449–10453
19. Jakob, U., Gaestel, M., Engel, K., and Buchner, J. (1993) J. Biol. Chem. 268, 1517–1529
20. Ellis, R. J., and van der Vies, S. M. (1991) Annu. Rev. Biochem. 60, 321–347
21. Wiech, H., Buchner, J., Zimmerman, R., and Jakob, U. (1992) Nature 358, 328–330
22. Hartl, F.-U., IJdo, R., and Langer, T. (1994) Trends Biochem. Sci. 19, 20–25
23. Sulston, J. (1988) in The Nematode Caenorhabditis Elegans (Wood, W. B., ed) pp. 125–155, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
24. Sulston, J., Horvitz, H. R., and Kimble, J. (1988) in The Nematode Caenorhabditis Elegans (Wood, W. B., ed) pp. 547–498, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
25. Kenyon, C. (1988) Science 240, 1448–1453
26. Han, M., and Sternberg, P. (1990) Cell 65, 921–931
27. Clark, G. S., Stern, M. J., and Horvitz, H. R. (1992) Nature 356, 340–344
28. Wood, W. B. (1988) in The Nematode Caenorhabditis Elegans (Wood, W. B., ed) pp. 215–241, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
29. Russnak, R. H., and Candido, E. P. M. (1985) Mol. Cell. Biol. 5, 1268–1278
30. Jones-D, Russnak, R. H., Kay, R. J., and Candido, E. P. M. (1986) J. Biol. Chem. 261, 12006–12015
31. Stringham, E. G., Dixon, D. K., Jones, D., and Candido, E. P. M. (1992) Mol. Biol. Cell 3, 221–233
32. Xu, E., and Rubin, C. S. (1990) J. Biol. Chem. 265, 5072–5080
33. Wood, W. B., Hecht, R., Carr, S., Vanderslice, R., Wolf, N., and Hirsch, D. (1980) Dev. Biol. 74, 446–469
34. Sulston, J., and Hodgkin, J. L. (1985) in The Nematode Caenorhabditis Elegans, pp. 603–605, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
35. Slice, L. W., Freedman, J. H., and Rubin, C. S. (1990) J. Biol. Chem. 265, 2556–2563
36. Smith, P. J., Wise, L. S., Berkowitz, R., Wan, C., and Rubin, C. S. (1988) J. Biol. Chem. 263, 9402–9408
37. Lu, X., Gross, R. E., Bagchi, S., and Rubin, C. S. (1990) J. Biol. Chem. 265, 3283–3288
38. Pearson, W. R., and Lipman, D. J. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 2444–2448
39. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) J.
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40. Freedman, J. H., Slice, L. W., Dixon, D., Fire, A., and Rubin, C. S. (1993) J. Biol. Chem. 268, 2554–2564
41. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
42. Reeder, R. H., Roan, J. G., and Dunaway, M. (1983) Cell 35, 449–456
43. Li, Y., and Rubin, C. S. (1995) J. Biol. Chem. 270, 1935–1944
44. Bregman, B., Bhattacharyya, N., and Rubin, C. S. (1989) J. Biol. Chem. 264, 4648–4656
45. Land, M., Islas-Trejo, A., Freedman, J. H., and Rubin, C. S. (1994) J. Biol. Chem. 269, 9234–9244
46. Hirsch, A. H., Glantz, S. B., Li, Y., You, Y., and Rubin, C. S. (1992) J. Biol. Chem. 267, 2131–2134
47. Iwaki, A., Iwaki, T., Goldman, J. E., and Liem, R. K. H. (1990) J. Biol. Chem. 265, 22197–22203
48. Deleted in proof
49. Wadsworth, W. G., and Riddle, D. L. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 8435–8438
50. Snutch, T. P., Heschl, M. F. P., and Baillie, D. L. (1988) Gene (Amst.) 64, 241–255
51. Heschl, M. F. P., and Baillie, D. L. (1989) DNA (N. Y.) 8, 233–243
52. Coulson, A., Sulston, J., Brenner, S., and Karn, J. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 7821–7825
53. Hu, E., and Rubin, C. S. (1991) J. Biol. Chem. 266, 19796–19802
54. Lu, X., Gross, R. E., Bagchi, S., and Rubin, C. S. (1990) J. Biol. Chem. 265, 3293–3303
55. Ross, L. H., Freedman, J. H., and Rubin, C. S. (1995) J. Biol. Chem. 270, 22066–22075
56. Wilson, R., Ainscough, R., Anderson, K., Baynes, C., Berks, M., Bonfield, J., Burton, J., Connell, M., Copsey, T., Cooper, J., Coulson, A., Craxton, M., Dear, S., Du, Z., Durbin, R., Favello, A., Fraser, A., Fulton, L., Gardner, A., Green, P., Hawkins, T., Hillier, L., Jier, M., Johnston, L., Jones, M., Kershaw, J., Kirsten, J., Lai, J., Lister, N., Lister, P., Lightning, J., Lloyd, C., Morton, B., O’Callaghan, M., Parsons, J., Perci, C., Rikken, L., Rhoopr, A., Saunders, D., Showkette, R., Sims, M., Smaldon, N., Smith, A., Smith, M., Sonnhammer, E., Staden, R., Sulston, J., Thierry-Mieg, J., Thomas, K., Vaudin, M., Vaughan, K., Waterston, R., Watson, A., Weinstock, L., Wilkinson-Sproat, J., and Wohldman, P. (1994) Nature 368, 32–38