

On-line review: Hsps, energy, and aging in *C. elegans*

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Hsps in *C. elegans*

Induction of a set of stress genes in response to hyperthermia has been studied in a number of different systems including yeast and fruit flies (*Drosophila melanogaster*) [1]. In 1983, Snutch and Baillie first demonstrated that exposing *C. elegans* to elevated temperatures (29-35°C) could induce the synthesis of at least eight different proteins, some of which were constitutive and some of which were inducible. These proteins ranged in size from approximately 81kDa down to 16kDa M.W., and included hsp16, hsp19, hsp29, hsp 38, hsp41, hsp70, and hsp81. Hsps were also found to be inducible in dauer larvae (which also synthesize a 50k mw protein not seen in other stages).

Several mutations have been identified in *C. elegans* which lead to the formation of dauer larva, a specialized non-feeding, non-aging life cycle stage which can be maintained for several weeks. Dauer formation usually occurs in response to unfavorable environmental conditions (see [2] and [3] for review), but dauer forming mutants enter the dauer stage even when food supplies and other conditions are adequate for normal growth. Interestingly, dauer larvae have been reported to be enriched in hsp90 mRNA [4]

Among these mutants, *daf-21* is a temperature sensitive mutation that induces constitutive dauer formation at the restricted temperature [5], regardless of favorable environmental conditions. Analysis of this mutation has confirmed that *daf-21* is actually hsp90, with a glutamate changed to lysine [6]. To date, this is the only known strain of *C. elegans* that contains a mutation in one of the stress proteins. The role of hsp90 in nematodes is not entirely clear, however, Powell-Coffman et al., [7] have demonstrated that hsp90 binds to the aryl hydrocarbon receptor (a ligand activated transcription factor that mediates carcinogenic and teratogenic effects of some pollutants). Hsp90 has also been shown to be expressed at higher levels in dauer larvae [4]

Much work on heat shock in *C. elegans* has focused on hsp70, one of the major stress proteins induced as a result of exposure to hyperthermia. Hsp70 in *C. elegans* is a

multigene family consisting of constitutively expressed genes, inducible genes, and a pseudogene [8] [9] [10]. This is consistent with the observation that other organisms such as *Drosophila melanogaster* [11], *Saccharomyces cerevisiae* [12], and *Homo sapiens* [13] have also been shown to have hsp70 multigene families with differential patterns of expression. The hsp70 family in *C. elegans* has at least nine members spread across at least three subfamilies [10]. These include:

- hsp70-1, which is expressed throughout development with expression being enhanced under stress. This gene has introns (which is unusual for hsp70s—perhaps nematodes have a stress-resistant splicing mechanism?)
- hsp70-2s which is closely related to hsp-1
- hsp70-3 which is constitutively expressed and is not inducible. This is probably related to grp 78 in mammals.
- hsp70-4 which has low constitutive expression but is highly inducible
- hsp70-6 which is both constitutively expressed and heat inducible

C. elegans has also been shown to have another protein with an hsp70-like structure. The hsp-70 ATPase domain can be found in the constitutively expressed protein coded for by the *Stch* genes in *C. elegans*, humans, and rats [14].

The molecular structure of the hsp70s has been determined, with the “DnaK” hsp70 from *E. coli* serving as the basis for comparison (for a review, see [15]). These molecules have a highly conserved N-terminal ATP binding domain, and a C-terminus domain that is both more divergent and responsible for substrate binding. They are found in both the cytoplasm and in mitochondria, in addition to being located within the lumen of the endoplasmic reticulum.

Leroux and Candido ([16] and [17]) have been responsible for the initial characterization of hsp60s from *C. elegans*. This eukaryotic cytosolic chaperonin complex is related to the *E. coli* protein known as GroEl, and the mitochondria heat shock protein known as hsp60 [18]. In *C. elegans*, this complex consists of at least seven different subunits, two of which are highly conserved, and range in molecular weight from 52kDa - 65kDa M.W. Leroux and Candido have purified these subunits and then cloned their genes. There are at least five genes (cct1, 2, 4, 5, and 6) that have now been identified and their resulting amino acid sequences are nearly identical. Of interest

here is the fact that this particular heat shock protein shows a high binding affinity for denatured actin. Other stress-related genes in the worm include one coding for a ubiquitin-conjugating enzyme (*ubc-2*) [19] and two metallothioneins (*mtl-1* and *mtl-2*) [20].

Another group of stress proteins that have been identified and characterized in *C. elegans* are the set of low molecular weight stress proteins including hsp16 [21] [22] and hsp12.6 [23]. These hsp16s show conservation of the location of an intron when compared to a murine α -crystallin gene, and profiles of a 17.5kDa and 17.6kDa M.W. heat shock protein from soybeans show a high degree of similarity when compared to stress proteins from *Drosophila*, *Xenopus*, *C. elegans*, and *G. max* [24]. Transgenic worms carrying the hsp16 promoter have been created and used to demonstrate the induction of the hsp16 transgene in individual cells using a sublethal exposure to a laser microbeam [25] and [26]. Candido's laboratory has also identified another small heat shock protein: hsp12.6 [23]. This protein is not stress inducible and is only expressed in the L1 larval stage of worm development. It has an α -crystallin domain but it does not form higher molecular weight complexes like the other small stress proteins do. The function of hsp12.6 is not known but it does not play the traditional role of a chaperonin since it cannot prevent the aggregation of unfolded proteins.

Recently, another small hsp, hsp25 has been identified in *C. elegans*. This protein is expressed under normal conditions, and is found in muscle tissue [27]

Other stress-related genes of interest in *C. elegans*:

Forms of the p-glycoprotein molecules that play a significant role in cancer resistance are also found in the nematode [28]. *pgp-1*, *pgp-2*, *pgp-3*, and *pgp-4* genes are found on chromosomes IV, I, X, X respectively. These PGP proteins are expressed in intestinal tissues. Heat shock leads to slight induction of *pgp-1* and *pgp-3*, but tan and cis-splicing of first exon of 1 is disturbed, probably leading to dysfunctional mRNA.

Recently, *cep-1*, a homolog of mammalian p53 has been identified in nematodes [29]. *C. elegans* also expresses metallothioneins, particularly in intestinal cells [20].

Energy metabolism in *C. elegans*

Studies have examined the changes in regulation of energy metabolism with growth in the nematode [30]. During the first larval stage, activity of the glyoxylate pathway (which is initially high) decreases. TCA metabolism dominates during the L2, L3, and L4 stages, and oxygen consumption increases. Dauer mutants exhibit decreased enzyme activities, and decreases in ATP levels.

C. elegans does, however, have a high tolerance for oxygen deprivation, probably due to small body size (allowing diffusion to play a significant role in oxygen distribution) [31]. In addition, although *C. elegans* contain the succinate oxidase system (just as vertebrates do), they may also possess a fumarate reductase system (an anaerobic respiratory chain) [32]. This would also contribute to the ability of nematodes to tolerate anoxia [33].

Interestingly, LDH in the nematode has only two of the six introns found in vertebrate LDH [34].

Aging in *C. elegans*

Several mutations have been identified in *C. elegans* that affect aging in the organism. Many mutations shorten lifespan, but a few rare mutations have had the effect of lengthening lifespan, and are said to confer the “Age” phenotype upon the worm [35]. For a review of genes involved in production of Age mutants see [36]. Some of these genes (and the amount by which the mutant form increases maximum life span include:

- *age-1* (65%)
- *spe-26* (65%). May code for an actin-binding protein, produces infertility
- *daf-2* (100%) A *daf-c* mutant (forms dauer even in presence of food; develops normally at low temperatures). Requires activity of *daf-16* gene [37]
- *daf-23* (190%) Another *daf-c* mutant
- *daf-28* (35%) Another *daf-c* mutant
- *clk-1* (35%)
- *rad-8* (35%)

Evidence indicates that these genes are involved in at least two different pathways which impact life-span [38]. One pathway involves *daf-2*, *age-1*, *akt-1*, *akt-2*, *daf-16*, and *daf-18*, and consists of an insulin-like signaling cascade; the second pathway

involves *clk-1*, *clk-2*, *clk-3*, and *gro-1*. Germ-line stem cells also play a role in this process, possibly through affecting production of a steroid which binds to the DAF-16 protein [38].

Recently, it has been discovered that mutations in *clk-1* (which inactivates an biosynthetic enzyme and forces worms to obtain coenzyme Q from their diet) produce life extension through a delay in larval development rather than an increase in adult life span [39]. In fact, this study shows that the reliance on dietary CoQ may actually reduce life span, as worms that were fed diets lacking in CoQ (and were thus forced to rely on endogenous production) showed extended life spans.

Many of these genes are also involved in the stress response, with some studies indicating increased survival following moderate heat stress [40]. Activities of both superoxide dismutase and catalase are elevated in the *age-1(hx546)* and *daf-2(e1370)* genes [41]. These mutants also have higher metabolic activity than the wild-type worms, and also show a reduction in the type of mitochondrial damage (deletions in mitochondrial DNA) that are normally associated with aging. All Age mutations tested also show an intrinsic increase in thermotolerance (as do other non-Age *daf-c* mutations such as *daf-4* and *daf-7*). Both *age-1* and *spe-26* have higher than normal levels of HSP-16. This increase in HSP protein levels may prevent the accumulation of altered proteins that may contribute to aging, and in fact exposure of wild-type worms to non-lethal heat shock tends to increase life-span. The *clk-1* protein is localized to mitochondria, and *clk-1* mutants show reduced respiration as well as cell cycle lengthening and alterations in timing of developmental processes. Overexpression of normal *clk-1*, on the other hand, leads to increases in respiration as well as a shortened life span [42].

A number of studies have also been done looking at the relationship between altered energy metabolism and lifespan. Honda et al. [43] determined that low levels of O₂ (<1%) lengthened the worm's lifespan while high levels of O₂ (>2%) shortened it. Lithgow et al. ([44] [35] and for review see [45]) have studied a specific mutation in *C. elegans*, *age-1*. These animals show an increased life span and an increased ability to survive heat shock.

Interestingly, a recent study using *Drosophila melanogaster* demonstrated that the induction of *hsp70* is not without cost. Feder and Krebs [46] created transgenic strains of

Drosophila carrying hsp70 transgenes. They then exposed their flies to temperature fluctuations that mimicked those which *Drosophila* are normally exposed to in the environment, finding that thermally stressed flies which induce hsp70 have a shorter lifespan than control flies.

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