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Gene expression pattern

dtrap-1 encodes a novel member of the heat shock super family of proteins and is expressed in derivatives of all three germ layers during *Drosophila* embryogenesis

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Abstract

Heat shock proteins (Hsps) comprise a highly conserved superfamily of proteins that are required for stress tolerance in living cells. At physiological conditions, these proteins act as chaperones during protein folding and protein assembly processes (Parsell and Lindquist, 1993. Annu. Rev. Genet. 27, 427–496). Members of the subfamily of Hsp90 proteins were shown to be additionally involved in the presentation and structural modification of components of diverse cellular signal transduction pathways including steroid hormone reception and regulatory kinase activities (Pratt, 1993. J. Biol. Chem. 268, 21455–21458); Cutforth et al., 1994. Cell 77, 1027–1036; van der Straten et al., 1997. EMBO J. 16, 1961–1997; Hunter and Poon, 1997. Trends Cell Biol. 7, 157–161). We have identified a *Drosophila* gene, called *dtrap-1*, which encodes a Hsp of a novel subfamily that is related to the Hsp90 family of proteins. During oogenesis *dtrap-1* is expressed in nurse cells and its transcripts accumulate in the oocyte. The maternal transcripts remain in the egg but rapidly degrade during early embryogenesis, except in the posterior pole region. Zygotic expression is initiated after the onset of gastrulation showing dynamic patterns of transcripts in the developing mid- and hindgut as well as a subset of mesoderm derivatives.

Keywords: Hsp90; Chaperones; Organogenesis; *dtrap-1*

1. Results

Members of the Hsp 90 family of proteins are evolutionary conserved from bacteria to humans (Parsell and Lindquist, 1993). They act as abundant molecular chaperones both in the cytosol and in the endoplasmatic reticulum. Human Hsp90A, the prototype member of this family, was shown to facilitate structural changes and presentation of key regulatory components of certain signal transduction pathways such as steroid receptors, kinases and p53, acting in concert with a number of specific partner proteins (Prodromou et al., 1997a,b). Chaperone function is ATP-dependent, mediated by an ATP binding site within the highly conserved aminoterminal region of the Hsp (Panaretou et al., 1998). Further analysis has shown that Hsp90 possesses two chaperone sites located in the amino- and carboxyterminal regions, respectively (Scheibel et al., 1998). The aminoterminal fragment binds preferentially to non-native peptides and prevents aggregation whereas the carboxyterminal region recognizes structured substrates and cochaperones with tetratricopeptide repeats (Owens-Grillo et al., 1996). These functional domains of human Hsp90 are conserved in its *Drosophila* homolog Hsp83 (Cutforth and Rubin, 1994).

During the analysis of a lethal P-element insertion, l(2)04535 (Karpen and Spradling, 1992), which resides within the second intron of the *Vha16* (also called *ductin*; Finbow et al., 1994) transcript in region 42C on the right arm of the second chromosome, we noticed an open reading frame 5′ to the *Vha16* transcription unit. We used a genomic DNA fragment of the newly identified open reading frame as a molecular probe to isolate a total of 9 overlapping cDNA clones from an embryonic cDNA library. Sequence analysis of these clones and the corresponding genomic DNA fragment revealed a transcription unit composed of three exons. The physical map of the transcription unit, the P-element insertion site as well as the *Vha16* transcription unit are shown in Fig. 1A.

The newly identified transcription unit contains a single open reading frame, also represented in the LD14417 cDNA

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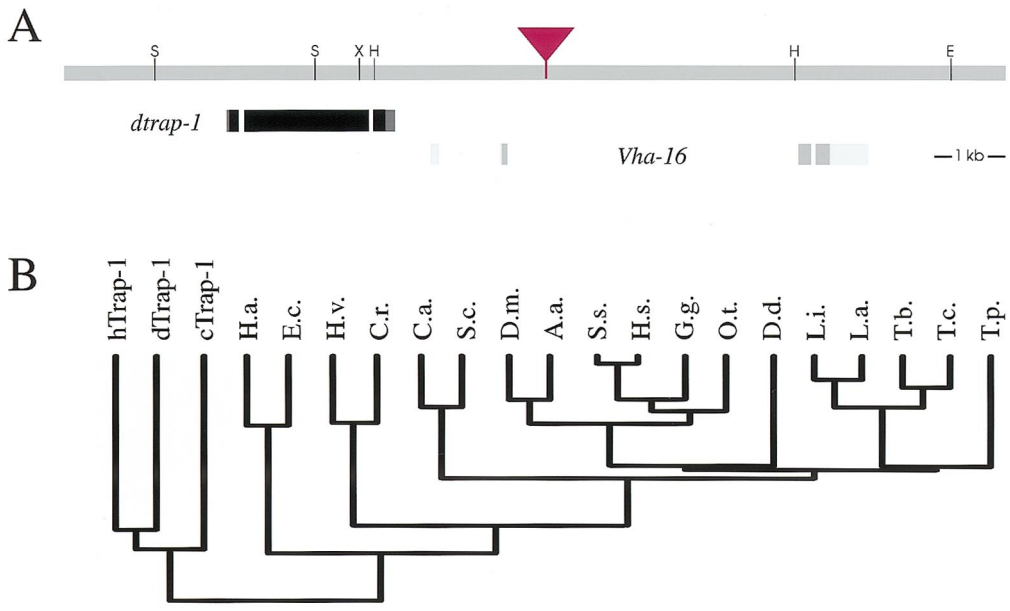

Fig. 1. Physical map of the *dtrap-1* transcription unit. (A) Genomic organization of *dtrap-1* (black) and *Vha-16* (gray). Untranslated leaders and trailers are shaded lighter. Diagnostic restriction sites are indicated; *Eco*RI (R), *Hind*III (H), *Sal*I (S) and *Xba*I (X). The insertion site of P l(2)04535 is marked by a triangle. Exon-intron boundaries were determined by sequence comparison of cDNA versus genomic sequence. (B) Unrooted tree of several Hsp83/90 proteins, dTrap-1, Trap-1 and a *C. elegans* genomic sequence homologous to dTrap-1. It was constructed from the full peptide sequences using the pileup program from the Wisconsin Package (Genetics Computer Group; version 8.0) with gap creation and extension penalties of 12 and 4, respectively. Abbreviations (accession number): hTrap-1 = human Trap-1 (A55877), dTrap-1 = *Drosophila melanogaster* Trap-1 (AF115775), cTrap-1 = *Caenorhabditis elegans* Trap-1 from Cosmid R151 (U00036), H.a. = *Haemophilus actinomyceticomites* HtpG (U26968), E.c. = *Escherichia coli* HtpG (P10413), H.v. = *Hordeum vulgare* Hsp90 (X67960), C.r. = *Catharanthus roseus* Hsp90 (L14594), C.a. = *Candida albicans* Hsp90 (X81025), S.c. = *Saccharomyces cereviseae* Hsp82 (P02829), D.m. = *Drosophila melanogaster* Hsp83 (P02828), A.a. = *Anopheles albimanus* Hsp82 (L47285), S.s = *Sus scrofa* Hsp90 (U94395), H.s. = *Homo sapiens* Hsp90A (P07900), G.g. = *Gallus gallus* Hsp90 (X70101), O.t. = *Oncorhynchus tshawytscha* Hsp90 (U89945), D.d. = *Dictyostelium discoideum* Hsc90 (L43591), L.i. = *Leishmania infantum* Hsp83 (P87770), L.a. = *Leishmania amazonensis* Hsp83 (P27741), T.b. = *Trypanosoma brucei* Hsp83 (X14176), T.c. = *Trypanosoma cruci* Hsp85 (P06660) and T.p. = *Trypanosoma parva* Hsp90 (M57386).

obtained from the Berkeley *Drosophila* Genome Project. Sequencing revealed that this single open reading frame could be conceptually translated into a polypeptide of 691 amino acid residues which shows a high degree of similarity to the human Trap-1 protein (Song et al., 1995), establishing that we have identified the putative *Drosophila* homolog referred to as dTrap-1. The *dtrap-1* gene is identical to the recently identified Trap1dr isolated by Felts and coworkers (Felts et al., 2000), that gives rise to a protein (accession number AF115775) for which chaperone-like properties could not be shown. However, it accumulates in mitochondria and has putatively functions different from those of Hsp83 (Felts et al., 2000). Nevertheless, the degree of sequence similarity between human Trap-1, dTrap-1 and the founding member of the Hsp 90 family of proteins is striking (Fig. 2A in Felts et al., 2000 and alignment underlying Fig. 1B). Furthermore, phylogenetic analysis indicates that dTrap1, human Trap-1 and a hypothetical protein deduced from a *Caenorhabditis elegans* genomic sequence comprise a new family of the superfamily of heat shock proteins (Fig. 1B). This analysis implies in addition that the founding member of this subfamily has diverged early from the large body of Hsp90 family members during eukaryotic evolution.

In order to determine *dtrap-1* expression during *Drosophila* embryogenesis, we used a RNA antisense transcript probe for in situ hybridization of whole-mount preparations of staged embryos. Fig. 2B shows that transcripts are evenly distributed in the preblastoderm stage embryo in the absence of nuclear transcription. Thus, these transcripts must be of maternal origin consistent with the finding that *dtrap-1* is strongly expressed during oogenesis and that transcripts are transported from nurse cells into the growing oocyte (Fig. 2A and inset). As development proceeds, and nuclei appear in the periphery of the embryo (late syncytial stage; see Campos-Ortega and Hartenstein, 1997) transcripts are degraded throughout the embryo. However, they remain in the posterior pole region of the embryo (Fig. 2C) up until the transition between syncytial and cellular blastoderm stage. A similar pattern of transcript degradation and remaining of transcripts in the posterior region of the embryo has been reported for the transcripts of *Drosophila hsp83* (Bashirullah et al., 1999).

First zygotic expression of the gene is initiated after gastrulation has started, weakly in the mesoderm primordium and more prominently in the developing endoderm (white arrows, Fig. 2D). Transcripts continue to accumulate in the developing muscles, remain prominent in the midgut

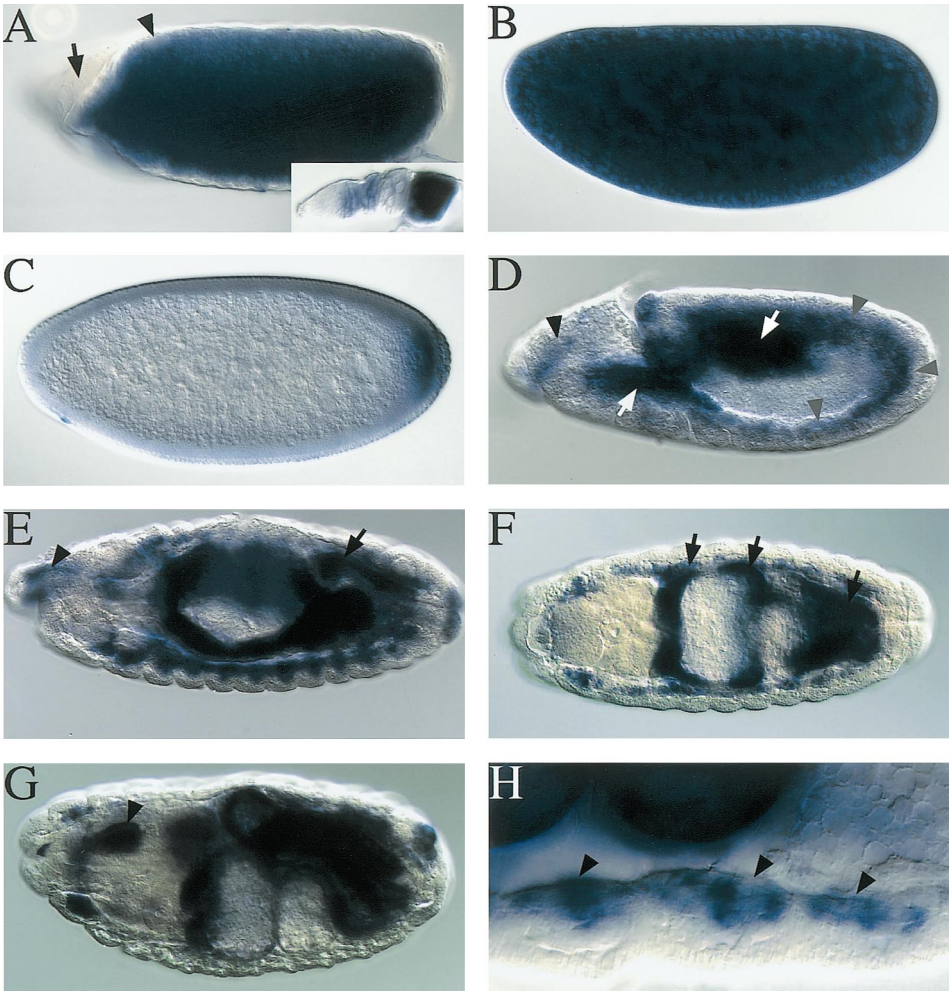

Fig. 2. Transcript pattern of the *dtrap-1* gene visualized by whole-mount in situ hybridization. Follicles are shown at stages 4 and 13 (A), embryos at embryonic stages 2 (B), 4 (C), 10 (D), 13 (E), 15 (F), 17 (G) and a blow up of body wall muscles at stage 16 (H). Labeled antisense RNA probe was derived from the XS800-fragment (see Fig. 1; stages according to Campos-Ortega and Hartenstein, 1997). The arrow in (A) points to the nurse cell remnants and the arrowhead marks the oocyte nucleus. The inset shows the start of prominent germ cell expression in a stage 4 follicle. Whereas the freshly laid egg has an uniform distribution of transcript (B), the cellularizing embryo shows rapid degradation of *dtrap-1*-RNA throughout its whole length with an exception at the very posterior pole (C). After gastrulation (stage 10, D) new transcripts are synthesized in the endoderm (white arrows) and in the mesoderm (arrowheads). At stage 14 (E) a prominent domain in the developing hindgut (arrow, ectoderm) complements the persisting expression in derivates of the endoderm (fusing midgut primordia) and mesoderm (fatbody and muscle precursors; e.g. arrowhead: pharynx muscles precursers). During organogenesis the endodermal expression splits into three domains (F; arrows) and at the end of embryogenesis (G; stage 17) the most prominent expression domains reside in some muscles and in the mid- and hindgut. In the precluding stages expression in muscles was extremely short timed with the exception of the primordia and the thereof resulting pharyx muscles, in which the expression of *dtrap-1* persisted (black arrowheads in (D,E,G)). The arrowheads in (H) point to ephemeral expression in lateral longitudinal muscles, where most prominent staining is detected next to the nuclei within the fused muscles. Embryos are shown in lateral (A–C,F), dorso-lateral (D) or dorsal view (E). In all images anterior is left and in (A–D,F) dorsal is up.

and in addition in the hindgut (arrow, Fig. 2E). During later stages of development, *dtrap-1* is maintained in the midgut but splits into three expression domains where the transcripts are expressed at high levels (arrows, Fig. 2F). Towards the end of embryogenesis, transcripts are found in a highly dynamic pattern in muscles (Fig. 2G,H). Among these, the pharynx muscles are the most prominent sites of transcript accumulation (Fig. 2G) and the only ones where expression is continuos from the precursors onward (black arrowheads in Fig. 2D,E,G). In conclusion, the *dtrap-1* gene encodes a novel member of a subfamily of Hsp 90-related Hsps and is transcribed in distinct expression domains in all three germ layers during *Drosophila* embryogenesis.

2. Materials and methods

Cloning of the *dtrap-1* gene was initiated by plasmid rescue (Wilson et al., 1989) using the l(2)04535 P element. The resulting fragment was used to screen a genomic phage library (Stratagene). Sequence comparison of subclones

identified the *Vha16* locus and an additional putative open reading frame (pORF) 5′ to *Vha16*. With a genomic fragment corresponding to this pORF (XS800) an embryonic cDNA library was screened (Clontech) and 9 independent clones were obtained. They were assembled to a cDNA with 2235 bp that was fully sequenced as cDNA and genomic DNA. This cDNA overlaps in the ORF completely with the clone LD14417 from the Berkley *Drosophila* Genome Project and was confirmed by 5′-RACE with mRNA prepared from CantonS embryos (protocol according to the manufacturers manual, Clontech). The screening probe XS800 was additionally used to prepare digoxygenin-labeled sense and antisense RNA probes as outlined by the manufacturer (Boehringer, Mannheim). In situ hybridizations were performed as described by Hauptmann and Gerster (1996).

Acknowledgements

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