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genome-wide analysis and expression profiling of the small heat shock proteins in zebrafish

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Abstract

Small Heat Shock Proteins (sHSPs) have important roles in preventing disease and promoting resistance to environmental stressors. Mutations in any one of a number of sHSPs, including *HSP27* (*HSPB1*), *HSP22* (*HSPB8*), *αA-crystallin* (*HSPB4*), or *αB-crystallin* (*HSPB5*) can result in neuronal degeneration, myopathy, and/or cataract in humans. Ten sHSPs are known in humans, and thirteen have been identified in teleost fish. Here we report the identification of thirteen zebrafish sHSPs. Using a combination of phylogenetic analysis and analysis of synteny, we have determined that ten are likely orthologs of human sHSPs. We have used quantitative RT-PCR to determine the relative expression levels of all thirteen sHSPs during development and in response to heat shock. Our findings indicate that most of the zebrafish sHSPs are expressed during development, and five of these genes are transcriptionally upregulated by heat shock at one or more stages of development.

Keywords

sHSP; α-crystallin; synteny; real time PCR; development

1. Introduction

Many Heat Shock Proteins (HSPs) are molecular chaperones that bind to and prevent aggregation of proteins. Small HSPs (sHSPs) are low-molecular weight HSPs that are present in nearly every species (Narberhaus, 2002). They range in size from 12-43kD and are characterized by a single conserved domain of approximately 80 residues known as the α-crystallin domain. While the functions of sHSPs presumably have their evolutionary roots in chaperoning proteins, many have additional functions. For example, HspB1 (Hsp27) regulates actin filament dynamics, its precise role depending on its phosphorylation state (reviewed by Liang and MacRae, 1997; Mounier and Arrigo, 2002). Mutations in several members of the sHSP family cause degenerative diseases: axonal Charcot-Marie Tooth Disease (CMT) or Distal Hereditary Motor Neuropathy (dHMN) in the cases of *HSPB1* (Evgrafov et al., 2004; Kijima et al., 2005; Tang et al., 2005a) and *HSPB8* (Irobi et al., 2004; Tang et al., 2005b), and desmin-related myopathy in the case of *HSPB5* (Vicart et al., 1998). In addition, mutations in *HSPB4* (Litt et al., 1998) or *HSPB5* (Berry et al., 2001) result in cataract formation.

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While the evolution of the sHSP gene family has been described in some detail (Kappe et al., 2002; Narberhaus, 2002; Kappe et al., 2003; Franck et al., 2004), regulation of expression has been less well characterized. The tissue distributions of individual sHSPs has been analyzed somewhat systematically in adults of various species (e.g. (Bhat and Nagineni, 1989; Dubin et al., 1989; Gastmann et al., 1993; Lee et al., 1993; Iwaki et al., 1997; Plumier et al., 1997; Posner et al., 1999; Dean and Tytell, 2001; Kappe et al., 2001; Runkle et al., 2002), and while some studies have examined expression during organismal development (e.g. (Ali et al., 1993; Gernold et al., 1993; Tam and Heikkila, 1995; Lang et al., 1999; Loones et al., 2000; Armstrong et al., 2001; Tallot et al., 2003; Verschuure et al., 2003; Hawkes et al., 2004; Monastirli et al., 2005; Mao and Shelden, 2006; Tuttle et al., 2006), the majority of these have focussed on HspB1. A systematic characterization of the expression of the entire gene family in a single species is needed in order to better assess the relative importance of each of the family members during development and in response to stress.

The zebrafish (*Danio rerio*) is an ideal model vertebrate in which to analyze gene expression and function, in particular during developmental stages due to its external development, small size, transparency, and tractability to genetic manipulation. We are using zebrafish to perform a systematic characterization of the entire sHSP family during development. While humans have ten sHSPs (Fontaine et al., 2003; Kappe et al., 2003), it has recently been suggested that the common ancestor to tetrapods and teleosts had as many as fifteen (Franck et al., 2004). We have identified thirteen sHSPs in the zebrafish, ten of which are likely orthologs of human sHSPs, and each of which corresponds to one of the thirteen teleost sHSPs recently described (Franck et al., 2004). We have also used quantitative real time RT-PCR to perform a comprehensive characterization of the normal and heat shock-induced expression of all thirteen zebrafish sHSP genes during embryonic and larval development. Our results demonstrate that, though their expression levels differ widely, most of the zebrafish sHSPs are expressed during embryonic and/or larval development, and, in contrast to results from previous studies in other species, five are upregulated by heat shock.

2. Methods

2.1. Sequence, phylogenetic, and genomic analysis

We used one representative of each vertebrate sHSP or a consensus sequence derived from all vertebrate sHSPs to query Genbank using BlastX (http://ncbi.nlm.nih.gov/Blast). The finished and unfinished genome databases were searched using the provided Blast algorithm (http://Sanger.co.uk). ESTs for new sHSPs were obtained (*hspb2* and *hspb3* from Jinrong Peng of the Institute of Molecular and Cell Biology, Singapore; *hspb6*, *hspb9*, and *hspb11* from Open Biosystems; and *hspb7*, *hspb8*, and *hspb15* from the American Type Culture Collection) and sequenced completely in both directions. GenBank accession numbers for newly-identified or newly-completed zebrafish sHSPs are: *hspb2* (**EF583628**), *hspb3* (**EF583629**), *hspb6* (**EF614998**), *hspb7* (**BC083373**), *hspb8* (**BC057441**), *hspb9* (**EF583630**), *hspb11* (**EF583631**), *hspb12* (partial cds; **EF636699**), and *hspb15* (**EF583632**). Confirmed sequences were conceptually translated in all six frames to find longest open reading frame.

Alignments of crystallin domains were performed using ClustalX 1.81 (Thompson et al., 1997). Pairwise and multiple alignment penalties for gap opening were 10.0, 0.10 for gap extension, and Gonnet 250 for protein weight matrix. Phylogenetic trees were created using Neighbor-Joining and Bayesian posterior probability algorithms using ClustalX or Mr. Bayes 2.1 (Huelsenbeck et al., 2000), respectively. Neighbor-Joining trees were bootstrapped 100 times. For Bayesian analysis, two simultaneous chains were run for 100,000 generations using Metropolis-coupled Markov chain Monte Carlo sampling. Trees were sampled every 100 generations and a burn-in value of 250 was used. All trees were rooted based on the results of a complete analysis performed using *Saccharomyces cerevisiae HSP26* as an outgroup.

Determination of gene locations and structures, and syntenic analysis were based on the UC Santa Cruz assembly (http://genome.ucsc.edu/) based on version Zv6 of the zebrafish genome (The Wellcome Trust Sanger Institute).

2.2. Fish care

Fish were maintained and bred using standard procedures and in accordance with Williams College animal welfare assurance certificate A3133-01. Embryos were staged according to Kimmel et al. (1995) as equivalent hours post-fertilization (hpf) at 28.5°C

2.3. Real time RT-PCR

Embryos were raised at 28.5°C until the 16-cell stage, 12hpf, 24hpf, and 48hpf, or 5 days post-fertilization (dpf). For all stages except the 16-cell stage, clutches were divided into two equal groups, with half being heat shocked at 37°C for one hour followed by recovery for one hour. This heat shock paradigm has been shown to induce heat shock proteins without some of the problematic effects of higher-temperature heat shock (Krone et al., 1997). Heat shock prior to blastula stages is extremely damaging to embryos; we therefore did not heat shock 16-cell stage embryos. Heat shocked and non-heat shocked embryos were homogenized in Trizol, total RNA was purified using the RNeasy mini-kit (Qiagen) protocol, and cDNA was synthesized from total RNA using Superscript III (Invitrogen) Reverse Transcriptase and random hexamer primers. Primers were designed using Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3.cgi) and purchased from Sigma-Genosys (The Woodlands, TX, USA). Primer sequences and their annealing temperatures are listed:

gene	forward	reverse	T_{ann} (°C)
hspb1	ATGAACACGGCTTCATTTCC	GCGGAGCTTCAACAGTTAG	56.3
hspb2	GCATGGCTTTGTGAGTCGTA	AGTTCTTCGTGGAGCCTGAA	57.3
hspb3	TTTCCCACTGGATTTCAAGC	CAGGGCAAAGAGTCGATGT	56.3
hspb4	ATGGCCTGCTCACTCTTTGT	CCCACTCACACCTCCATACC	56.3
hspb5a	CCCAGGCTTCTTCCCTTATC	GTGCTTCACATCCAGGTTGA	60.7
hspb5b	CCTACTGACGGCCAAATGTT	GGCATCAGCAGCAGACAAA	60.7
hspb6	GGATCCCAAATGGAGTCAC	TTGTCTGTGTCAGAGGTGCG	56.3
hspb7	TAAATGCCGGCTACCAGAG	TGTGCATGCTCCAGTTTAGC	57.3
hspb8	ACCGACCAAAGTGAGAGGG	GCATTTACGGAGCTCTGAGG	57.3
hspb9	TGGACGACCCTTTCTTTGAG	GCATTATTTGGGCTCTACGG	55.0
hspb11	AGAGCTCGCCGTTAAACAG	AATAGGATCCCTTCCCATCG	56.3
hspb12	ACCCACAAGTCTGTGCTTCC	CGTCGTTCACACACAGGTTC	55.0
hspb15	ACAAGCTTCCGGCTGACTTA	GTTCATCCGTTTTTGCAGGT	56.3
EF-1α	CAGCTGATCGTTGGAGTCAA	TGTATGCGCTGACTTCCTTG	56.3

The iCycler iQ Real Time Detection System (Bio-Rad) was used for PCR. For each primer pair, a temperature gradient was performed to determine optimal annealing temperature, and this temperature was used for analysis. Amplification efficiency for each primer pair was determined according to the protocol recommended by the iCycler manufacturer. For analysis, cDNA was diluted ten-fold in water and used for PCR. In addition to cDNA, all PCR reactions contained 0.5μM each forward and reverse primers and SybrGreen (Bio-Rad) reaction mix. Melting curves for each PCR product were performed automatically by the system. In one case where a primer pair failed to amplify single, specific product, new primers were designed and specificity was verified for the new pair. Following PCR, C_t values were determined automatically by the software. Expression levels were based on analysis of cDNA from each of two or three independent RNA samples, each of which were run in triplicate. Gene expression levels, normalized to *EF-1α* (a standard internal control), were estimated using the equation: $F = (1+\varepsilon)^{(-\Delta C_t)}$ where $\Delta C_t = C_t(\text{EF-1}\alpha) - C_t(\text{sHSP})$ and is ε efficiency for genes whose amplification efficiencies were within 1.0% of that of *EF-1α*. For those genes whose amplification efficiencies were greater than 1.0% different from that of *EF-1α*, values were

corrected to reflect these differences. Relative expression values were converted to and displayed as light intensity values on a scale from 0 to 255.

3. Results

3.1. the sHSP gene family in zebrafish

Seven zebrafish sHSPs, *hspb1, hspb2, hspb3, hspb4, hspb5a, hspb5b*, and *hspb12* have been previously identified (Posner et al., 1999; Franck et al., 2004; Thisse and Thisse, 2004; Mao et al., 2005; Smith et al., 2006). Through searching all available expressed gene and genomic sequence databases, we have identified six additional zebrafish sHSPs (Table 1). From the aligned crystallin domains (Fig. 1), we constructed phylogenetic trees using either Neighbor-Joining or Bayesian posterior probability algorithms to determine the evolutionary relationships of the sHSPs. While the trees derived using these algorithms were not precisely identical, they differed in only minor details (Fig. 2).

Of the six new zebrafish sHSPs, we found one each orthologous to the tetrapod genes *HSPB7*, and *HSPB8*; one to *HSPB11* (*HSP30*), which appears to exist in all vertebrates except mammals; and one each corresponding to the apparently fish-specific genes *hspb13, hspb14*, and *hspb15*. The nearest human homologs to the latter three genes, as based on the topology of the phylogram in Figure 2, are *HSPB6*, *HSPB9*, and *HSPB1*, respectively. Assuming that fish do not have *HSPB6* or *HSPB9*, this suggests that the common ancestor to teleosts and tetrapods had all four genes (*HSPB6, HSPB13, HSPB9*, and *HSPB14*), with *HSPB6* and *HSPB9* having been lost during the evolution of the teleost lineage, and *HSPB13* and *HSPB14* having been lost during the evolution of tetrapods. However, an alternative explanation, that each of these gene pairs derived from a common ancestor, but that the protein-coding sequences diverged quickly following the teleost-tetrapod split as a result of differences in selective pressure, is also plausible.

Because synteny between zebrafish and human genomes is relatively well conserved (Postlethwait et al., 2000; Woods et al., 2000), we were able to compare the syntenic relationships of every probable or possible orthologous pair in order to provide a more definitive assessment of orthology. All of the zebrafish sHSPs were represented in the most recent zebrafish genome assembly (version Zv6) except for *hspb12*. We found that synteny is reasonably strongly conserved between four zebrafish and human sHSP genes (two or more immediate gene neighbors in common), *hspb1*, *hspb2*, *hspb5b*, and *hspb7* (Fig. 3A for *hspb5b*; data not shown for others). Conservation of synteny is less strong for *hspb3, hspb4, hspb5a*, and *hspb8* (Fig. 3A for *hspb5a*; data not shown for others). For example, while zebrafish *hspb5b* and human *HSPB5* share the same two immediate neighbors, zebrafish *hspb5a* and human *HSPB5* share only one of two nearby neighbors. Of the six genes that map to within 100kbp of zebrafish *hspb5a*, however, five are within 6.7Mbp of *HSPB5*, supporting the argument for their orthology.

The closest human homolog to zebrafish *hspb13* is human *HSPB6*. We found that a 265kbp region spanning zebrafish *hspb13* has four to five genes in common with the chromosomal region spanning human *HSPB6*, suggesting a common ancestry for these genes (Fig. 3B). This level of conservation is even stronger than it is for several accepted orthologous pairs; therefore, the zebrafish *hspb13* gene is most likely an *HSPB6* ortholog. Zebrafish *hspb14* and human *HSPB9* show a similar pattern. While they lack common neighbors within 100kbp (of zebrafish *hspb14*) and 90kbp (of human *HSPB9*), three genes that map to within 100kbp of the zebrafish *hspb14* are within 3.3Mbp of human *HSPB9* on Chromosome 17 (Fig. 3C). Zebrafish *hspb14* and human *HSPB9* share another similarity; unlike the majority of sHSPs, both zebrafish *hspb14* and human *HSPB9* are most frequently found unspliced (see Table 1). These results suggest that zebrafish *hspb14* is likely to be the fish ortholog of human *HSPB9*. Based

on these data, we will henceforth refer to this gene as *hspb9*. By way of comparison, zebrafish *hspb15* shows no evidence for orthology with human *HSPB1*, its closest human homolog; they do not have a single neighbor in common within 250kbp of either gene, and none of the human genes that map to this interval in zebrafish are located on the same human chromosome as the human *HSPB1* gene. Although we cannot absolutely rule out the possibility that this pair is orthologous, there is currently insufficient evidence to support it.

3.2. expression of sHSPs – normal development and upregulation by heat shock

We used quantitative real time RT-PCR to determine the approximate relative expression levels of all thirteen sHSPs during development and in response to heat shock (Fig. 4 and Table 2). Based on our results, *hspb1* appears to be expressed at higher levels than the other sHSPs throughout embryonic development, although *hspb8* is also expressed at quite high levels. With the exception of *hspb3*, *hspb5a*, and *hspb15*, all of the remaining sHSP genes appear to expressed during normal development, albeit at much lower levels than either *hspb1* or *hspb8*. As we controlled for PCR efficiency in determining expression levels, these data are at least roughly comparable from gene to gene. However, the high degree of variability in the PCR limits our ability to define expression levels with absolute certainty.

In response to a one-hour heat shock at 37°C, we found the expression of several sHSPs, *hspb1*, *hspb4*, *hspb8*, *hspb9*, and *hspb11*, to be upregulated more than five-fold at one or more stages of development: (Fig. 4B and Table 3). While the extent of upregulation of *hspb4* was not statistically significant, the magnitude of upregulation (17.3-fold at 12hpf and 5.2-fold at 24hpf) suggests that these differences are real. *hspb1* showed the greatest maximal degree of upregulation (90.5x normal levels at 5dpf). In general, the heat shock-responsiveness of the sHSPs appears to increase with age with the notable exception of *hspb4*.

Because it would be nearly impossible to exhaustively describe the expression of all thirteen sHSPs, we have inventoried all zebrafish sHSP ESTs currently in Genbank to provide a more global view of the expression of these genes during development and in the adult (Table 3). Interestingly, *hspb1* is found at highest levels in myoblast libraries, consistent with its reported expression in developing somites (Thisse et al., 2001;Mao and Shelden, 2006). Not surprisingly, the lens α-crystallins, *hspb4*, *hspb5a*, and *hspb5b* are found predominantly in lens libraries. Three sHSPs, *hspb6*, *hspb7*, and *hspb11*, are primarily found in adult heart libraries. This finding is not surprising in the case of *hspb7*, which was originally identified as a protein expressed highly and selectively in human heart (Krief et al., 1999). On the other hand, it is a bit surprising that *hspb6*, which is relatively broadly expressed in other species (Verschuure et al., 2003), appears to be more or less restricted to heart in zebrafish. To our knowledge, expression of *hspb11* has not yet been characterized in adults of any species. Finally, *hspb8* is relatively abundant in a broad spectrum of embryonic and adult libraries, consistent with expression data from other systems (Kappe et al., 2001;Verschuure et al., 2003), while *hspb2*, *hspb3*, *hspb9*, *hspb12*, and *hspb15* are not particularly abundant in any library represented in Genbank.

4. Discussion

4.1. evolution of the sHSP gene family

sHSPs comprise a diverse and rapidly-evolving family of proteins. Duplication and subsequent expansion of sHSP genes seems to have occurred frequently throughout evolution. For example, the nematode *Caenorhabditis briggsae* has as many as ten sHSPs that are in the same clade as the vertebrate *HSPB9* and *HSPB11* genes (Hong et al., 2004) and data not shown), and *Xenopus laevis* has as many as seven *HSPB11* genes (Krone et al., 1992). Some sHSPs have also apparently been lost during evolution. For example, *HSPB12*, which has been found

in chicken and fish, seems to have been lost in mammals (Franck et al., 2004). Could this diversity have contributed to species evolution? By way of analogy, it has been proposed that duplication of the ancestral genome may account for the diversity of teleosts (Postlethwait et al., 2000; Venkatesh, 2003; Volff, 2005). Indeed, the complement of sHSPs present in an organism is likely to confer specific selective advantages to individuals in specific situations and could therefore have contributed to speciation.

While humans are thought to have ten sHSPs, zebrafish (and other teleosts examined to date) have thirteen. Here we are able to show that the fish gene previously referred to as *hspb13* is in fact orthologous to human *HSPB6*, and fish *hspb14* is, in all likelihood, orthologous to human *HSPB9*. The only remaining mammalian sHSP for which no fish ortholog has been found is therefore *HSPB10*. The two identified fish genes that are most similar to human *HSPB10* are *hspb9* and *hspb11*. However, there is no evidence from our analysis to suggest that either of these genes is an *HSPB10* ortholog. Because *HSPB10* in mammals is a sperm-specific protein, and sperm is likely to be underrepresented in zebrafish EST sequencing projects, it may be that fish *hspb10* has simply yet to be found. Alternatively, *hspb10* may have been lost during fish evolution due to different constraints on sperm evolution, or it may have diverged to such an extent that it is no longer recognizable.

It is rather interesting, in light of estimates that approximately one quarter of all human genes has a pair of orthologs in teleost fish (Postlethwait et al., 2000; Woods et al., 2005), that only *HSPB5* has paired orthologs in the zebrafish. In order to determine whether we overlooked the second of some duplicated pairs, we attempted to examine the syntenic relationships between each of the remaining zebrafish genes, *hspb12* and *hspb15*, and their nearest mammalian counterparts, *HSPB7* and *HSPB1*. Unfortunately, *hspb12* is not yet represented in the finished zebrafish genome, so we were not able to resolve this relationship. Regardless, the presence of *HSPB12* in the chicken strongly suggests that it did not arise as a result of the teleost genome duplication, and thus is not a second ortholog of *HSPB7*. In the case of *hspb15*, there is currently no evidence to support its being a second *HSPB1* ortholog. However, as the zebrafish genome is not one hundred percent complete, we cannot unequivocally rule out the possibility.

4.2. expression of zebrafish sHSPs

As stated previously, this is the first study to systematically characterize the expression of the entire sHSP gene family in a single species. Our results show that many of the sHSPs are expressed at appreciable levels during embryonic and larval development. Of these, we found *hspb1* to be expressed at the highest levels, reaching maximal expression around 12hpf, similar to previous findings (Mao et al., 2005). Five of the thirteen sHSPs, *hspb1, hspb4, hspb8, hspb9*, and *hspb11*, were found to be upregulated by heat shock. These results are largely consistent with results in other systems, where *hspb11* was reported to upregulated by heat shock in *Xenopus* (Ali et al., 1993; Heikkila, 2003), and *HSPB1, HSPB5* (Mounier and Arrigo, 2002) and *HSPB8* (Chowdary et al., 2004) have been reported to be upregulated by heat shock in some mammalian systems. However, this is the first report of *hspb4* being upregulated by heat shock in any system, although Hawse et al. (Hawse et al., 2003) found that *HSPB4* was upregulated specifically by Cu^{2+} in culture human lens epithelial cells. This apparent discrepancy may be explained by the fact that, in our hands, *hspb4* was upregulated during only a fairly narrow window of development, a window that could have been easily missed by other studies. Indeed, it has been reported that the heat shock-responsiveness of genes can change throughout the life of the organism (Davis and King, 1989; Heikkila, 2004). We also found *hspb9* to be upregulated by heat shock which had not been reported in other systems. This discrepancy could again be accounted for by differences between developmental and adult stages. An alternative explanation is that real time PCR is more senstive at detecting small differences in expression than traditional methods. Regardless of the explanation, the results

of these studies provide an important foundation for both further analysis of the evolution and regulation of sHSPs, as well as efforts to determine whether the effects of sHSP mutations in humans may be related to defects arising during development.

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abbreviations

sHSP small heat shock protein

HSP heat shock protein

CMT Charcot-Marie Tooth Disease

dHMN Distal Hereditary Motor Neuropathy

EF-1α elongation factor-1α

hpf hours post-fertilization

dpf days post-fertilization

μM micromolar

kD kilodaltons

RT-PCR reverse transcription polymerase chain reaction

C_t threshold cycle

EST expressed sequence tag

kbp kilobase pairs

Mbp megabase pairs

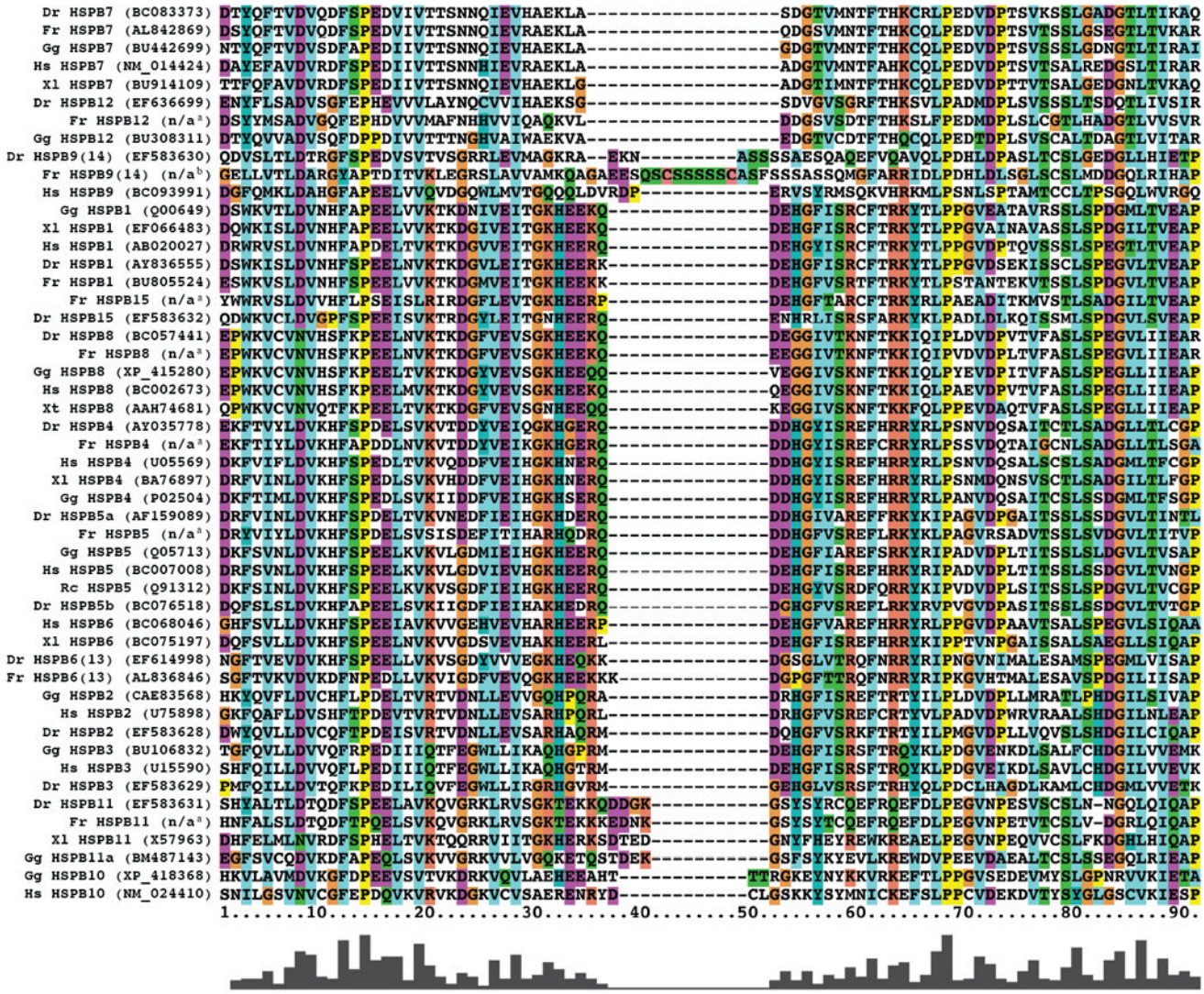

Figure 1.

Alignment of vertebrate sHSP crystallin domains. Color shading indicates type of amino acid residue, where these residues are conserved across species'. Sequences from the following species were used as representatives of each class of vertebrates: *Homo sapiens* (Hs) for mammalian, *Gallus gallus* (Gg) for avian, and *Xenopus laevis* (Xl), *Xenopus tropicalis* (Xt), or *Rana catesbiana* (Rc) for amphibian. An exception was made for teleost fish, where, in addition to zebrafish *Danio rerio* (Dr), sequences for the Japanese pufferfish *Takifugu rubripes* (Fr) were included where possible. The *X. laevis* gene *HSP30D* gene was used to represent the amphibian *HSPB11* family, and the *G. gallus* gene *HSPB11a* was used to represent the avian *HSPB11* family. Height of gray bars below alignment represent the degree of conservation at each position. For HSPB6 and HSPB6, the number in parenthesis refers to previous gene name. Accession numbers for cDNA or protein sequences are provided (parenthesis).

[a]ORF predicted from Fugu genome, fourth assembly (http://www.fugu-sg.org)

[b]from Franck et al. (2004)

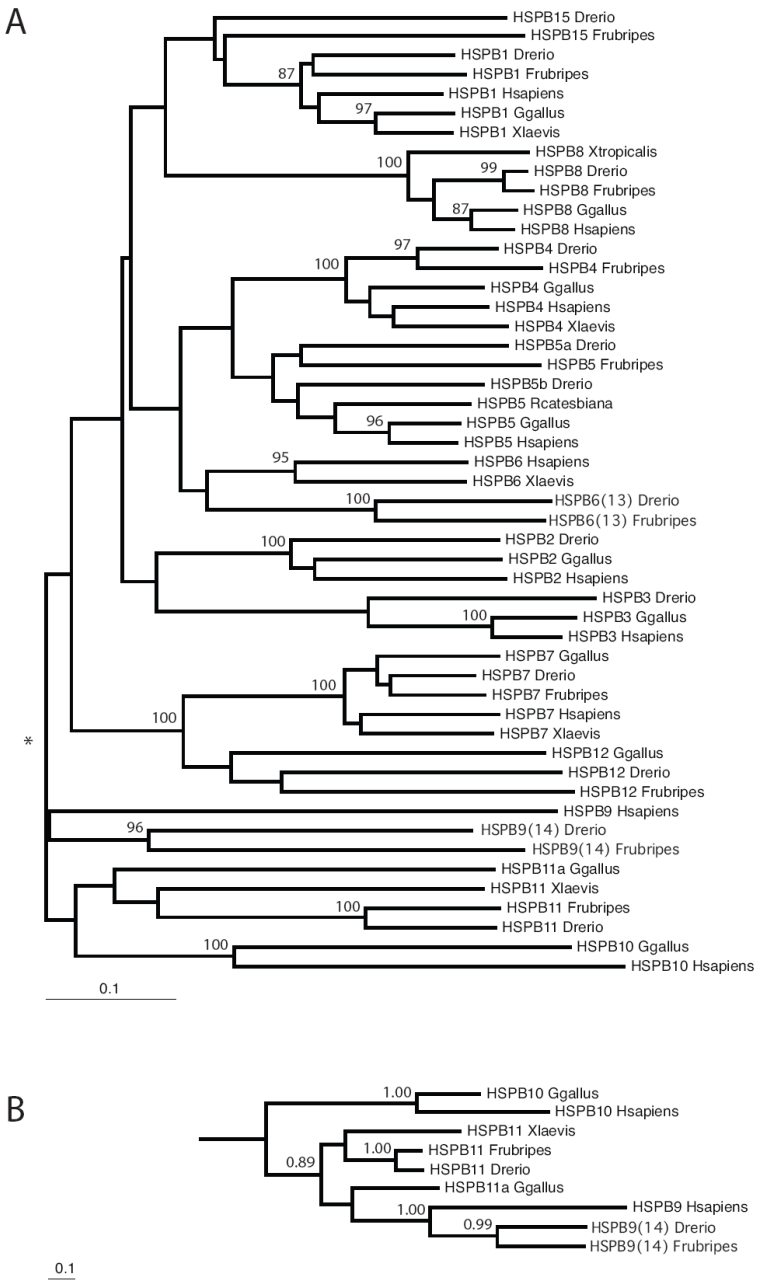

Figure 2.

Phylogenetic trees of vertebrate sHSPs based on the alignment shown in Figure 1 and constructed using Neighbor-joining (A) or Bayesian posterior probability (B) algorithms. A. Root position (asterisk) was determined from phylogenetic tree using *S. cerevisiae HSP26* as outgroup. Bootstrap values ≥87% are provided. B. Portion of Bayesian tree. Posterior probability values ≥0.89 are provided. Scales refer to the inferred number of sequence changes per site. Numbers in parenthesis refer to the prior names of these genes.

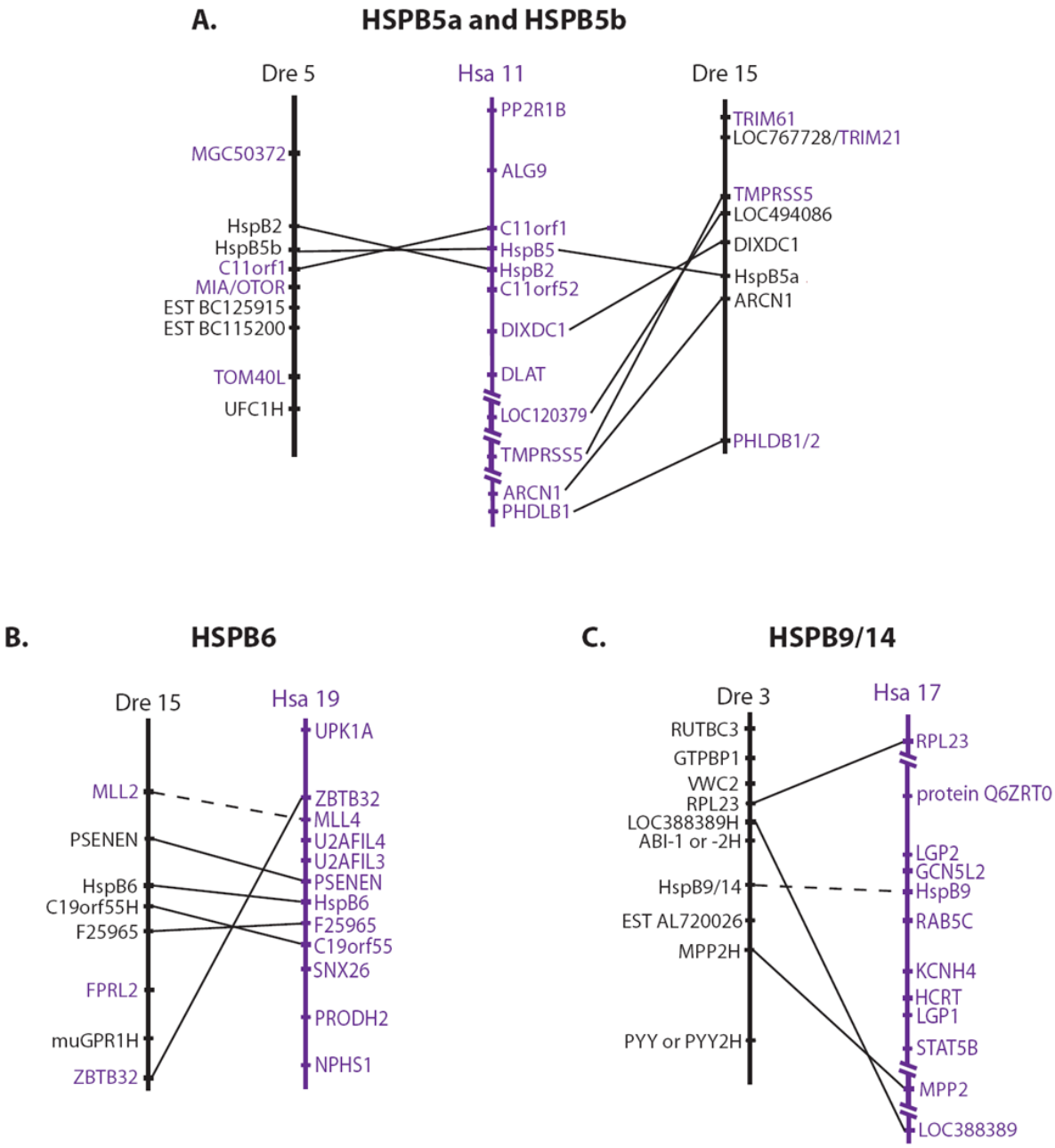

Figure 3.

Syntenic relationships between representative zebrafish sHSPs and their putative human orthologs. Zebrafish (Dre) data are shown in black. Human (Hsa) data are shown in violet. In instances where no zebrafish gene is known to exist at a particular location in the zebrafish genome, but where a putative ortholog to a human gene has been mapped, the human gene name is provided in violet. Unannotated zebrafish mRNAs and ESTs were subject to a Blast search, and given a provisional name of their nearest human homolog (E-value cutoff-20) followed by an 'H' (e.g. LOC388389H). mRNAs or ESTs with no significant matches to anything in Genbank are identified by accession number. A. Syntenic relationships between zebrafish *hspb5a* and *hspb5b* and human *HSPB5*. Approximately 200kbp of zebrafish

Chromosomes 5 and 15, and 300kbp of human Chromosome 11, are shown. Human genes located outside the 300kbp interval, and their approximate distances from human *HSPB5*, are *LOC120379* (160kbp), *TMPRSS5* (1.8Mbp), *ARCN1* (6.7Mbp), and *PHLDB1* (6.7Mbp). B. Conserved synteny between zebrafish *hspb6* and human *HSPB6*. Approximately 265kbp of zebrafish Chromosome 15, and 165kbp of human Chromosome 19, are shown. C. Syntenic relationship between zebrafish *hspb9* and human *HSPB9*. Approximately 200kbp of zebrafish Chromosome 3, and 180kbp of human Chromosome 17, are shown. Human genes that map outside this region and their approximate distances from human *HSPB9* are: *RPL23* (3.3Mbp), *MPP2* (1.8Mbp), and *LOC388389* (2.8Mbp). Dashed lines in B and C indicate homologous genes not previously identified as orthologs.

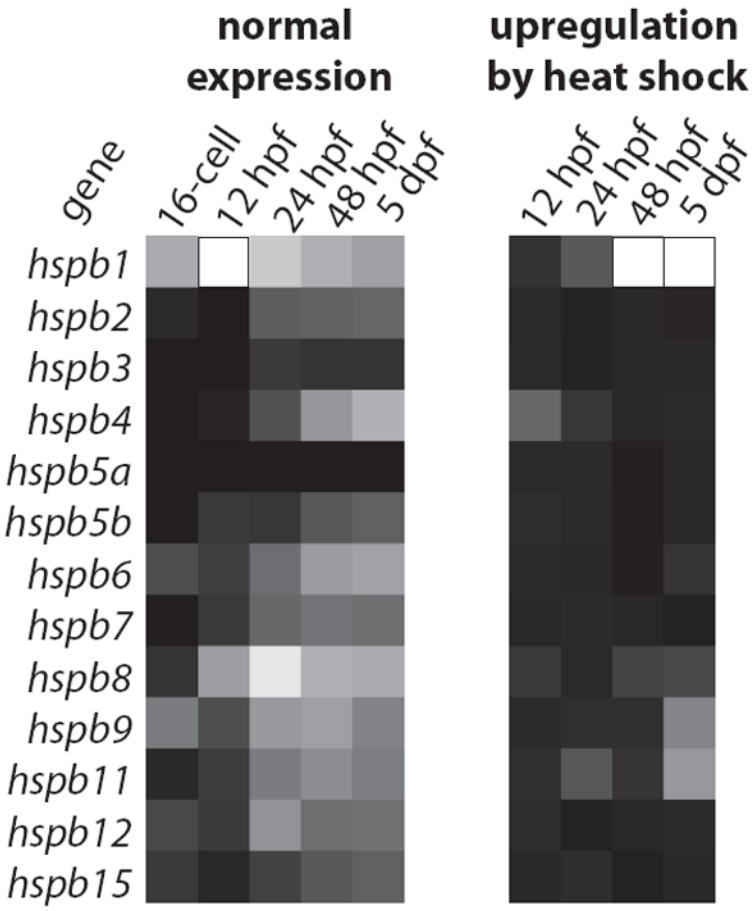

Figure 4.

Expression of zebrafish sHSPs during development and in response to heat shock. At left is shown relative expression levels during normal development. Because of the huge range in expression levels between *hspb1* and the remainder of the sHSPs, each value was multiplied by 10^5 and the natural log of this number was converted to greyscale intensity values, where white corresponds to the maximum observed expression level (that of *hspb1* at 12hpf). At right is heat shock-induced expression as fraction of normal. White in this case corresponds to 50-fold *or greater* upregulation (see Table 2 for actual values). Values are displayed on a linear scale.

Table 1

Summary of sHSP genes in zebrafish and human

Gene		Zebrafish		Human	
Gene	**Other names**	**Chromosome:location[a]**	**Exons[b]**	**Map position**	**Exons[b]**
hspb1	Hsp27	16:40148126-40154368	3	7q11.23	3
hspb2	MKBP	5:64297779-64313514	2	11q22-23	2
hspb3		5:53445179-53445937	1	5q11.2	1
hspb4	aA-crystallin, CRYAA	1:41386888-41390243	3 or 4[c]	21q22.3	3 or 4[d]
hspb5a	aB-crystallin, CRYABA	15:12257017-12261795	3	11q22.3-q23.1	3
hspb5b	aB-crystallin, CRYABB	5:64319420-64323810	3	see above	3
hspb6(13)[e*]	Hsp20, HspB13	15:49659903-49661283	3	19q13.12	3
hspb7[*]	cvHsp	23:26600535-26605689	3	1p36.23-p34.3	2-4[f]
hspb8[*]	Hsp22, E2IG1, H11	5:10081155-10100866	3	12q24.23	3
hspb9(14)[e*]	HspB14	3:40596891-40597575	1 or 2[g]	17q21.2	1
hspb10	ODF1	not found		8q22.3	2
hspb11[*]	Hsp30	21:17886029-17886771	1	NA	NA
hspb12		NA[h]	4	NA	NA
hspb15[*]		5:30370587-30371692	3	NA	NA

[a] based on version Zv6 zebrafish genome assembly

[b] based on mRNAs and ESTs mapped to genome within UC Santa Cruz genome assemblies

[c] some splice variants include a 4th 3'UTR exon

[d] some splice variants skip a 3'UTR exon

[e] number in parenthesis refers to previous numerical designation (Franck et al, 2004)

[f] a variety of splice forms contain between two and four exons, though most contain three

[g] 89% of mRNAs mapped to genome are unspliced

[h] is not represented in most recent genome assembly

[*] indicates newly-identified genes

Table 2

Relative expression of zebrafish sHSPs during embryonic and larval development[a]

	16-cell	12 hpf			24 hpf			48 hpf			5 dpf		
gene	**normal**	**normal**	**HS[b]**	**fold diff. *c***	**normal**	**HS**	**fold diff.**	**normal**	**HS**	**fold diff.**	**normal**	**HS**	**fold diff.**
hspb1	497 ± 150	9242 ± 3351	34470 ± 3558	3.7*	1586 ± 252	21250 ± 1792	13.9*	625 ± 227	20470 ± 1412	51.2*	332 ± 15.3	29880 ± 1031	90.5*
hspb2	1.4 ± 0.6	0.1 ± 0.0	0.2 ± 0.1	1.9	15.7 ± 11.0	7.3 ± 3.5	0.5	17.8 ± 7.0	20.0 ± 10.6	1.1	22.2 ± 10.6	16.8 ± 8.0	0.8
hspb3	0.0 ± 0.0	0.2 ± 0.1	0.3 ± 0.1	2.0	2.9 ± 2.2	1.4 ± 0.7	0.5	2.2 ± 1.1	2.1 ± 0.9	1.0	2.2 ± 0.4	3.0 ± 0.7	1.4
hspb4	1.0 ± 0.6	1.1 ± 0.6	18.4 ± 10.2	17.3	8.6 ± 3.6	44.5 ± 23.4	5.2	212 ± 155	304 ± 158	1.4	618 ± 225	1115 ± 77.0	1.8
hspb5a	0.8 ± 0.9	0.7 ± 0.7	0.7 ± 0.6	1.1	0.1 ± 0.1	0.3 ± 0.3	2.1	0.3 ± 0.3	0.0 ± 0.0	0.0	0.6 ± 0.3	0.4 ± 0.1	0.7
hspb5b	0.1 ± 0.1	2.9 ± 1.0	7.3 ± 4.7	2.5	2.6 ± 1.7	4.4 ± 3.3	1.7	12.4 ± 10.8	2.2 ± 1.3	0.2	18.0 ± 1.9	22.1 ± 2.3	1.2
hspb6	7.3 ± 2.0	3.6 ± 1.3	7.6 ± 2.3	2.1	34.5 ± 0.0	44.2 ± 2.3	1.3	263.3 ± 54.0	76.7 ± 2.7	0.3	353 ± 95.5	1532 ± 263	4.3
hspb7	0.2 ± 0.2	2.9 ± 0.1	3.7 ± 0.0	1.3	26.0 ± 25.3	43.2 ± 46.5	1.7	46.5 ± 14.1	30.3 ± 7.2	0.7	33.9 ± 5.8	16.1 ± 0.6	0.5
hspb8	2.2 ± 1.9	295 ± 78	1583 ± 585	5.4	4542 ± 3705	8036 ± 4229	1.8	602 ± 245	5188 ± 3239	8.6	513 ± 122	4782 ± 659	9.3*
hspb9	63.3 ± 35.1	7.1 ± 1.7	12.6 ± 3.8	1.8	253 ± 17.5	843 ± 255	3.3	324 ± 215	1182 ± 0.0	3.6	90.8 ± 18.6	2290 ± 237	25.2*
hspb11	1.3 ± 1.0	5.5 ± 2.0	16.7 ± 4.5	3.0	65.0 ± 9.0	868 ± 316	13.3	142 ± 57.3	552 ± 0.0	4.8*	74.2 ± 5.1	2368 ± 0.0	31.9*
hspb12	5.3 ± 0.4	3.1 ± 0.9	7.3 ± 3.3	2.4	190 ± 19.7	119 ± 39.7	0.6	33.9 ± 5.8	32.3 ± 2.2	1.0	39.8 ± 2.8	84.5 ± 20.1	2.1
hspb15	3.0 ± 0.5	1.2 ± 0.5	1.6 ± 0.7	1.3	4.3 ± 0.0	11.6 ± 1.2	2.7	12.4 ± 6.9	4.4 ± 2.2	0.4	17.5 ± 10.9	23.4 ± 0.8	1.3

[a] based on results of real time PCR, fraction of *EF-1α* expression (x10^5); values are reported ± standard error of the mean

[b] expression following one-hour heat shock relative to *EF-1α* expression (x10^5)

[c] fold difference; ratio of HS-induced expression to unstressed expression

* $p<0.05$, Student's t-test

Table 3

Summary of zebrafish sHSP ESTs*

gene	"embryo"	0-72hpf	2-8hpf	myoblast	1dpf	2dpf	3dpf	3dpf heart	5dpf	whole body[a]	whole body[b]	male whole body	shield, 26s, liver[c]	adult heart	bone	brain	eye	gills	kidney	kidney marrow	lens	post. segment eye[d]	retina[e]	skin	testis	unknown
hspb1			1	62										37												
hspb2										3				3							1					
hspb3										2									1							
hspb4									2	8			1				13				43		2			
hspb5a																	1				24					
hspb5b											6			1			12				23	1			1	
hspb6											6	5		10										2		
hspb7		1							1		1			81												1
hspb8	13	2		4	1					1	2		6	3				1	7	8		1			10	
hspb9		1			2	1	1																			1
hspb11					2	1		1	4		1			72	1							2			1	
hspb12		1											1													
hspb15									2							1										

[a] embryos and adults

[b] adults only

[c] shield stage embryos, 26-somite stage embryos, and adult liver

[d] does not include retina

[e] posterior segment; includes lens, retinal pigment epithelium, and skin

* shading indicates most abundant source(s) of each gene