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Review

The expression and function of *hsp30*-like small heat shock protein genes in amphibians, birds, fish, and reptiles

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ABSTRACT

Small heat shock proteins (sHSPs) are a superfamily of molecular chaperones with important roles in protein homeostasis and other cellular functions. Amphibians, reptiles, fish and birds have a *shsp* gene called *hsp30*, which was also referred to as *hspb11* or *hsp25* in some fish and bird species. *Hsp30* genes, which are not found in mammals, are transcribed in response to heat shock or other stresses by means of the heat shock factor that is activated in response to an accumulation of unfolded protein. Amino acid sequence analysis revealed that representative HSP30s from different classes of non-mammalian vertebrates were distinct from other sHSPs including HSPB1/HSP27. Studies with amphibian and fish recombinant HSP30 determined that they were molecular chaperones since they inhibited heat- or chemically-induced aggregation of unfolded protein. During non-mammalian vertebrate development, *hsp30* genes were differentially expressed in selected tissues. Also, heat shock-induced stage-specific expression of *hsp30* genes in frog embryos was regulated at the level of chromatin structure. In adults and/or tissue culture cells, *hsp30* gene expression was induced by heat shock, arsenite, cadmium or proteasomal inhibitors, all of which enhanced the production of unfolded/damaged protein. Finally, immunocytochemical analysis of frog and chicken tissue culture cells revealed that proteotoxic stress-induced HSP30 accumulation co-localized with aggresome-like inclusion bodies. The congregation of damaged protein in aggresomes minimizes the toxic effect of aggregated protein dispersed throughout the cell. The current availability of probes to detect the presence of *hsp30* mRNA or encoded protein has resulted in the increased use of *hsp30* gene expression as a marker of proteotoxic stress in non-mammalian vertebrates.

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1. Introduction

The heat shock response is the stress-induced accumulation of heat shock proteins (HSPs) that aids in cell survival by protecting vital components against damage (Morimoto, 1998, 2008; Richter et al., 2010). Major HSP families include HSP90, HSP70, HSP60, HSP47 and the small HSPs (sHSPs). The mechanism of stress-inducible expression of *hsp* genes is similar in most eukaryotic systems (Voellmy, 2004; Morimoto, 1998, 2008). *Hsp* gene expression is regulated primarily at the transcriptional level and is mediated by heat shock factor 1 (HSF1), which interacts with the heat shock element (HSE), an enhancer found in stress-inducible *hsp* genes. Various stresses can activate the heat shock response including elevated temperature, heavy metals, proteasomal inhibitors and disease states by increasing the cellular level of unfolded or misfolded protein which leads to the activation of HSF1 (Voellmy, 2004; Morimoto, 2008; Gidalevitz et al., 2011). In unstressed cells HSP90 binds to, and thereby maintains, HSF1 in the monomeric, inactive state. In response to proteotoxic stressors, HSP90 is recruited away from HSF1 in order to prevent the aggregation of other unfolded proteins that accumulate in the cell. This permits HSF1 monomers to trimerize followed by their hyperphosphorylation, translocation to the nucleus, binding to HSEs and facilitation of *hsp* gene transcription (Voellmy, 2004; Anckar and Sistonen, 2011).

Initially, sHSPs were ignored but research during the past 20 years has clearly established that these molecular chaperones have important roles in cellular homeostasis. For example, sHSPs were reported to have roles in actin capping/decapping, cellular differentiation, prevention of apoptosis, and acquisition of thermotolerance (Arrigo and Landry, 1994; MacRae, 2000; van Montfort et al., 2001; Heikkila, 2004, 2010; Mymrikov et al., 2011; Acunzo et al., 2012; Garrido et al., 2012; Treweek et al., 2015). Furthermore, sHSP synthesis or their mutations are associated with diseases such as Alzheimer's, Charcot-Marie-Tooth, hereditary motor neuropathy type 2, Parkinson's disease, cancer, and desmin-related myopathy (Sun and MacRae, 2005; Garrido et al., 2012; Toth et al., 2013). Most sHSPs examined to date consist of divergent N- and C-terminal sequences, which flank a highly conserved β-sheet-rich α-crystallin domain of 80–100 amino acids (MacRae, 2000; van Montfort et al., 2001; Kampinga et al., 2009; Heikkila, 2010; Kappe et al., 2010; Mymrikov et al., 2011; Haslbeck and Vierling, 2015; Treweek et al., 2015). An extensive analysis of 8714 sHSPs determined that the evolutionarily conserved α-crystallin domain evolved independently of the N-terminal and C-terminal sequences (Kriehuber et al., 2010). Constitutively expressed sHSPs are present as an ensemble of large and small oligomers including dimers, which are the basic building units of sHSP oligomers (Haslbeck and Vierling, 2015; Treweek et al., 2015). In response to the presence of unfolded protein, the cellular sHSP ensemble shifts to a higher concentration of smaller complexes such as dimers, which bind and stabilize unfolded substrate followed by a possible reassociation of the sHSP/substrate complexes to higher oligomeric forms. The binding of unfolded protein substrate to sHSP oligomeric complexes maintains their solubility and inhibits their aggregation until they can be refolded by other ATP-dependent molecular chaperones or are targeted for degradation. In humans, a total of 11 distinct sHSPs have been reported including HSPB1 (HSP27), HSPB2, HSPB3, HSPB4 (αA-crystallin), HSPB5 (αB-crystallin), HSPB6 (HSP20), HSPB7 (cvHSP), HSPB8 (H11), HSPB9, HSPB10 (ODF1) and HSPB11 (Kappe et al., 2003, 2010; Kampinga et al., 2009). All of the aforementioned human sHSPs are evolutionarily related and have a conserved α-crystallin domain except for HSPB11 (Kappe et al., 2010). Also, most of these human sHSPs have homologs in other organisms.

In 1984, electrophoretic analysis of in vitro translation products of mRNA isolated from heat shocked *Xenopus laevis* cultured cells revealed the presence of a 30 kDa protein band that was subsequently referred to as HSP30 (Bienz, 1984a). After isolation of partial *hsp30* cDNAs, a cluster of two *hsp30* genes were cloned and named *hsp30A* and *B* (Bienz, 1984b). However, *hsp30A* had a 21 bp insertional mutation containing a stop codon that produced only a 10-kDa protein whereas *hsp30B*, a possible pseudogene, had a deleted nucleotide near the C-terminal end that resulted in a frameshift producing an additional 19 amino acids. A second *hsp30* gene cluster isolated by Krone et al. (1992) contained two complete and functional stress-inducible genes, *hsp30C* and *D*, which encoded proteins with a theoretical molecular mass of approximately 24 kDa. The discovery of *X. laevis hsp30C* and *D* genes laid the groundwork for the identification of *hsp30* gene orthologs in amphibians, fish, birds and reptiles.

The following review will examine the current knowledge regarding HSP30, including a review of HSP30 nomenclature, an examination of their gene structure and regulatory sequences, phylogeny, molecular chaperone properties, and their constitutive and stress-inducible expression in embryos, adults, and tissue culture cells. Finally, this review will examine the stress-induced localization of HSP30 in cells and their association with aggresome-like structures that function to minimize the toxic impact of aggregated protein. An understanding of HSP30 biology is of importance given the recent growth in the use of HSP30 or its message as a potential molecular biomarker of proteotoxic stress in non-mammalian vertebrates.

2. *Hsp30* genes

2.1. HSP30 nomenclature

Over the past 15 years there has been some confusion in the scientific literature with respect to naming HSP30-like sHSPs in non-mammalian vertebrates. While HSP30 was the term used to designate *X. laevis* sHSP, the designation of HSP30 was also used for an unrelated fungal plasma membrane sHSP (Bienz, 1984a; Plesofsky-Vig and Brambl, 1990; Tiwari et al., 2015). Franck et al. (2004) suggested that *X. laevis* HSP30C be given the formal name of HSPB11c employing human nomenclature in which *HSPB* referred specifically to the 10 human *shsp* genes known at that time and that the numeral following the *HSPB* was arbitrary, having nothing to do with the size of the protein. The use of HSPB11 to name HSP30 orthologs was also employed with zebrafish and several bird species (Table 1; Franck et al., 2004; Elicker and Hutson, 2007; Marvin et al., 2008; Kluver et al., 2011; Buttner et al., 2012; Shahid et al., 2016). Unfortunately, a human sHSP that was previously known as HSP16.2, Clorf41, IFT25 or HSPC034 was renamed as HSPB11 (Kampinga et al., 2009). It is important to note that human HSPB11 does not share any amino acid sequence identity with fish or avian HSPB11/HSP30 (Kampinga et al., 2009; Kappe et al., 2010). Furthermore the chicken ortholog of *X. laevis* HSP30, which was named HSP25 in a number of publications, should not be confused with rat or mouse HSP25, which is actually a homolog of HSPB1/HSP27 (Kawazoe et al., 1999; Katoh et al., 2004; Kampinga et al., 2009; Mymrikov et al., 2011). While it is beyond the scope of this review to suggest a formal name for the HSP30/HSPB11/HSP25 sHSPs in non-mammalian vertebrates, it is important for researchers to be aware of the alternate names of the homologs in other model or non-model