

Identification of *Xenopus* heat shock transcription factor–2: conserved role of sumoylation in regulating deoxyribonucleic acid–binding activity of heat shock transcription factor–2 proteins

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Abstract Heat shock transcription factor (Hsf)–1 and Hsf2 are members of the heat shock factor (HSF) protein family involved in heat shock protein (*hsp*) gene regulation, a regulation that is critical for the ability of cells to survive exposure to stress conditions. Although the role of Hsf1 in binding and activating transcription of *hsp* gene promoters in response to cell stress is well established, how Hsf2 enhances stress-induced *hsp* expression is not understood. To gain an insight into the critical conserved features of the regulation and function of Hsf2, we have identified and characterized the Hsf2 protein from *Xenopus laevis*. We found that, similar to its human counterpart, *Xenopus* Hsf2 is sumoylated at lysine 82 and that, as it does in human Hsf2, the modification event of the small ubiquitin–related modifier 1 functions to increase the deoxyribonucleic acid–binding activity of this transcription factor in *Xenopus*. These results indicate that sumoylation is an evolutionarily conserved modification of Hsf2 proteins, supporting the position of this modification as a critical regulator of Hsf2 function.

INTRODUCTION

Heat shock transcription factor (Hsf)–2 is a member of the heat shock factor (HSF) family of transcription factors (Sarge et al 1991; Schuetz et al 1991). Another member of this family, Hsf1, has been well characterized as the transcriptional inducer of heat shock protein (*hsp*) gene expression in response to cell stress (Morano and Thiele 1999; Pirkkala et al 2001; Christians et al 2002). Overexpression of Hsf2 enhances stress-induced *hsp* gene transcription (He et al 2003), and Hsf2 is found in nuclear stress bodies with Hsf1 (Alastalo et al 2003), but precisely

how Hsf2 is important for inducible *hsp* gene transcription is still a mystery. In light of the critical role of *hsp* gene expression in protecting cells from stress, it is essential to elucidate the mechanisms that control Hsf2 activity and the mechanism by which Hsf2 stimulates *hsp* gene expression.

We previously discovered that Hsf2 is covalently modified at lysine 82 by a protein called small ubiquitin–related modifier 1 (SUMO-1) and that this sumoylation functions to stimulate Hsf2 deoxyribonucleic acid (DNA) binding (Goodson et al 2001). SUMO-1 is a 97-amino protein with homology to ubiquitin whose conjugation to lysine residues of proteins appears to be involved in regulating functional properties of these proteins, including subcellular localization and protection against degradation (Hay 2001; Hochstrasser 2001; Muller et al 2001; Seeler and Dejean

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2001; Pichler and Melchior 2002). A comparison of protein sequences flanking the lysine residues where SUMO-1 groups are attached to different proteins led to the identification of the sumoylation consensus sequence ΨKXE, where Ψ is a residue bearing a hydrophobic side chain (Hay 2001; Hochstrasser 2001; Muller et al 2001; Seeler and Dejean 2001; Pichler and Melchior 2002). The functionality of this consensus sequence has been validated by mutational analyses (Rodriguez et al 2001; Sampson et al 2001).

Only 1 HSF has been identified from *Xenopus laevis* and corresponds to the heat-inducible Hsf1 (Stump et al 1995). This Hsf1 is present in both pretranscriptionally active and transcriptionally active oocytes and is heat activatable (Ovsenek and Heikkila 1990). The *Xenopus* Hsf1 is present in oocytes as a nuclear protein, which gains DNA-binding ability on heat shock, suggesting that localization to the nucleus is not a part of the multistep process of Hsf1 activation (Mercier et al 1997).

As an avenue for increasing our understanding of Hsf2, we sought to determine whether *Xenopus* expressed an Hsf2 homologue, which thus far had only been identified in humans, mouse, and chicken (Sarge et al 1991; Schuetz et al 1991; Nakai and Morimoto 1993). Our reasoning was that identifying an Hsf2 homologue in a species widely divergent from humans would provide a wealth of important information including, (1) extending the boundary of when the HSF gene family split occurred during evolution, (2) giving us a system with which to determine how early Hsf2 sumoylation arose during evolution and whether its regulation and function are the same or different between widely divergent species, which (3) because *Xenopus* is a well-characterized model organism, could then be used for developmental and other studies to shed new light on Hsf2 sumoylation and the role of Hsf2 in the regulation of *hsp* gene expression.

We found that *Xenopus* does indeed have an Hsf2 homologue. It undergoes sumoylation at the same lysine position as does human Hsf2, and this modification stimulates the DNA-binding activity of *Xenopus* Hsf2 as it does in its human counterpart. These results demonstrate that the HSF gene family arose earlier than previously thought and suggest that Hsf2 sumoylation arose before the divergence of these evolutionary lines, indicating the importance of this modification as an integral mechanism of Hsf2 regulation.

MATERIALS AND METHODS

Identification of *X laevis* Hsf2

Candidate full-length *X laevis* Hsf2 expressed sequence tag (EST) clones were identified through BLAST search of the National Center for Biotechnology Information database using the human Hsf2 sequence. Two putative *X*

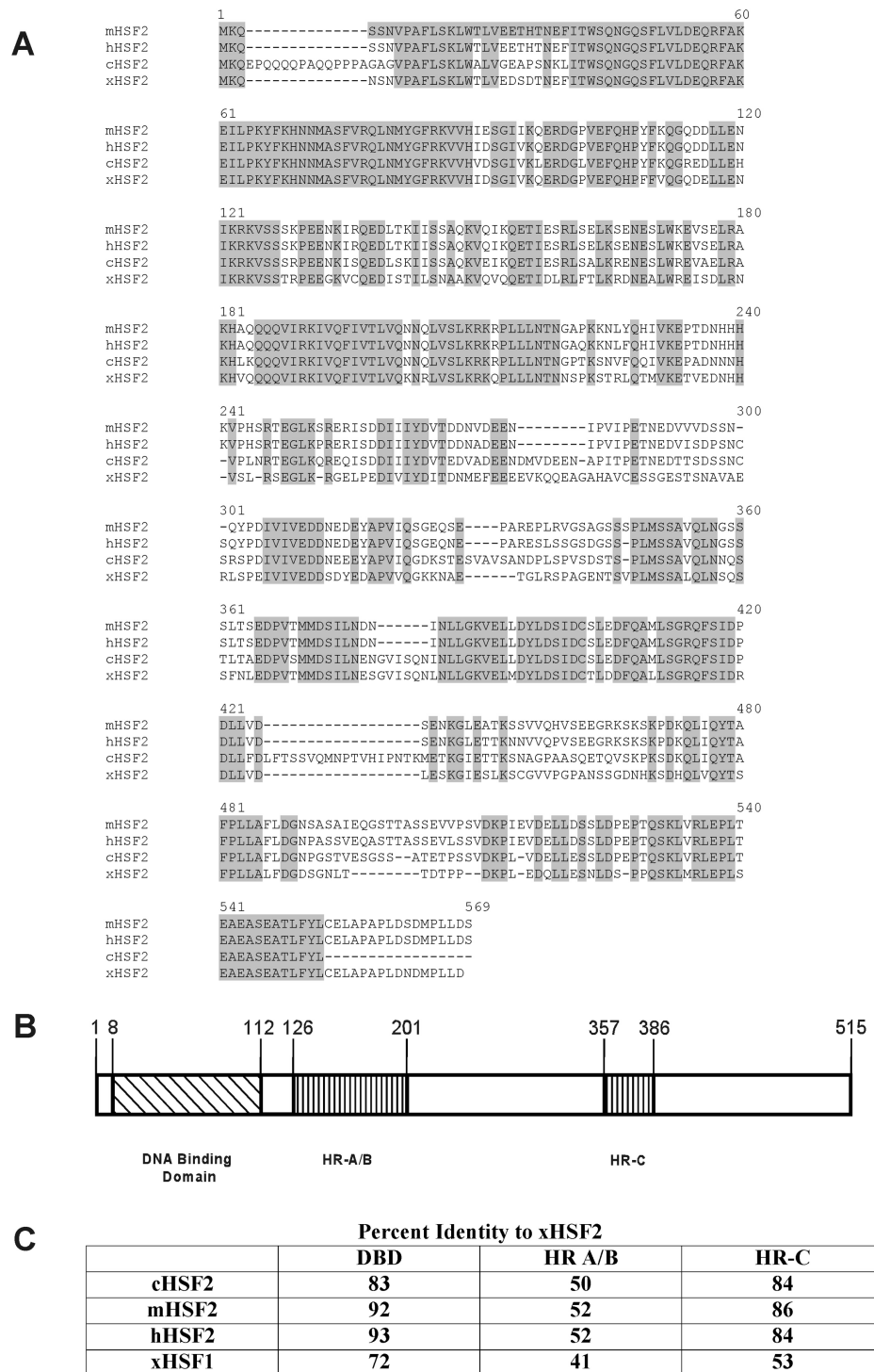
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R D N E A L W R E I S D L R N K H V Q Q 171
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Q Q V I R K I V Q F I V T L V Q K N R L 191
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Fig 1. Nucleotide and amino acid sequence of *Xenopus* heat shock transcription factor-2. The putative polyadenylation signal is indicated by an underline.

laevis Hsf2 EST clones XL032d11 and XL059j06 (GenBank accession numbers BJ042603 and BJ058495) were obtained from Dr Naoto Ueno at the National Institute for Basic Biology (Okazaki, Japan). Subsequent sequencing of the EST clones revealed that the EST clone XL059j06 represented a partially spliced transcript by the identification of an intron still present in the EST. All experiments in this study were performed using the EST XL032d11, which represented a mature transcript based on the absence of any introns in the sequence.

Fig 2. Sequence comparisons of heat shock transcription factor-2 (Hsf2) proteins of different species. (A) Homology among *Xenopus*, mouse, chicken, and human Hsf2 proteins. Positions identical in all 3 proteins are shaded. (B) Three conserved functional domains of Hsf2 proteins. Indicated are the locations of the deoxyribonucleic acid (DNA)-binding domain, trimerization domain (HR-A, HR-B), and a C-terminal region that contains another leucine zipper motif (HR-C). (C) Percent identity comparison of DNA binding, trimerization (HR-A, HR-B), and C-terminal leucine zipper domains (HR-C) among Hsf2 proteins of different species as well as *Xenopus* heat shock transcription factor-1.



Mutagenesis of Hsf2

To determine whether lysine 82 in the *X laevis* Hsf2 was the site of sumoylation, an Hsf2 mutant was generated using the QuickChange mutagenesis kit (Stratagene, La Jolla, CA, USA) according to the manufacturer's protocol. Mutation of lysine 82 to arginine was confirmed by DNA sequencing.

In vitro SUMO-1 modification assay

X laevis wild-type and mutant proteins were in vitro translated using the Promega TNT T7 Quick for polymerase chain reaction DNA rabbit reticulocyte and then subjected to in vitro SUMO-1 modification assay, essentially as described previously (Duprez et al 1999). Mod-

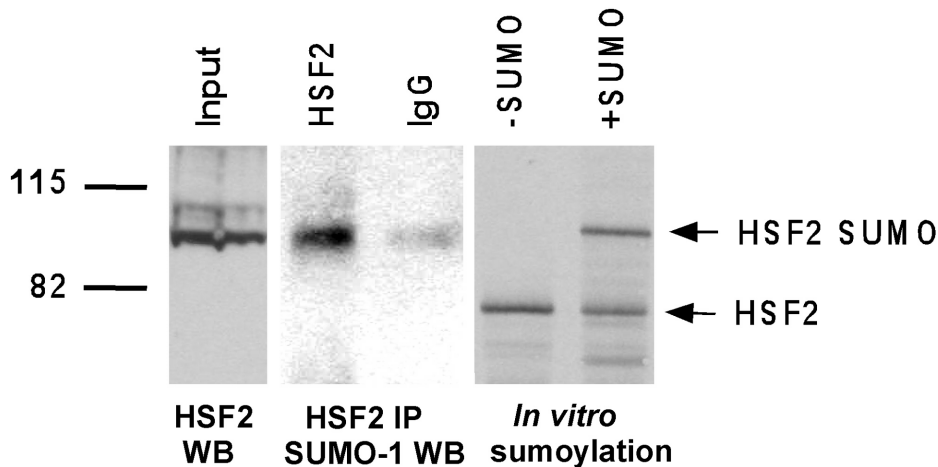


Fig 3. *Xenopus* heat shock transcription factor-2 (Hsf2) is sumoylated. Extracts of *Xenopus* tadpoles were subjected to immunoprecipitation using anti-Hsf2 antibodies or nonspecific immunoglobulin G (as a negative control) and then subjected to Western blot analysis using anti-SUMO-1 antibodies. To show that the mouse Hsf2 antibody detected *Xenopus* Hsf2, the input was subjected to Western blot using anti-mouse Hsf2. Size comparisons of the endogenous *Xenopus* Hsf2 were made to the in vitro-translated, sumoylated, and nonsumoylated forms of *X.l.* Hsf2. Direct size comparisons were made because all samples were run on the same SDS-PAGE gel and transferred to nitrocellulose. SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SUMO, ubiquitin-related modifier 1.

ification reactions were terminated using sodium dodecyl sulfate (SDS)-sample buffer containing β -mercaptoethanol and fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (10%) followed by gel fixation in 20% isopropanol: 10% acetic acid for 15 minutes. After fixation, gels were soaked in Amplify (Pharmacia, Piscataway, NJ, USA) according to the manufacturer's instructions and then dried and subjected to autoradiography.

Immunoprecipitation analysis

For immunoprecipitation, frozen *X laevis* tadpoles were lysed in 0.15 M Tris-HCl (pH 6.7), 5% SDS, and 30% glycerol in a tissue homogenizer. The lysate was then diluted 1:10 in phosphate-buffered saline containing 0.5% Nonidet P-40 containing complete protease inhibitor (Roche Molecular Biochemicals, Indianapolis, IN, USA). Subsequent immunoprecipitation analysis was performed using mouse Hsf2 polyclonal antibody (Sarge et al 1993), as described previously (Hong et al 2001). Immunoprecipitated proteins were then analyzed by SDS-PAGE and Western blot using anti-SUMO-1 monoclonal antibodies (21C7) (Matunis et al 1996).

Electrophoresis mobility shift assay

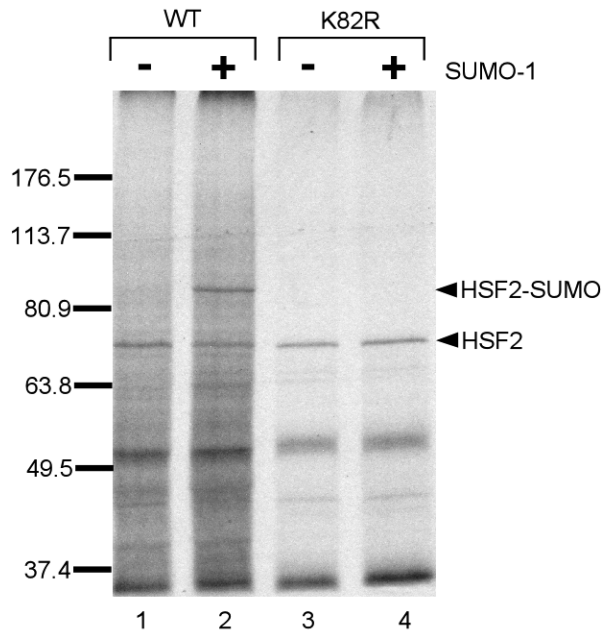
X laevis Hsf2 wild type and Hsf2 K82R were in vitro translated and sumoylated as described above. The translated proteins were then subjected to electrophoresis mobility shift assay. The binding reaction contained 20 μ L of binding buffer (10 mM Tris-HCl, pH 7.4, 50 mM NaCl, 1 mM ethylenediamine-tetraacetic acid [EDTA], 0.5 mM dithiothreitol, and 5% glycerol), 0.1 ng of 32 P-end labeled-DNA probe, 0.5 μ g of poly(dI-dC)poly(dI-dC), and 10 μ g of bovine serum albumin and was incubated at 20°C for

10 minutes. Binding reactions were then subjected to electrophoresis on native 4% polyacrylamide gels in 0.5 \times Tris-borate-EDTA, and Hsf2 DNA-binding complexes were observed by autoradiography. The oligonucleotide probe contained 4 inverted repeats of the heat shock element consensus sequence 5'-nGAAAn-3'.

RESULTS

A BLAST search using the human Hsf2 protein sequence as a query revealed a match with an EST clone (GenBank accession number BJ042603) whose sequence is different from that of the known *Xenopus* Hsf1 protein. We obtained this clone and sequenced the regions not already present in the database to yield the nucleotide-protein sequence shown in Figure 1. The predicted open-reading frame predicts a polypeptide of 515 amino acids. An alignment of this *Xenopus* sequence with Hsf2 sequences from humans and mouse is shown in Figure 2A. Figure 2B shows the 3 major regions of homology in HSF proteins, which are the DNA-binding domain, trimerization domain containing the leucine zippers that interact between monomers to form trimers (HR-A, HR-B), and a more C-terminal region that also contains a leucine zipper motif (HR-C). A comparison of the percent identities in these 3 regions among *Xenopus*, mouse, and human Hsf2 proteins, as well as the *Xenopus* Hsf1 protein, is shown in Figure 2C. This analysis supports the contention that it is a member of the Hsf2 subfamily because it is much more distantly related to Hsf1.

Our previous studies showed that human Hsf2 is covalently modified by the SUMO-1 protein at lysine 82. To test whether Hsf2 sumoylation is conserved in *Xenopus*, we immunoprecipitated Hsf2 from extracts of *Xenopus* tadpoles and then analyzed the immunoprecipitates by Western blot using anti-SUMO-1 antibodies. The results



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	ΨKXE
xHSF2	GIVKQERDG
mHSF2	GIIKQERDG
hHSF2	GIVKQERDG
RanGAP1	GLLKSEDKV
PML	KVIKMESEE
SP100	VDIKKPKPF
IκB	DGLKKE RLL
p53	LMFKTEGPD

Fig 4. *Xenopus* heat shock transcription factor-2 (Hsf2) is sumoylated at lysine 82. (A) In vitro-translated, ³⁵S-labeled wild-type, and K82R *Xenopus* Hsf2 were incubated in a reaction containing purified SUMO-1 and SUMO-1 E1 and E2 enzymes and an ATP-regenerating system. After the reaction, the protein samples were subjected to SDS-PAGE followed by autoradiography to observe nonsumoylated and sumoylated Hsf2 proteins. The positions of nonsumoylated and sumoylated Hsf2 are indicated by arrowheads. (B) Alignment showing conservation of sumoylation sites in Hsf2 proteins of different species and other known sumoylated proteins. Conserved residues of the SUMO-1 modification consensus sequence (ΨKXE) are indicated as outlined letters. ATP, adenosine triphosphate; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SUMO, ubiquitin-related modifier 1.

show a band of the expected size of sumoylated *Xenopus* Hsf2 (90 kDa), suggesting that Hsf2 in this species is indeed modified by SUMO-1 (Fig 3, middle panel). To strengthen the conclusion that this band indeed repre-

sents sumoylated *Xenopus* Hsf2, the products of an in vitro sumoylation reaction using in vitro-translated *Xenopus* Hsf2 protein were separated on the same SDS-PAGE gel, and the results (Fig 3, right panel) show that the size of the in vitro-sumoylated *Xenopus* Hsf2 does indeed match that of the immunoprecipitated band. To compare the size of these Hsf2-sumo products with that of the endogenous *Xenopus* Hsf2 protein, on this same SDS-PAGE gel we also separated a sample of the lysate used for the immunoprecipitation, which was then subjected to Western blot using Hsf2 antibodies. The results show that the predominant band of the endogenous *Xenopus* Hsf2 (Fig 3, left panel) comigrates with that of the sumoylated Hsf2 proteins in the middle and right panels, suggesting that a significant portion of the endogenous *Xenopus* protein is sumoylated.

Xenopus Hsf2 has a match to the SUMO modification consensus sequence at lysine 82 within its DNA-binding domain. The consensus SUMO-1 modification site comprises the sequence ΨKXE, where the lysine is the site of covalent attachment and Ψ indicates the requirement for a hydrophobic amino acid at this position (Rodriguez et al 2001; Sampson et al 2001). To test whether SUMO modification is occurring at this lysine, we performed in vitro sumoylation reactions on wild-type *Xenopus* Hsf2 and *Xenopus* Hsf2 in which lysine 82 was changed to a non-sumoylatable arginine. The results, shown in Figure 4, indicate that this mutation completely blocks sumoylation of the Hsf2 protein, indicating that this is indeed the site of sumoylation. Human Hsf2 is also sumoylated at lysine 82, indicating that the site of sumoylation has been strictly conserved between human and *Xenopus* Hsf2 proteins, strongly supporting the importance of sumoylation for Hsf2 function (Goodson et al 2001). A comparison of the *Xenopus* Hsf2 site with the sumoylation sites of other proteins is shown in Figure 4B (Sarge et al 1991; Schuetz et al 1991; Hay 2001; Hochstrasser 2001; Muller et al 2001; Seeler and Dejean 2001).

We demonstrated previously that sumoylation stimulates the DNA-binding activity of human Hsf2 (Goodson et al 2001). To assess whether SUMO-1 modification has the same or a different regulatory role with respect to *Xenopus* Hsf2, we compared the DNA-binding activity of nonsumoylated *Xenopus* Hsf2 with that of Hsf2 we sumoylated using the reconstituted in vitro SUMO-1 modification reaction. The results reveal that SUMO-1 modification results in a significant increase in the DNA-binding ability of *Xenopus* Hsf2 (Fig 5). This increase in DNA binding was not observed when the K82R mutant was tested, indicating that sumoylation is required for this functional effect.

DISCUSSION

In this article we have identified and characterized the Hsf2 protein homologue in the African clawed toad *X*

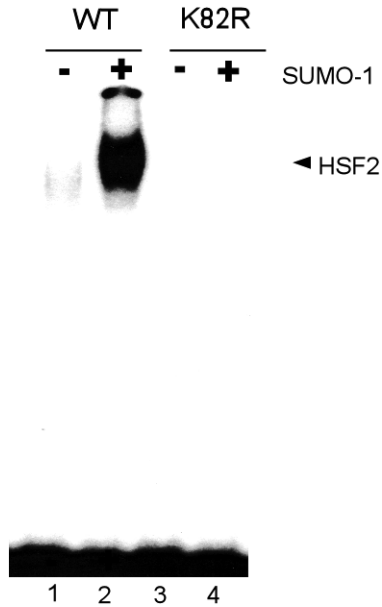


Fig 5. Sumoylation stimulates the deoxyribonucleic acid-binding activity of *Xenopus* heat shock transcription factor-2 (Hsf2). Non-sumoylated and sumoylated (using the *in vitro* sumoylation reaction) wild-type and nonsumoylatable K82R mutant *Xenopus* Hsf2 was subjected to gel shift assay using an heat shock element-containing probe. Input Hsf2 samples were aliquots of the Hsf2 samples shown in Figure 4.

laevis. The close identity of this sequence with Hsf2 sequences of other species clearly places this *Xenopus* sequence in the Hsf2 subfamily of the HSF transcription factors. In addition, analysis of *Xenopus* ESTs suggests the existence of at least 2 other HSF family members in this species that are distinct from Hsf1 and Hsf2 and await characterization.

We have determined that, similar to its human counterpart, the Hsf2 protein in *Xenopus* is covalently linked with the SUMO-1 protein and that this occurs at the identical lysine residue in the Hsf2 proteins of both species, which is lysine 82. Our results also indicate that for both human and *Xenopus* Hsf2, sumoylation stimulates the DNA-binding activity of these transcription factors. The conservation of Hsf2 sumoylation at lysine 82 between such widely divergent species, and the conserved role of this modification in regulating Hsf2 DNA binding, underscores the importance of this modification event for Hsf2 function. Being able to compare Hsf2 proteins from these 2 species will be very useful for studies probing the mechanism(s) by which SUMO-1 modification regulates the DNA-binding and perhaps other activities of Hsf2 proteins. It also opens the door to comparative studies testing whether Hsf2 sumoylation may be developmentally regulated in mammals or amphibians (or both).

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REFERENCES

- Alastalo TP, Hellesuo M, Sandqvist A, Hietakangas V, Kallio M, Sistonen L. 2003. Formation of nuclear stress granules involves HSF2 and coincides with the nucleolar localization of Hsp70. *J Cell Sci* 116: 3557–3570.
- Christians ES, Yan LJ, Benjamin IJ. 2002. Heat shock factor 1 and heat shock proteins: critical partners in protection against acute cell injury. *Crit Care Med* 30: S43–S50.
- Duprez E, Saurin AJ, Desterro JM, et al. 1999. SUMO-1 modification of the acute promyelocytic leukaemia protein PML: implications for nuclear localisation. *J Cell Sci* 112: 381–393.
- Goodson ML, Hong Y, Rogers R, Matunis MJ, Park-Sarge OK, Sarge KD. 2001. Sumo-1 modification regulates the DNA binding activity of heat shock transcription factor 2, a promyelocytic leukemia nuclear body associated transcription factor. *J Biol Chem* 276: 18513–18518.
- Hay RT. 2001. Protein modification by SUMO. *Trends Biochem Sci* 26: 332–333.
- He H, Soncin F, Grammatikakis N, et al. 2003. Elevated expression of heat shock factor (HSF) 2A stimulates HSF1-induced transcription during stress. *Biol Chem* 278: 35465–35475.
- Hochstrasser M. 2001. SP-RING for SUMO: new functions bloom for a ubiquitin-like protein. *Cell* 107: 5–8.
- Hong Y, Rogers R, Matunis MJ, Mayhew CN, Goodson ML, Park-Sarge OK, Sarge KD, Goodson M. 2001. Regulation of heat shock transcription factor 1 by stress-induced SUMO-1 modification. *J Biol Chem* 276: 40263–40267.
- Matunis M, Coutavas E, Blobel G. 1996. A novel ubiquitin-like modification modulates the partitioning of the Ran-GTPase-activating protein RanGAP1 between the cytosol and the nuclear pore complex. *J Biol Chem* 271: 1457–1470.
- Mercier PA, Foksa J, Ovsenek N, Westwood JT. 1997. *Xenopus* heat shock factor 1 is a nuclear protein before heat stress. *J Biol Chem* 272: 14147–14151.
- Morano KA, Thiele DJ. 1999. Heat shock factor function and regulation in response to cellular stress, growth, and differentiation signals. *Gene Exp* 7: 271–282.
- Muller S, Hoegel C, Pyrowolakis G, Jentsch S. 2001. SUMO, ubiquitin's mysterious cousin. *Nat Rev Mol Cell Biol* 2: 202–210.
- Nakai A, Morimoto RI. 1993. Characterization of a novel chicken heat shock transcription factor, heat shock factor 3, suggests a new regulatory pathway. *Mol Cell Biol* 13: 1983–1997.
- Ovsenek N, Heikkila JJ. 1990. DNA sequence-specific binding activity of the heat-shock transcription factor is heat-inducible before the midblastula transition of early *Xenopus* development. *Development* 110: 427–433.
- Pichler A, Melchior F. 2002. Ubiquitin-related modifier SUMO1 and nucleocytoplasmic transport. *Traffic* 3: 381–387.
- Pirkkala L, Nykanen P, Sistonen L. 2001. Roles of the heat shock transcription factors in regulation of the heat shock response and beyond. *EASEB J* 15: 1118–1131.
- Rodriguez MS, Dargemont C, Hay RT. 2001. SUMO-1 conjugation

- in vivo requires both a consensus modification motif and nuclear targeting. *J Biol Chem* 276: 12654–12659.
- Sampson DA, Wang M, Matunis MJ. 2001. The small ubiquitin-like modifier-1 (SUMO-1) consensus sequence mediates Ubc9 binding and is essential for SUMO-1 modification. *J Biol Chem* 276: 21664–21669.
- Sarge KD, Murphy SP, Morimoto RI. 1993. Activation of heat shock gene transcription by heat shock factor 1 involves oligomerization, acquisition of DNA-binding activity, and nuclear localization and can occur in the absence of stress. *Mol Cell Biol* 13: 1392–1407.
- Sarge KD, Zimarino V, Holm K, Wu C, Morimoto RI. 1991. Cloning and characterization of two mouse heat shock factors with distinct inducible and constitutive DNA-binding ability. *Genes Dev* 5: 1902–1911.
- Schuetz TJ, Gallo GJ, Sheldon L, Tempst P, Kingston RE. 1991. Isolation of a cDNA for HSF2: evidence for two heat shock factor genes in humans. *Proc Natl Acad Sci U S A* 88: 6911–6915.
- Seeler JS, Dejean A. 2001. SUMO: of branched proteins and nuclear bodies. *Oncogene* 20: 7243–7249.
- Stump DG, Landsberger N, Wolffe AP. 1995. The cDNA encoding *Xenopus laevis* heat-shock factor 1 (XHSF1): nucleotide and deduced amino-acid sequences, and properties of the encoded protein. *Gene* 160: 207–211.