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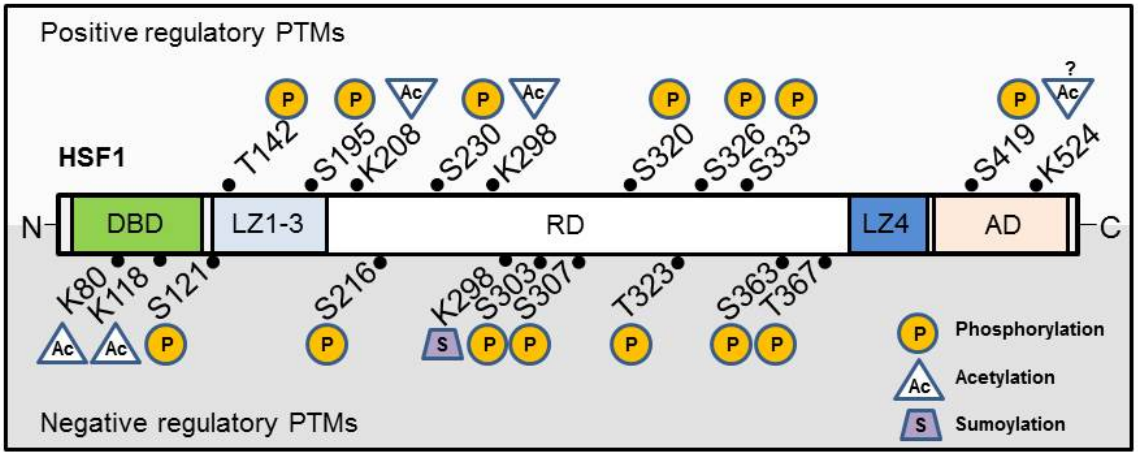
Supplementary information S3: Post-translational modifications of HSFs



Both HSF1 and HSF2 undergo a number of Post-translational modifications (PTM). Those that influence the function or stability of HSF1 have been most thoroughly investigated. Here we will summarize these PTMs in terms of their type, location and whether they have been experimentally determined to have a positive or negative impact on HSF1 activity (see Figure).

Phosphorylation

HSF1, but not HSF2 activity and stability is regulated, in part, by Ser (S)/Thr (T) phosphorylation [1,2] (see Figure and Table 1). Because of the changes observed in the phosphorylation state of HSF1 in response to proteotoxic conditions, this PTM has been widely studied [1,3]. At 37˚C, basal phosphorylation at S121, S303, S307 and S363 repress HSF1 transcriptional activity [4,5]. More recent studies have connected some of these

phosphorylation events to changes in HSF1 activity and stability in disease. For example phosphorylation of S121, mediated by AMPK, has an important role in HSF1 repression during metabolic stress[6].

Two recent reports link HSF1 phosphorylation at S303 and S307 to its opposing activity and stability in cancer and in Huntington's disease. HSF1 phosphorylated at S303 and/or 307 shows increased interaction with the E3 ligase F box protein FBXW7, which targets HSF1 for ubiquitin-dependent proteasomal degradation [7,8]. In Huntington's disease these phosphorylations are mediated by casein kinase II alpha prime (CK2α´) (**Figure 4b, 5b**). Both CK2α´ and FBXW7 are present at high levels in Huntington's disease, whereas decreased levels of FBXW7 in melanoma cells results in increased HSF1 abundance (**Figure 5b**) [7]. HSF1 is also activated in cancer cells by its association with IER5 (Immediate Early Response gene 5), which forms a complex with protein phosphatase PP2A, which leads to decreased phosphorylation of S121, S307, S314, T323 and T367, thereby generating a hypo-phosphorylated, active form of HSF1 [9].

Among the positive regulatory phosphorylation events on HSF1, S230, S320, and S326 are strongly associated with HSF1 activation under stress conditions and hyper-phosphorylation of these residues is commonly used as a surrogate for HSF1 activation [10-13,14]. Increased phosphorylation of S326 mediated by MEK1 and other kinases is associated with HSF1 activation in cancer cells and tissues [15,16]. However, an important study using an HSF1 mutant (HSF1ΔPRD), in which 15 known phosphorylation sites within the Regulatory Domain (RD) were mutated to preclude phosphorylation, have revealed no alteration in the subcellular localization, DNA-binding or trans-activation

potential of HSF1 in cell culture [17]. This suggests that the HSF1 hyper-phosphorylation signature alone is not an absolute marker for assessing HSF1 activity under all conditions, and further studies will be required to understand the role of these phosphorylation events.

SUMOylation

SUMOylation is an emerging protein modification with diverse roles in biology. HSF1 is SUMOylated at Lys298 (K298) by Ubc9 (see Figure and Table 1) in a mechanism that is dependent on prior phosphorylation at nearby S303 (this is referred to as phosphorylation dependent SUMOylation motif or PDSM) [10,18,19]. The negative regulatory role for HSF1 SUMOylation is supported by the observation that HSF1 de-SUMOylation occurs progressively with increasing temperatures, and that a HSF1 mutant (where K298 is exchanged to arginine), which cannot be SUMOylated, hyper-activates HSF1 target gene expression [20]. Since K298 SUMOylation is not known to affect HSF1 stability or subcellular localization, this modification may modulate HSF1 activity, perhaps by influencing interactions with regulatory factors [21]. Similar to HSF1, the HSF4β isoform possesses a PDSM motif where K293 SUMOylation is promoted by S298 phosphorylation. This SUMOylation appears to repress HSF4β activity [22].

HSF2 sumoylation increases during mitosis [22]. Although this does not affect HSF2 oligomerization, HSF2-K82 sumoylation within the wing domain (corresponding to K91 in

HSF1) inhibits HSF2 DNA binding activity [1]. Recently, the repressive role of HSF2-K82 sumoylation has been confirmed using an HSF1 DNA Binding Domain (DBD) chimera (HSF1W2) containing the HSF2 wing domain. While the HSF1 DBD is not SUMOylated, the HSF1W2 protein is SUMOylated *in vitro* and this chimeric protein has reduced HSF1 DBD-driven DNA binding and target activation *in vivo* [23]. The differential SUMOylation profile of HSF1 and HSF2 DBDs observed in vivo [23] may drive specific protein–protein interactions within the wing domain, and elsewhere, that impact target-gene binding and other regulatory steps *in vivo*. The SUMOylation sites for the HSF1 and HSF2 isoforms have been validated by proteome-wide analyses, which revealed additional SUMOylation sites with unknown biological consequences [24].

Ubiquitylation

Although HSF1 is subject to ubiquitylation (Ub)-dependent degradation under different physiological or pathophysiological conditions [25,26,27] no specific ubiquitylated residues have been identified to date that drive this degradation. HSF2 is also highly ubiquitylated during hyperthermia [28]. Treatment of cells with MG132 or the cancer chemotherapeutic bortezomib, both of which inhibit the proteasome, stabilize HSF2 [29]. A high-throughput ubiquitin-modified proteomics analysis revealed that HSF2-Ub occurs on K51, K151, K210 and K420 [30,31], though a role for these ubiquitylation events in HSF2 stability has not been reported.

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Acetylation

HSF1 is acetylated (Ac) at several lysine residues under both basal and stress conditions [32,26] (see Figure and Table 1). Acetylation of HSF1 at K80 results in the occlusion of direct phosphate backbone contacts that are critical for HSF1 DNA binding. This acetylation is mediated by the acetyltransferase p300 and is reversed by SIRT1 [1,32]. HSF1 is also acetylated at additional sites including K118, K208 and K298, which is driven by p300/CBP- (see Figure) [26]. Similar to HSF1 K80, acetylation of K118 regulates HSF1 DNA binding, while K208 and K298 acetylations inhibit HSF1 proteasomal degradation [26]. Acetylation of K298 would preclude SUMOylation at the primary amine of this residue, suggesting that dynamic changes in acetylation and SUMOylation could function together in the regulation of HSF1 stability (see Figure). Although HSF1 acetylation has not been studied in neurodegenerative disease models, the activity of the histone acetyltransferase p300/CBP is reduced in Huntington's disease cell models, which could lead to decreased HSF1 acetylation and stability, potentially contributing to the reduction in HSF1 protein levels observed in Huntington's disease models and postmortem tissues from patients [33,34,8].

Several other HSF1 PTMs have been described (http://www.phosphosite.org/proteinAction?id=1196&showAllSites=true) although their importance in modulating HSF1 activity or abundance is currently unknown.

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