

P001 Noncovalent SUMO binding specifies E2-mediated sumoylation of HSF2

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In contrast to many modes of ubiquitin conjugation, the covalent attachment of SUMO to substrate proteins is directly catalyzed by a single E2 enzyme. Nevertheless, sumoylation is known to be highly specific in terms of target lysines, but mechanisms of substrate and target lysine selection are poorly understood. We have previously shown that the structural context of the SUMO acceptor site is important for sumoylation of heat shock factor 2 (HSF2) in the DNA-binding domain. Here, we reveal that also noncovalent SUMO binding acts as an additional specificity determinant for HSF2 sumoylation. The regulatory domain of HSF2 contains a hydrophobic SUMO-binding motif (SBM) that interacts with SUMO proteins in a noncovalent manner. Surprisingly, disruption of the HSF2 SBM abolishes E2-mediated sumoylation of HSF2. However, sumoylation of HSF2 *in vitro* is not SBM-directed in the presence of PIAS proteins, indicating that modification by E3 ligases can bypass this mode of regulation. Mutation of the HSF2 SBM significantly reduces HSF2 sumoylation also in cells, suggesting that a significant portion of HSF2 SUMO conjugation processes may be achieved in an E3-independent manner.