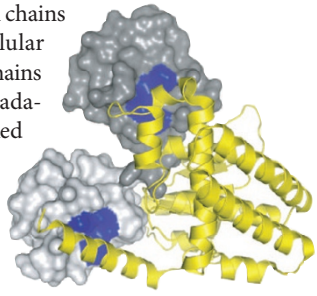
Conformational selection



It has long been known that ubiquitin chains modify proteins for distinct cellular function; for instance, Lys48-linked chains target proteins for proteasomal degradation, whereas Lys63- and Met1-linked chains function in cell signaling. These chain types are recognized by ubiquitin-interacting proteins, but how recognition is achieved is not well understood. Ye *et al.* analyzed different conformations of dual-labeled Lys48-, Lys63- and Met1-linked diubiquitin molecules by single-molecule fluorescence resonance energy transfer (smFRET) or by two-color coincidence detection. Each diubiquitin chain type existed in multiple conformational states in solution. Lys63- and Met1-linked diubiquitin adopted extended 'open' as well as more compact conformations, whereas Lys48-linked diubiquitin adopted predominantly compact conformations. Ubiquitin-interacting proteins, including deubiquitinases (DUBs), were found to bind preexisting conformations of Lys63- and Met1-linked chains, whereas DUBs recognized existing semiopen and open conformations but not the compact conformation. Access to the ubiquitin hydrophobic patches (to which all known DUBs bind) was obstructed in the prevalent compact structure of Lys48-linked diubiquitin, which suggests that DUBs may remodel ubiquitin chains to hydrolyze the isopeptide bond. In fact, mutating the Lys48-diubiquitin interface changed the conformational dynamics and affected DUB activity, which suggests that conformational selection takes place and is functionally important. The authors propose that distinct conformations in different ubiquitin chain types may contribute to the specificity of ubiquitin-interacting proteins and that conformational equilibria in ubiquitin chains provide an additional layer of linkage-dependent regulation within the ubiquitin system. Their research shows that the protein tertiary conformation, as determined by the ubiquitin domains, has a direct impact on the binding and activity of interacting proteins. (*Nature* **492**, 266–270, 2012) *AH*

Hybrid signals for BRCA1

To counteract the threat of genomic instability introduced by DNA double-strand breaks (DSBs), cells have evolved DNA-damage response pathways that rapidly deploy repair proteins when and where they are needed. Mobilization is mediated by post-translational modifications. In mammals, early events in the DNA-damage response include recruitment of the E3 ubiquitin ligases RNF8 and RNF168 to break sites, where they add K63-linked ubiquitin chains to the surrounding chromatin. Ubiquitination creates a binding target for RAP80, a protein that contains tandem ubiquitin-interacting motifs (UIMs); in turn, RAP80 recruits BRCA1 to the DNA break site. Now, Guzzo *et al.* show that RAP80 also contains a SUMO-interacting motif (SIM) and that BRCA1 is recruited through RAP80 interactions with hybrid SUMO-ubiquitin chains. The authors identified a consensus SIM in RAP80 that confers binding to SUMO-2 polymers *in vitro* and that is required in conjunction with the UIMs to recruit RAP80 to DSBs induced by ionizing radiation in U2OS cells. Because the RAP80 SIM is proximal to the UIMs, a dual SUMO-ubiquitin recognition mechanism was queried by assaying binding to chains of diubiquitin conjugated to SUMO-2. Indeed, RAP80-binding affinity for the hybrid chain was 80-fold greater than that observed for either SUMO or ubiquitin alone. Ubiquitin and SUMO signals are integrated *in vivo* by RNF4, a SUMO-targeted E3 ligase that generates hybrid chains. Endogenous RNF4 localizes to DNA-repair foci upon ionizing-radiation treatment, and recruitment of both RAP80 and BRCA1 is reduced when RNF4 is depleted by siRNA. Transfection of an RNF4 wild-type construct, but not a RING-domain mutant, restores RAP80 and BRCA1 recruitment to DSBs in depleted cells, which indicates that RNF4 ubiquitination activity is required to generate RAP80 binding signals. These results establish roles for both ubiquitin and SUMO in regulating BRCA1 localization to DNA breaks and provide a foundation to investigate the potential roles of SUMO-ubiquitin hybrid chains in signaling cell stress. (*Sci. Signal.* **5**, ra88, 2012) *BM*

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Protective degradation

A number of neurodegenerative disorders are associated with the formation and accumulation of protein aggregates in neurons. The underlying causes for aggregation are varied and include the expansion of CAG repeats within coding sequences that give rise to aberrant polypeptides containing polyglutamine (polyQ) tracts. In general, the pathological phenotype of polyQ disorders results from the disruption of several cellular processes, hence preventing the accumulation of misfolded proteins is likely to be the most effective therapeutic intervention. In the case of spinobulbar muscular atrophy (SBMA), an expanded polyQ tract encoded within the first exon of the androgen receptor (AR) gene results in hormone-dependent unfolding and formation of toxic AR oligomers. In a recent report, Wang *et al.* target the pathway through which polyQ-AR is normally degraded, with the intent of stimulating the process. It was previously shown that overexpression of the molecular chaperone Hsp70 promotes the clearance of polyQ-AR, although how exactly Hsp70 operates in this pathway remained poorly defined. In their study, the authors show that overexpression of Hip—a cochaperone that stabilizes Hsp70 in its ADP-dependent conformation and consequently increases substrate binding—promotes the clearance of polyQ-AR aggregates in a ubiquitin-dependent manner. Next, the ability of the small synthetic molecule YM-1, previously shown to bind the nucleotide-binding domain of Hsp70 in its ADP-bound (but not ATP-bound) state, was assayed for its ability to stimulate Hsp70 binding to unfolded substrates. The results demonstrate that YM-1, similarly to Hip, indeed favors Hsp70 in the ADP-bound, high–substrate-affinity state. Consistent with these observations, YM-1 diminished the cellular accumulation of polyQ-AR aggregates, and the induced clearance was inhibited by the proteasome inhibitor MG132, thus confirming the mechanism as proteasome dependent. Finally, the authors turned to a *Drosophila melanogaster* SMBA model to demonstrate the protective effect of YM-1 against polyQ-AR–induced toxicity in a physiologically relevant context. Although the potentially deleterious effects of YM-1 and similar molecules on other cellular processes remain to be assessed, this work suggests that allosteric activators of Hsp70 represent viable candidates for the treatment of several diseases with protein aggregation as their root cause. (*Nat. Chem. Biol.* doi:10.1038/nchembio.1140, published online 9 December 2012) *SL*