Ubc9 acetylation: a new route for achieving specificity in substrate SUMOylation

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In contrast to the dozens of proteins involved in ubiquitination pathways, relatively few proteins control SUMO modification. Generating specificity and diversity in pathway outcomes may therefore rely on post-translational modification of pathway components and/or substrates. A new paper in *The EMBO Journal* identifies one such modification event, the acetylation of Ubc9, as a key regulatory step in controlling substrate selection.

The SUMO modification pathway plays an important role in controlling the activity of a large number of cellular proteins, and in particular has been associated with chromatin structure, transcription and DNA repair processes (reviewed in Geiss-Friedlander and Melchior, 2007). The pathway that leads to SUMO conjugation resembles the ubiquitination pathways. Specificity in substrate modification by ubiquitin is largely driven by utilising distinct combinations of E2 conjugating enzymes and E3 ligases to recognise distinct substrates (reviewed in Pickart and Eddins, 2004). However, while the SUMO pathway consists of a similar enzymatic cascade, there are only a handful of E3 ligases identified, and there is only one E2 conjugating enzyme, Ubc9. In contrast to ubiquitin E2 enzymes, which require E3 ligases to efficiently modify substrates, Ubc9 appears to be less reliant on E3 ligases, at least *in vitro* (reviewed in Geiss-Friedlander and Melchior, 2007). This therefore leaves us with a conundrum as to how a single E2 conjugating enzyme can generate specificity in substrate modification with SUMO. At the substrate level, it was initially thought that the ψ KxE/D sequence motif was sufficient for recognition and subsequent modification of the embedded lysine residue by Ubc9 (Rodriguez *et al*, 2001). However, several different variations of this core motif have now been identified, where flanking residues help specify modification by SUMO. For example, N-terminal clusters of hydrophobic residues (hydrophobic cluster sumoylation motif; HCSM) (Matic *et al*, 2010) and C-terminal clusters of acidic residues (negatively charged amino acid-dependent sumoylation motif; NDSM) (Yang *et al*, 2006) surrounding the core ψ KxE/D sequence define extended versions of this motif and hence provide more specificity to allow Ubc9 to discriminate among the multitude of potential target lysine residues. Moreover, in some cases, even lysine residues outside a core ψ KxE/D motif have been found to be modified, suggesting that alternative consensus motifs or targeting mechanisms might

exist (reviewed in Geiss-Friedlander and Melchior, 2007). Thus, an emerging picture is that there are an increasing number of different types of SUMO conjugation motifs, and yet only a sole E2 Ubc9, again emphasising the conundrum of how specificity of SUMO modification is generated.

In the present study (Hsieh *et al*, 2013), Shih and colleagues have focussed on one variation of the core ψ KxE/D motif, the NDSM, and investigated whether acetylation might provide a signal that can help Ubc9 discriminate between substrates containing this extended motif and ones containing the minimal core ψ KxE/D motif. Using a series of elegant molecular approaches, the authors demonstrate that Ubc9 is acetylated and that this acetylation can be reversed by the activity of the deacetylase SIRT1, which normally dampens down the levels of Ubc9 acetylation. Importantly, Hsieh *et al* identify hypoxia as one stimulus that increases the activity of SIRT1 and drives Ubc9 deacetylation. This in turn leads to increases in substrate sumoylation by Ubc9 (Figure 1). However, while this is itself

Figure 1 Schematic representation of the acetylation-dependent Ubc9 control circuit. Acetylated Ubc9 has broad specificity for sumoylating all substrates with lysine residues embedded in the core ψ KxE motif. Upon deacetylation Ubc9 preferentially targets lysine residues in substrates with the NDSM, due to increased interactions driven by the acidic residues in the NDSM (dotted line). Changes in Ubc9 acetylation can be triggered by hypoxia through increasing SIRT1 activity and potentially through as yet unknown signals that converge on lysine acetyl transferase(s) (KAT) that acetylates Ubc9.

already an interesting finding, the most significant discovery is that not all Ubc9 substrates are affected equally. Acetylation specifically controls the activity of Ubc9 towards substrates such as ELK1 and CBP that contain the extended NDSM rather than just the core ψ KxE/D motif, thereby providing insight into how the activity of Ubc9 can be modified to target it to a subset of its substrates.

Molecularly, deacetylation of Ubc9 causes it to bind to NDSM-containing substrates with higher affinity, hence explaining why these become better substrates. The defining feature of these targets is the presence of acidic residues C-terminal to the ψ KxE/D motif, and Hsieh *et al* demonstrate that mutation of these residues abrogates the stimulatory effect of deacetylation on Ubc9-mediated sumoylation of these substrates. One tempting mechanism to explain these effects is that as neutralisation of the positive charge on K65 on Ubc9 (the site of acetylation) is reversed by deacetylation, this might therefore promote electrostatic interactions with the negatively charged acidic patch in the NDSM. However, preliminary data alluded to by the authors seem to exclude this mechanism and instead point to an acetylation-induced conformational change in Ubc9, which would propagate into a change in binding affinity for the NDSM. Further biophysical and structural studies in this area are eagerly awaited to prove this hypothesis.

Overall, this study provides an important advance in our understanding of how specificity in the SUMO pathway is generated, and how post-translational modifications can influence this. While this study has focussed on the local context of the SUMO acceptor site, other studies have shown that SUMO-interacting motifs (SIMs) on substrates can also influence their sumoylation by helping recruit SUMO-modified Ubc9. Conversely, this result implies that additional modifications of Ubc9, such as sumoylation (Knipscheer *et al*, 2008), can also influence its activity towards a subset of substrates, thereby contributing to specificity mechanisms. Importantly, phosphorylation of SIMs can enhance substrate sumoylation, providing another example of how additional post-translational modifications may selectively affect substrate targeting (Chang *et al*, 2011). Interestingly, phosphorylation in the vicinity of core ψ KxE/D motifs has also been shown to promote SUMO modification and provides yet another example of an extended core motif, in this case one which is transiently established

following phosphorylation (the phosphorylation-dependent sumoylation motif; PDSM) (Hietakangas *et al*, 2006). Intriguingly, phosphorylation causes the transient appearance of a negative charge C-terminally to the ψ KxE/D motif, thereby creating an environment similar to the NDSM. Thus, it is tempting to speculate that acetylation of Ubc9 will also influence its targeting of substrates containing the PDSM. This example illustrates the potential for combinatorial control of substrate sumoylation by pathways that influence both acetylation and phosphorylation status. A further twist on this theme is the observation that acetylation of the lysine residue in the core ψ KxE/D motif in proteins such as the transcription factor HIC1 can block sumoylation (Stankovic-Valentin *et al*, 2007). Since this acetylation is also controlled by SIRT1, one could envisage a situation where both a substrate and Ubc9 become deacetylated at the same time, thereby unlocking a hitherto dormant SUMO acceptor site for modification. The potential intersection of additional regulatory modifications could serve to further diversify the specificity of substrate sumoylation. It will be interesting to determine whether Ubc9 is subjected to more post-translational modifications that influence its activities.

As ever with an important study, there are several outstanding questions left unanswered. For example, we do not know the identity of the enzyme that acetylates Ubc9. We also do not know whether acetylation of Ubc9 is enhanced under particular conditions and if so, whether this occurs in a global or subcellular context. Indeed, one could envisage such transient acetylation (or deacetylation) events taking place in the context of chromatin-bound complexes, and these might change as signals influencing the activities of these complexes change. Furthermore, it is likely that more signalling pathways and post-translational modifications will be identified that impact on either SUMO pathway components themselves or their substrates to influence the outcomes of the SUMO pathway. One of the key future challenges is to understand how the multiple different modifications interact in a temporal manner to orchestrate regulatory events downstream from the SUMO pathway.

Conflict of interest

The authors declare that they have no conflicts of interest.

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