

**P060** Characterization of transcription factor modification by SUMO

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We use Elk-1 as a model to investigate how transcription factor modification by SUMO can repress gene expression. Elk-1 contains a specific repression domain (R-motif), which reduces its ability to cause gene activation and hence downregulates gene expression. Within the R-motif are three lysines residues that are covalently modified by SUMO. SUMO modification of the acceptor lysines is required for the repressive properties of the R-motif. Western-blot analysis suggests that only a single SUMO moiety is conjugated to Elk-1 at a time. Mutation of a single lysine residue reduces, but does not abolish, the amount of SUMOylated Elk-1 suggesting a degree of redundancy.

An *in vitro* approach was used to identify which lysine is the primary site of SUMO modification. *In vivo* <1% of the total population of Elk-1 is SUMOylated and SUMOylation highly dynamic. To overcome this problem, we have used a system where the SUMOylation machinery has been transplanted into *E. coli*. The simultaneous expression of a protein, consisting of the Elk-1 R-motif fused to GST, creates a minimal substrate for SUMO conjugation, and allows purification of SUMO-modified Elk-1. This approach, in tandem with mass spectrometry analysis, provides a method for identifying which lysine residues are SUMO modified, and may help provide insights into how SUMOylation of transcriptional cofactors regulates gene expression. Specificity of deSUMOylation is also being investigated.