

**P021** Role of ubiquitin and SUMO molecules in the regulation of the inhibitory function of I $\kappa$ B $\alpha$  on NF- $\kappa$ B activity  
**Valerie Lang, Fabienne Aillet, Isabel Egaña and Manuel Rodriguez**

*From the Ubiquitin-like proteins and Cancer Group of the Proteomics Unit, CIC bioGUNE, Ed. 801A Parque Tecnológico de Bizkaia, Derio 48160, Spain*

The NF- $\kappa$ B pathway is involved in many cellular and biological functions and its deregulation have catastrophic consequences, therefore its activity needs to be specifically and highly regulated. This regulation is brought through multiple post-translational modifications (phosphorylation, ubiquitination or sumoylation), of the different core components of NF- $\kappa$ B signalling. For example, I $\kappa$ B $\alpha$  is modified both by SUMO-1 and ubiquitin. In contrast to ubiquitination, SUMOylation of I $\kappa$ B $\alpha$  has been proposed to repress NF- $\kappa$ B transcription. Because conjugation of ubiquitin-related molecules is a highly dynamic reversible process, one of the major difficulties encountered in studying the functions regulated by these post-translational modifications is to preserve the modified targets for further analysis. To overcome this problem one of the strategies that we are developing in the laboratory consist in the generation of chimeric proteins encoding ubiquitin and SUMO-1 fused to I $\kappa$ B $\alpha$  substrate. To avoid cleavage by ubiquitin-related proteases, double C-terminal glycines have been eliminated or replaced by double alanines. To monitor a possible cleavage of the fusion proteins, detection of HA-N-terminal and SV5-C-terminal tags has been performed. First, we analysed the stability and the localisation of these fusions proteins. We checked that the fusions proteins kept the same capacity to bind to NF- $\kappa$ B and we investigated the effect of these fusion proteins on NF- $\kappa$ B activity. Our latest results are presented here.