Abstract

Post-translational modifications are essential for initiating, regulating, propagating and terminating cellular stimuli resulting in the degradation and translocation of protein, signal transduction and multiple other cellular events. Promyelocytic leukaemia (PML) nuclear bodies (NB) are sub-nuclear structures involved in tumour suppression, DNA repair, transcription, apoptosis, and antiviral responses. The assembly of PML-NB is regulated by the covalent attachment of the small ubiquitin-related modifier (SUMO) protein to the PML protein. Post-translationally modified PML serves as a scaffold for the recruitment of other proteins containing short SUMO-interaction motifs (SIM). SIMs are also a characteristic feature of a novel class of RING-type E3 ubiquitin ligases which specifically recognize SUMOylated proteins, the so-called SUMO-targeted ubiquitin ligases (STUbLs). In humans, the RING-finger protein 4 (RNF4), which contains four putative SIMs, mediates the degradation of the promyelocytic leukaemia (PML) protein in response to arsenic treatment. Similar observations were obtained for the viral STUbL ICP0, which enables herpes simplex virus 1 (HSV-1) to evade the antiviral effect of PML-NBs. Therefore, removing of SUMOylated substrates, especially PML, seem to be a general feature in responding to cellular stresses and viral/bacterial invasions.

In this thesis, novel E3 ubiquitin ligases containing potential SUMO-interaction motifs were identified. The human RNF111 and a viral orthologue of ICP0, Orf61p from varicella zoster virus (VZV) were analysed to verify their function as STUbLs and to determine their mode of substrate recognition. The substrate specificities of these novel STUbLs were analysed by immunoprecipitation of SUMOylated substrates *in vivo* and *in vitro* and the reconstitution of the SUMO-dependent ubiquitylation *in vitro.* Both proteins interact specifically with SUMOylated proteins through their SIM domains. Further, both proteins have been shown to modify their target proteins with ubiquitin, but not necessarily in a Lys48-linked manner. Interestingly, RNF111 displays novel substrate specificity by preferentially binding to SU MO1-capped SUMO2/3-chains, while Orf61p prefers SUMO1- modified substrates irrespective of a chain. This is in contrast to the SUMO2/3-chain dependent recognition of the well-studied STUbL RNF4. Therefore, the specificity of the STUbLs was further analysed by comparing the interaction of their individual SIMs and of synthetic model SIMs with SUMO isoforms using isothermal titration calorimetry (ITC).