

**P003** A novel mechanism for NF- $\kappa$ B regulation by A20, an anti-inflammatory protein

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NF- $\kappa$ B activity is suppressed by A20, an anti-inflammatory protein. A20 contains an E3 ubiquitin ligase domain that catalyses the generation of polyubiquitin chains. It also contains an N-terminal cysteine protease domain, which cleaves ubiquitin monomers from branched polyubiquitin chains. Ubiquitination is essential at several stages during the activation of NF- $\kappa$ B. It regulates the degradation of inhibitory I $\kappa$ B molecules. In addition, activation of RIP, TRAF and NEMO signal adaptors relies on their modification with 'non-classical' forms of polyubiquitin.

We demonstrated that the ubiquitin editing functions of A20 were not essential for suppression of NF- $\kappa$ B. Overexpression studies revealed that native A20 and mutated forms, in which either the deubiquitinating or E3 ligase activities were inactivated, were equally effective in down-regulating nuclear translocation of NF- $\kappa$ B or the activity of an NF- $\kappa$ B reporter in response to TNF $\alpha$  or IL-1 (in HeLa cells or in A20-deficient fibroblasts). Our studies also revealed that A20 localizes to punctuate intracellular bodies that were electron-dense, highly mobile and were bound to lysosomes. These characteristics suggest that A20 localizes to aggresomes. We also observed targeting of pro-inflammatory signal adaptors to A20 aggresomes. We suggest that A20 aggresomes may play a key role in NF- $\kappa$ B inhibition by modulating the compartmentalization of pro-inflammatory signaling molecules.