Down-Regulation of Immune Signalling by a MAP-Kinase-triggered Phosphoswitch Activating the E3 ligase PUB22

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Signalling circuits are continuously being triggered and the intensity, as well as the duration of signalling events must be tightly controlled. Negative regulation plays an essential role to ensure cellular homeostasis. PUB22 negatively regulates immune signaling in concert with related E3s by targeting components of the vesicle trafficking. However, little is known about the mechanisms controlling E3 activity.

We show that activation of a specific MAPK results in the interaction and phosphorylation of PUB22. Two phosphosites were identified within and adjacent to the U-box domain, which mediates the interaction with the E2-ubiquitin complex. Analysis of PUB22 phosphomimetic mutants, revealed an increased stability *in vivo,* correlating with decreased *in vitro* autoubiquitination activity. Notably, the PUB22 phosphomimetic mutant maintained its ability to ubiquitinate a substrate *in vitro*, uncoupling the regulation of auto- and target ubiquitination. Moreover, it fully complemented immunity related phenotypes, while PUB22 phosphonull mutants were inactive. We also show that PUB22 forms oligomers via its U-box, mediating self-ubiquitination in *trans*. A structural model supported by mutational analysis, show that the interaction is mediated by conserved hydrophobic residues and that introduction of a negative charge mimicking phosphorylation, inhibits oligomer formation.

We propose a model in which MAPK-mediated phosphorylation of PUB22 upon activation of the immune response disrupts oligomerization, rescuing PUB22 from degradation and allowing it to engage targets to dampen cellular signaling. Together, we show that a MAP kinase, which is a central signalling component of the immune response, also triggers a negative feed-back loop by stabilizing the E3 ubiquitin ligase PUB22 through a novel mechanism.