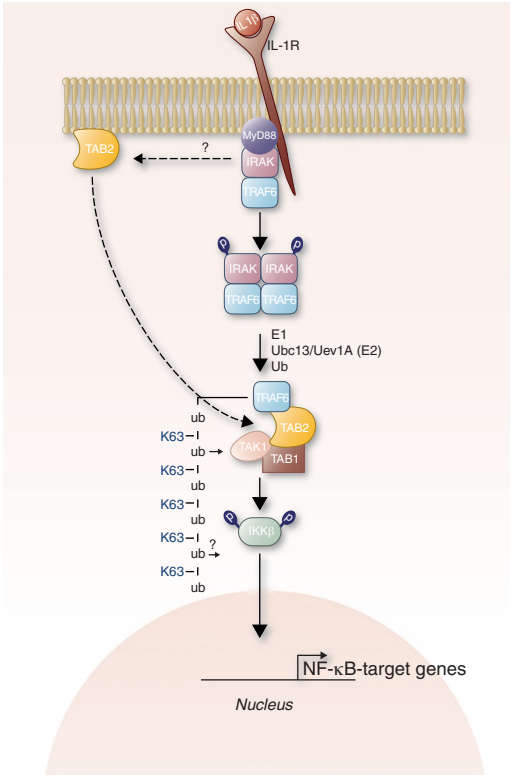
Getting activated with poly-ubiquitination

Ubiquitination is an established signal that targets proteins for degradation by the proteasome. However, recent studies have shown that there's ubiquitination and there's ubiquitination. First, mono-ubiquitination has different consequences to poly-ubiquitination, and can function as a signal for endocytosis. Furthermore, although poly-ubiquitin chains that polymerize through lysine 48 of ubiquitin seem to target tagged proteins for degradation by the proteasome, poly-ubiquitin chains that polymerize through lysine 63 of ubiquitin seem to be involved in proteasome-independent function, such as signal transduction of the inflammatory cytokine interleukin-1-β (IL1β).

Upon engagement of the IL1 receptor (IL-1R) by IL1β, an adaptor protein, tumour necrosis factor-associated factor 6 (TRAF6), is recruited to the IL1 receptor, that somehow activates the IκB kinase (IKK) and c-Jun N-terminal kinase (JNK) kinases. This in turn leads to the activation of the NF-κB and c-Jun transcription factors, respectively, and to the onset of a transcriptional response. Last year, Zhijian Chen and colleagues purified two protein complexes that mediate IKK activation by TRAF6. They showed that one of these complexes consists of an E2 ubiquitin-conjugating enzyme (Ubc13/Uev1A). TRAF6, through its RING finger domain, functions as an E3 ubiquitin ligase. The tripartite complex thus catalyses the synthesis of poly-ubiquitin chains linked though lysine 63 of ubiquitin, which is required for TRAF6-dependent activation of IKK.

However, poly-ubiquitin-dependent activation of IKK by TRAF6 was found to be independent of proteasome activity (Deng, L. *et al. Cell* **103**, 351–361; 2000), confirming that chains of ubiquitin polymerized through lysine 63 have proteasome-independent functions. Chen and colleagues went on to identify the components of the second complex, which consists of the TAK1 kinase, and the TAB1 and TAB2 proteins (Wang, C. *et al. Nature* **412,** 346–351; 2001). TAB2 recruits TAK1 to TRAF6. TAK1 is known to be involved in IKK activation by an unknown mechanism. Chen and colleagues found TAK1 to be required, together with TRAF6 and Ubc13/Uev1A, to activate IKK by phosphorylation (see figure). After activation, TAK1 can similarly mediate phosphorylation of MKK6, which results in the activation of JNK and c-Jun. In both cases, the activation of TAK1 requires poly-ubiquitination through lysine 63. And TRAF6 was shown to be oligomerized and poly-ubiquitinated upon exposure to IL1β. These findings indicate a new function of poly-ubiquitin chains in protein modification and activation, and the next step will be to understand the underlying mechanism for how poly-ubiquitinated TRAF6 activates TAK. Is it through a conformational change of the TRAF6 and the localization of TAB2? Is the poly-ubiquitin chain transferred to TAK1 to activate it?



Courtesy of Z. Chen

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phosphoinositide-binding sites in PH and FYVE domains, some general features are shared with the FYVE domain: ligand recognition involves a set of basic residues, conformational changes seem to accompany ligand binding, and a hydrophobic loop seems to interact with the lipid bilayer.

Why has there been an evolutionary pressure to conserve several structurally different phosphoinositide-binding domains? The FYVE domain has the advantage of being small and compact (about 70 residues), whereas the larger PX domain (100–140 residues) may provide the opportunity to modulate ligand affinity. In fact, the loop between the two α-helices is crucial for the interaction of the PX domain of Vam7p with PI(3)P-containing micelles[4]. In addition, this region is the target of phosphorylation and in $p47^{phox}$ it has recently been shown to interact with a Src homology 3 (SH3) domain of $p47^{phox}$ (ref. 16). It is conceivable therefore that phosphorylation or binding to SH3 domains may directly modulate the phosphoinositide-binding of PX domains. Furthermore, as with PH domains, PX domains are structurally diverse, whereas FYVE domains show greater sequence conservation. This indicates that PX and PH domains are more adaptable to evolutionary changes in ligand specificities, whereas the FYVE domains, which are known to be very sensitive to point mutations, are more 'conservative'.

The identification of the PX domain as a phosphoinositide-binding domain is exciting because it immediately indicates a mechanism for the subcellular targeting and regulation of a set of highly diverse proteins. Besides the proteins described above, other PX-domain-containing proteins of interest include phospholipase D, which regulates membrane trafficking, a kinesin-like motor protein of unknown function (KIAA1590) and the cytokine-independent survival kinase (CISK), which controls cell survival[17]. Knowing that PX domains bind to phosphoinositides is going to be very useful when analysing the functions of these proteins as well as those of the many PX-domain-containing proteins that have been identified through the genome-sequencing programmes. The fact that the human genome encodes about 250 PH-