STRUCTURE WATCH

One sign for many signals

Tumour-necrosis factor receptor (TNFR)-associated factor 6 (TRAF6) — which is important for innate and adaptive immunity and for bone homeostasis — is the only member of the TRAF family that is involved in signalling from both the TNFR and the interleukin-1 receptor/Toll-like receptor (IL-1R/TLR) superfamilies. TRAF6 acts mainly through its TRAF-C domain, which does not interact with peptide motifs that are recognized by many other TRAFs, and the molecular basis of TRAF6 specificity has remained unclear. Now, however, in *Nature*, Wu and colleagues describe the crystal structures of TRAF-C alone and in complex with peptides from members of the TNFR superfamily (CD40 or TRANCE-R).

The structures revealed that there are marked differences in peptide binding to TRAF6 and to other TRAFs. Using structure-based sequence alignment of TRAF6-binding sites in human and mouse CD40 and TRANCE-R, the authors identified a TRAF6-binding motif — Pro-X-Glu-X-X-(Ar/Ac), where Ar is an aromatic residue and Ac is an acidic residue. The best-characterized TRAF6 signalling pathway for the IL-1R/TLR superfamily involves IRAK, an adaptor kinase that is found upstream of TRAF6, and Wu and co-workers also found TRAF6-binding motifs in three IRAKs. These results indicate that TRAF6 uses a single structural mechanism to regulate several signalling cascades. Also, because the authors found that peptides derived from the TRAF6-binding motif could inhibit TRAF6-mediated signalling, this work highlights new potential therapeutic modulators for diseases such as osteoporosis.

REFERENCE Ye, H. *et al.* Distinct molecular mechanism for initiating TRAF6 signalling. *Nature* **418**, 443–447 (2002)

A new snapshot

Increases in cytoplasmic Ca^{2+} levels cause skeletal muscle cells to contract. After excitation, normal Ca^{2+} levels are restored by actively pumping these ions from the cytoplasm into the sarcoplasmic reticulum (SR). The transporter that carries out this process is Ca^{2+}-ATPase (a P-type ion-transporting ATPase), and the structure of Ca^{2+}-ATPase in the Ca^{2+}-bound form ($E1Ca^{2+}$) has previously been determined. Now, however, a new snapshot of this transporter is available. Toyoshima and Nomura have determined the 3.1-Å-resolution crystal structure of this pump in its Ca^{2+}-free (E2) state, and this second structure of the pump in its reaction cycle has allowed us to begin to understand how such ion pumps work.

The authors saw large conformational differences between the $E1Ca^{2+}$ and E2 states. The cytoplasmic part of the pump has three domains that are widely separated in the $E1Ca^{2+}$ state, and these domains form a compact structure in the E2 state. In addition, six of the ten transmembrane helices undergo large-scale rearrangements, and these changes are not limited to the helices that form the Ca^{2+}-binding sites. The authors believe that such large, complicated movements are needed for counter-transport (two Ca^{2+} ions can be transported per ATP hydrolysed, and two or three H^+ ions are counter-transported). In addition, these rearrangements ensure that Ca^{2+} is released into the SR lumen and that new Ca^{2+} ions can enter the pump from the cytoplasmic side.

REFERENCE Toyoshima, C. & Nomura, H. Structural changes in the calcium pump accompanying the dissociation of calcium. *Nature* **418**, 605–611 (2002)

SIGNAL TRANSDUCTION

Master switch

Smad3 protein is a mediator of transforming growth factor-β (TGF-β) signalling. It is recruited by SARA ('Smad anchor for receptor activation') to the TGF-β-receptor kinase, where it is phosphorylated. Smad3 then dissociates from SARA and the receptor, forms a heterotrimeric complex with cofactor Smad4, and translocates to the nucleus, where the Smad3–Smad4 complex binds to the promoters of target genes. Ski, a co-repressor, can bind directly to Smad3 to cause transcriptional repression. Interestingly, SARA and Ski bind to overlapping sites in a carboxy-terminal domain of Smad3, called MH2. Kai Lin and colleagues now report in *Genes & Development* how these different interactions might be regulated.

The authors used analytical ultracentrifugation to show that unbound Smad3 trimerizes in a concentration-dependent way. By contrast, when complexed with SARA, Smad3 does not oligomerize. Might SARA therefore inhibit Smad3-trimer formation? When the authors modelled the Smad3–SARA complex on the trimeric scaffold of a related Smad protein (Smad2), they found that the Smad3–SARA complex makes poor intersubunit contacts. This is because the MH2-domain three-helix bundle — a conserved structure for intersubunit contacts — does not tilt enough towards the neighbouring Smad3 subunit. SARA might therefore be able to inhibit Smad3 trimerization by preventing the three-helix bundle from tilting. On Smad3 phosphorylation, the conformational change caused by tilting of the three-helix bundle represents an allosteric switch that disrupts the interaction of Smad3 with both SARA and the receptor.

In a series of biochemical experiments, Lin and co-workers found that phosphorylated Smad3 binds to SARA with a low affinity, but that its binding drives phosphorylated Smad3 towards the monomeric state. This suggests that Smad3–SARA and homotrimeric-Smad3 interactions are competing and mutually exclusive.

Lin and colleagues then showed that the interaction between Ski and Smad3 is greatly reduced when conserved residues at the trimer interface are mutated, stabilizing Smad3 in the monomeric form. So, although SARA and Ski bind to overlapping surfaces on Smad3, they recognize the monomeric and trimeric forms, respectively.

In conclusion, phosphorylation-induced Smad3 trimerization functions as a master switch in TGF-β signalling, converting Smad3–receptor interactions into Smad3–transcriptional-comodulator interactions. This initial study might have far wider implications, according to the authors, who say that "trimerization-dependent interaction between Smad3 and Ski may represent a more general paradigm of Smad–nuclear interactions".

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References and links

ORIGINAL RESEARCH PAPER Qin, B. Y. *et al.* Smad3 allostery links TGF-β receptor kinase activation to transcriptional control. *Genes Dev.* **16**, 1950–1963 (2002)

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