AXIN shapes Tankyrase ARChitecture

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The poly(ADP-ribose)polymerase (PARP) Tankyrase uses ankyrin repeat modules to capture substrates via Tankyrase-binding peptide motifs. In this issue of Structure, Eisemann et al. (2016) describe how the signaling protein AXIN can access and conformationally adapt the multivalent ankyrin repeat region of Tankyrase, and discuss potential implications for enzymatic substrate modification.

Poly(ADP-ribose)polymerases (PARPs) use NAD^+ to construct long poly(ADP-ribose) (PAR) chains (Gibson and Kraus, 2012). Tankyrase is one such PARP and is represented by two paralogs in humans and many other vertebrates (Tankyrase 1, TNKS/ARTD5; Tankyrase 2, TNKS2/ARTD6). Highly similar at the structural level (Figure 1A), Tankyrases fulfill essential, diverse and largely overlapping roles during development and in physiology, and are candidate therapeutic targets in cancer, fibrosis and neurodegeneration (Hsiao and Smith, 2008; Riffell et al., 2012).

Tankyrase is also a positive regulator of Wnt-β-catenin signaling (Huang et al., 2009). It binds and PARylates AXIN (AXIN1/2), the central scaffolding subunit of the β-catenin destruction complex, thereby initiating its PAR-dependent proteasomal degradation (Huang et al., 2009; Zhang et al., 2011). Moreover, AXIN PARylation promotes its interaction with LRP6, a Wnt co-receptor (Yang et al., 2016).

Tankyrase's multi-domain architecture includes an N-terminal ankyrin repeat region, followed by a polymerizing sterile alpha motif (SAM) domain and a catalytic PARP domain (Figure 1A). Rather than forming continuous solenoids, the ankyrin repeats in Tankyrase are segmented into five so-called ankyrin repeat clusters (ARCs) (Guettler et al., 2011; Seimiya et al., 2004). Their main role is to recruit binders and PARylation substrates and to target Tankyrase to particular subcellular sites (Riffell et al., 2012). With the exception of ARC3, whose function remains unclear, ARCs recognize degenerate peptide sequences, conforming to the consensus R-x-x-[small hydrophobic or G]-[D/E]-G-[no P]-[D/E], with invariant R and G residues at positions 1 and 6, respectively (Guettler et al., 2011; Seimiya et al., 2004). AXIN contains two such Tankyrase-binding motifs (TBMs) (Huang et al., 2009; Morrone et al., 2012) (Figure 1B). A crystal structure of murine Tnks ARC2-3 bound to the bivalent N-terminus of murine Axin1 revealed a dimeric arrangement in which Axin1 links two ARC2 units (Morrone et al., 2012). The crystallizability of ARC2-3 suggested a sufficiently rigid linkage of the two ARCs, challenging a simple "beads on a string" model. However, our understanding of the overall ankyrin repeat architecture remains limited. In this issue of Structure, Eisemann and colleagues (2016) make exciting inroads into the structural understanding of ARCs.

Turning to small angle X-ray scattering (SAXS), Eisemann et al. (2016) demonstrate that the ankyrin repeat region of TNKS is highly flexible. Guided by limited proteolysis, they produced two subfragments, spanning ARCs 1-3 and 4-5, respectively. SAXS showed that the former is relatively rigid, while the latter is more dynamic. An extended ARC3-4 linker appears to act as a flexible hinge (Figure 1A and C). The authors successfully crystallized TNKS ARC1-3, providing the largest ARC fragment crystal structure reported to date. The triple-ARC adopts an asymmetric U-

shape, also supported by SAXS data, and reveals the connectivity between ARCs. Whereas ARCs 1 and 2 are linked by a broken helix, enabling numerous mutual contact points, ARCs 2 and 3 are connected by a continuous helix as the sole bridge. The peptide binding pockets in ARCs 1 and 2 are placed on the same face on the U-shape while the 'pseudo ligand site' of ARC3 maps to the opposite surface.

The authors next employed fluorescence polarization (FP) to address how the various ARCs within the penta-ARC assembly act coordinately. They first explored binding of isolated TBMs from two substrates (TBM^{LNPEP}, $TBM1^{AXIN1}$, $TBM2^{AXIN1}$) to single ARCs. Mutationally inactivating all but one functional ARC, they showed that ARC2 is the main binding contributor, while the other ARCs bound much more weakly under the assay conditions. ARC preferences for $TBM1^{AXIN1}$ confirmed previous findings with TNKS2; however, for many other TBMs, ARC5 appears to play a more important role, at least in TNKS2 (Guettler et al., 2011). With ARC2 as the main binder, how do the other ARCs contribute? Using FP, the authors elegantly showed that pairs of either ARCs 1 and 2, 4 and 5 or 2 and 5 cooperate in binding bivalent AXIN1. Spatial restraints imposed by the accessibility of the ARC pockets and the linker length between TBMs likely guide this behavior. Indeed, cooperation of adjacent ARCs was lost when the $TBM1^{AXIN1}$-$TBM2^{AXIN1}$ linker was shortened. Notably, ARCs 2 and 5 still cooperated, in agreement with a flexible hinge between ARCs 3 and 4.

The relevance of the ARC dimer in the previously reported crystal structure of ARC2-3 from murine Tnks bound to Axin1 remained unclear (Morrone et al., 2012). Eisemann et al. employed analytical ultracentrifugation (AUC) of human TNKS ARC1-5, detecting only monomeric assemblies, even in the presence of bivalent AXIN1. Given the high degree of conservation, the absence of dimers is unlikely to be ortholog-specific. Instead, the additional ARCs may oppose ARC2-3 dimerization. Moreover, dimerization of ARC2-3, in which only ARC2 binds TBM peptides, appears to be promoted by bivalent Axin1 (Morrone et al., 2012). AUC and SAXS performed by Eisemann et al. (2016) illustrated that bivalent AXIN1 induces a compaction of ARC1-5, in particular when the $TBM1^{AXIN1}$-$TBM2^{AXIN1}$ linker was shortened (Figure 1C). The authors studied the structural states of ARC1-5 by generating an extensive ensemble of atomistic Monte Carlo models representing the conformational landscape of ARC1-5 and selecting models that agree with experimental SAXS data. In the compact state induced by bivalent AXIN1, the ARC4-5 and ARC1-3 units move closer to each other (Figure 1C). Collectively, the study demonstrated that the two TBMs in AXIN1 can simultaneously access different ARCs in the same Tankyrase molecule, and that the ARCs provide an adjustable docking platform with both rigid and flexible subdomains, whose conformation can be shaped by multivalent binders.

What are the implications for Tankyrase function? Multivalency in Tankyrase:substrate interactions will enable cooperative binding. The combination of both rigid and flexible regions within the adaptable ARCs, and ARC3 as a spacer, may diversify the possible range of Tankyrase binders, each with a different number of TBMs, topology and TBM-TBM spacing. Which ARC combinations are accessed preferably will likely be ligand-specific. The authors suggest that the multi-TBM presentation may encode another important binding determinant, in addition to the TBM 'sequence rules' (Guettler et al., 2011). Eisemann et al. (2016) speculate that the ARC compaction induced by the recruitment of certain multivalent binders could orient the substrate and PARP domain for productive PARylation (Figure 1C). Further work is required to test this idea. If true, it would offer an attractive explanation for why not all Tankyrase binders are PARylated. Other possibilities may include the availability of accessible PAR-accepting amino acids and the dynamics of substrate binding. The authors also highlight a possible function of substrate multimerization, which clusters TBMs and further increases the cooperativity in Tankyrase binding. Indeed, both Tankyrase and AXIN can form polymers, and we can anticipate the molecular

choreography of such assemblies to be even more complex, possibly also involving the interaction of AXIN with ARCs from different Tankyrase molecules (Morrone et al., 2012).

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Figures and Figure Legends

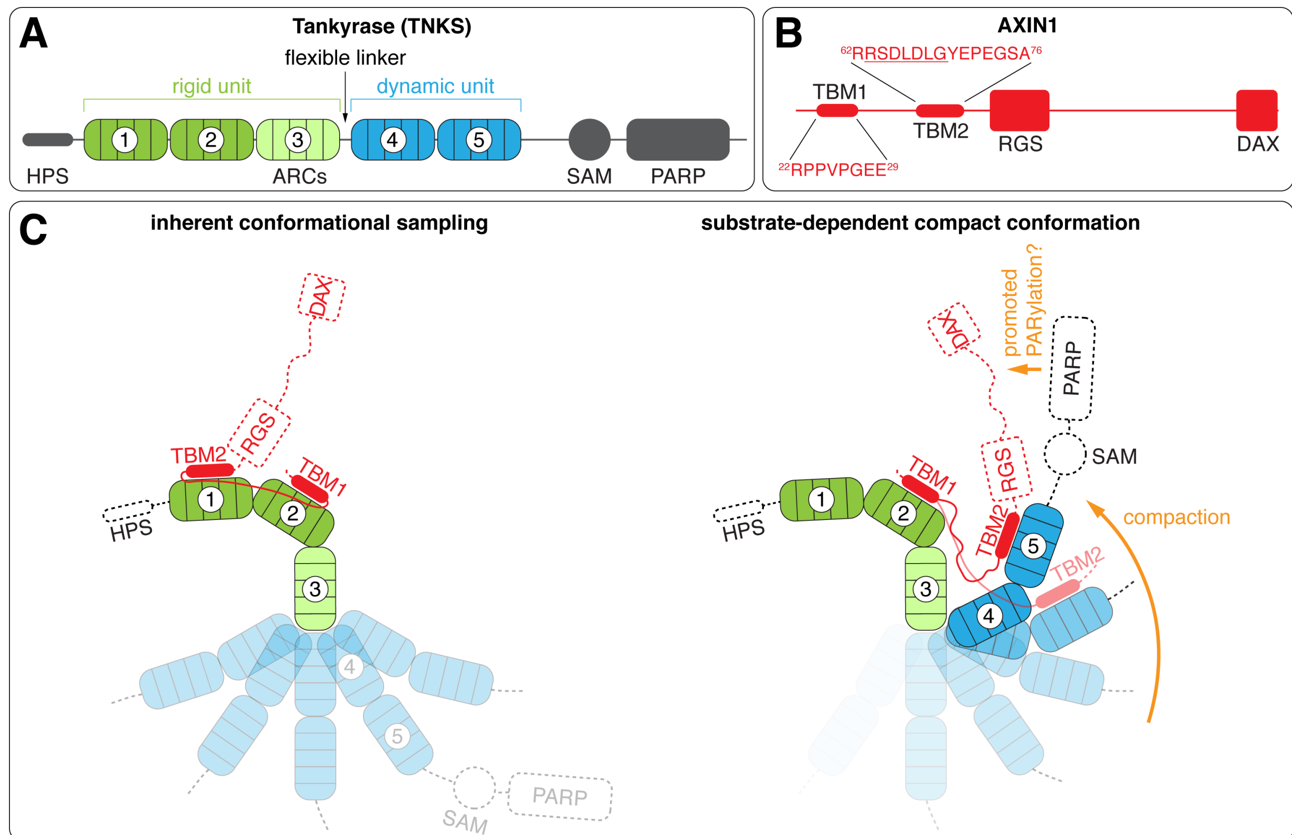

Figure 1: AXIN-induced conformational changes in Tankyrase.
(A) and (B) Schematics of Tankyrase (TNKS) and AXIN1 (HPS, His-Pro-Ser-rich region; ARCs, ankyrin repeat clusters; SAM, sterile alpha motif domain; PARP, catalytic domain; TBM, Tankyrase-binding motif, with TBM2 bearing an unusual insertion (underlined); RGS, regulator of G-protein signaling domain; DAX, Dishevelled and Axin domain). **(C)** Bivalent AXIN1 can simultaneously bind ARCs 1+2, 4+5 or 2+5. In the apo state, and presumably when ARCs 1+2 or 4+5 are engaged, the ARCs freely sample a wide conformational range (left). When AXIN1 links ARCs 2 and 5, the ARCs adopt a more compact state with ARC5 placed closely to ARC2 (right).