Structural Basis of Dynamic Polymerization of DIX Domains: Revised Model of Wnt Signaling

The Wnt signaling pathway controls numerous cell fates in animal development, and is also a major cancer pathway. A key negative cytoplasmic effector of this pathway is Axin, one of the components of the "destruction complex," which promotes phosphorylation of β-catenin and its subsequent degradation. Another key cytoplasmic effector, Dishevelled (Dvl), a positive effector of this pathway, binds to the Wnt transmembrane receptors, Frizzled and LRP5/6, and interacts with Axin to inhibit phosphorylation of β-catenin. Then, β-catenin is accumulated and translocated to the nucleus where it binds T-cell-specific transcription factor or lymphoid-enhancer factor to activate transcription of Wnt target genes. Axin functions as a tumor suppressor, and mutation of Axin often results in development of various human cancers.

Both Dvl and Axin contain a DIX domain, a functionally important domain whose molecular properties and structure have been unclarified. We have determined the first crystal structure of the Axin-DIX domain at 2.9 Å resolution by the single-anomalous diffraction method using data collected at **BL41XU** beamline [1]. DIX has a ubiquitin-like fold with five β-strands (β1-β5) and one α-helix. DIX interacts with neighboring molecules in a head-to-tail manner through a β-bridge between β2 and β4, forming filaments in the crystal (Fig. 1). Specifically, Y766 and I764 on β2 of one monomer interact with Y793 on β3 and F807 on β4 of the adjacent monomer (Fig. 2), thus forming a hydrophobic cluster.

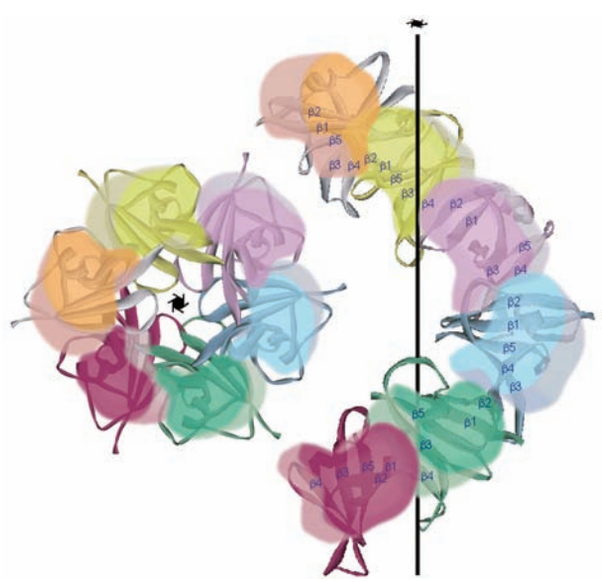

Fig. 1. Helical filament formed by Axin-DIX domain in crystal. Homomeric filament formed by Axin-DIX domain along crystallographic 6_1-axis. The filament is formed by head-to-tail interactions predominantly between β2 and β4 surfaces.

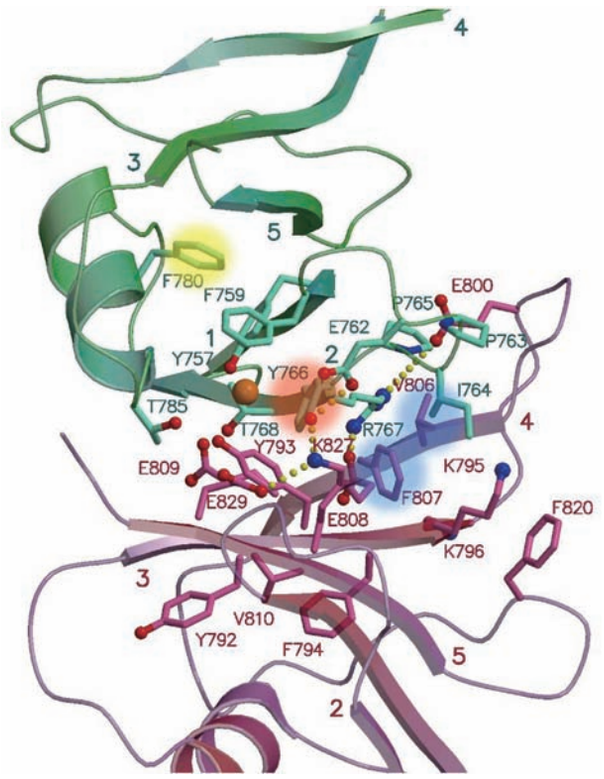

Fig. 2. Residues involved in intermolecular interactions between Axin-DIX domains. The residues corresponding to the mutated residues Y27, F43, and V67/K68 of Dvl2 mutants are highlighted in red, yellow, and blue, respectively. The orange sphere indicates an isolated electron density peak at the molecular interface.

The DIX domain of Dvl2 mediates dynamic polymerization, which is essential for the signaling activity of Dvl2 *in vivo* [1]. The purified DIX domain self-associates *in vivo*, and polymerizes gradually and reversibly in a concentration-dependent manner, ultimately forming fibrils [1]. The sequences of Dvl2 and Axin-DIX domains are highly homologous and both self-associate *in vitro* [2]. Mutation studies of Dvl2 in the DIX domain showed that Y27D (designed from the crystal structure), F43S, and V67A/K68A point mutations, which correspond to Y766, F780, and V806/F807 in Axin, respectively, abolished both Wnt signaling activity and dynamic polymerization ability [1]. Y766, V806, and F807 of Axin are on the

molecular interface between adjacent monomers in the crystal (Fig. 2). The mutation of these residues may weaken β2-β4 interaction. F780 is a core residue of the domain; therefore, it may affect the intra-filamental interaction indirectly.

These studies suggest the crucial role of polymerization of Dvl2 through its DIX domain in the Wnt signaling pathway. Increasing local concentration of Dvl2 by clustering may increase the avidity of Dvl2 for low-affinity binding partners such as Axin and Frizzled (Fig. 3). The DIX domain mediates the formation of a dynamic interaction platform with a high local concentration of binding sites for transient signaling partners.

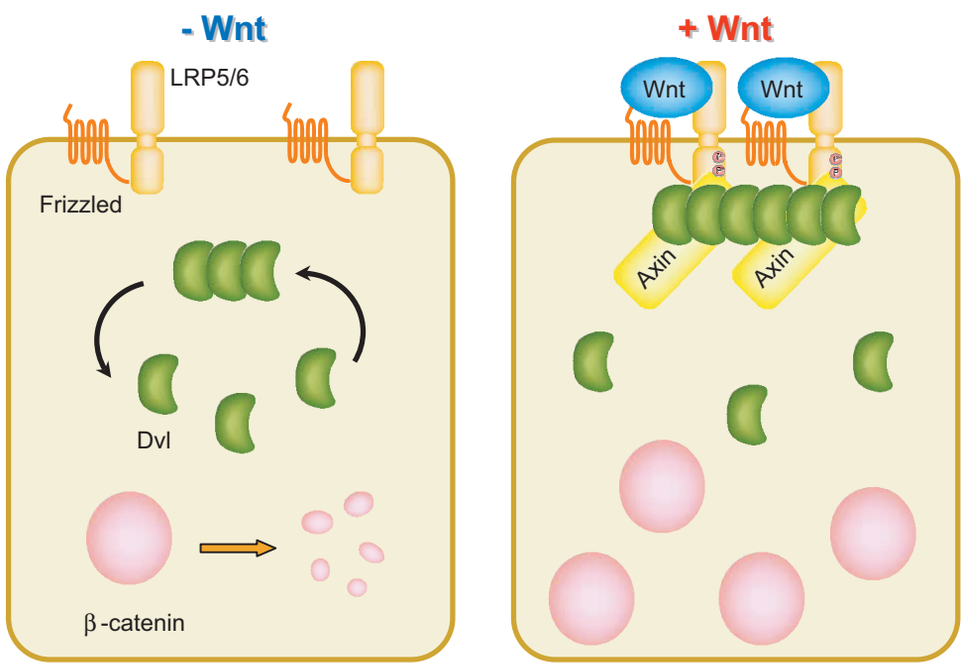

Fig. 3. Revised model of Wnt signaling. Left panel: In the absence of Wnt, Dvl remains cytoplasmic and in equilibrium between monomers and polymers. The destruction complex is active, and β-catenin is degraded. Right panel: Wnt stimulation triggers Dvl recruitment to the plasma membrane by Frizzled receptors, which function with LRP5/6 coreceptors. Dvl polymers at the membrane serve as a dynamic scaffold for Axin recruitment and inactivation. Wnt stimulation also leads to phosphorylation of LRP5/6. Phosphorylated LRP5/6 can interact with Axin, potentially providing another mechanism of recruitment and inactivation of Axin at the membrane.

Naoki Shibata [a,b] and Yoshiki Higuchi [a,b,*]

[a] Department of Life Science, University of Hyogo
[b] SPring-8 / RIKEN

*E-mail: hig@sci.u-hyogo.ac.jp

References

[1] *T. Schwarz-Romond, M. Fiedler, N. Shibata, P.J.G. Butler, A. Kikuchi, Y. Higuchi, M. Bienz: Nat. Struct. Mol. Biol.* ***14*** *(2007) 484.*
[2] C. Sakanaka and L.T. Williams: J. Biol. Chem. **274** (1999) 14090.