- 1P018 DIX ドメインを介する Wnt タンパク質の可逆的ポリマー化の構造基盤と Wnt シグナル制御モデル
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- Structural basis of dynamic polymerization of DIX domains: a revised model of Wnt signaling.
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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, 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 and interacts with Axin to transduce the Wnt signal. Both Dvl and Axin contain a DIX domain, a functionally important domain whose molecular properties and structure are unknown. We have determined the first crystal structure of the Axin-DIX domain at 2.9 Å resolution. The DIX domain has a ubiquitin-like fold with five β -strands (β 1- β 5) and one α -helix and interacts with neighboring molecules through a β -bridge between β 2 and β 4, forming filaments in the crystal by head-to-tail self-interaction through β -bridges. We also demonstrate that the DIX domain of Dvl2 mediates dynamic polymerization, which is essential for the signaling activity of Dvl2 *in vivo*. The purified DIX domain self-associates *in vitro*, and polymerizes gradually and reversibly in a concentration-dependent way, ultimately forming fibrils. Our studies point to a new mechanistic principle underlying Wnt signaling - namely signaling by reversible polymerization, whereby the DIX domain mediates the formation of a dynamic interaction platform with a high local concentration of binding sites for transient signaling partners.

- 1P020 放線菌由来 NRPS アデニレーションドメインの X 線結晶構造解析
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- 1aE09 Crystal structure of NRPS A domain from Streptomyces
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Cytotrienin A (CytA), an ansatrienin derivative, was isolated as an apoptosis inducer from culture broth of a soil *Streptomyces*. CytA has a 1-aminocyclopropane-1-carboxylic acid (ACC) moiety, although well known ansatrienins have a D-alanine moiety normally found in ansatrienins. We have already found a gene cluster composed of 4 genes, cytC1 ~ C4, which was seemed to be responsible for ACC biosynthesis. CytC1 is a free-standing adenylation domain (A domain) of NRPS (nonribosomal peptide synthetases). NRPS A domain catalyzes two steps toward formation of aminoacylated peptidyl carrier protein (PCP) domain. In the first step, the A domain selects an amino acid and activates it to generate an aminoacyl-AMP using ATP. In the next second step, the aminoacyl group is irreversibly transferred to the thiol of a 4'-phosphopantetheinyl group on PCP domain. Although most A domains in NRPS modules are highly specific for a single amino acid, CytC1 can convert several amino acids including L-valine, D-valine and ACC as well as its expected substrate, L-aminobutyric acid, to form aminoacyl-AMP intermediates. To elucidate the correlation between the substrate specificity and the structure of the active site of CytC1, we have determined the crystal structures of CytC1 in complex with ATP and some amino acids substrates at 1.8 Å ~ 2.2 Å resolution. These structures show that several phenylalanine residues in the binding pocket of CytC1 participate in the selectivity of substrates.

- 1P019 転写開始における RNA polymerase の活性型と不活性型のスイッチ機構
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- 1aE08 Mg²⁺ and Mn²⁺-triggered switching between productive and abortive synthesis in cyanobacterial RNA polymerase
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All prokaryotic and eukaryotic RNA polymerases (RNAPs) so far isolated have been known to perform "abortive initiation", which is an iterative synthesis and release of oligo-RNA products in the step of transcription initiation. In this study, we found that the RNAPs purified from cyanobacteria, which perform oxygenic photosynthesis rarely produce abortive RNA. If compared with *Escherichia coli* RNAP, the cyanobacterial enzyme produced almost 10⁻¹ order of magnitude of abortive RNA *in vitro*. The trigger switching from highly productive synthesis to abortive synthesis in the cyanobacterial RNAP was deficiency of catalytic metal ion, Mg²⁺ or the replacement of Mg²⁺ to Mn²⁺ in the reaction buffer. We demonstrated that the structural factor decreasing the amounts of abortive RNA is in the core enzyme rather than in the σ factor by constructing the chimeric holoenzyme. One of the unique structural features in cyanobacterial RNAPs is that the huge domain conserved in oxygenic photosynthetic organisms inserted between the catalytically essential structural motif, trigger helix in the β subunit. We are now trying to determine the crystal structure of the insertion domain in cyanobacterial RNAP and examining the relations between the strength of abortive initiation and the domain architecture connecting to the trigger helix.

- 1P021 アーキロドプシン-2 の 3 量体におけるバクテリオルベリンの役割
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- 1aE10 Bacterioruberin in the Trimeric Structure of Archaeorhodopsin-2
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Archaeorhodopsin-2 (aR2), a major protein found in the claret membrane of *Halorubrum sp. aus-2*, functions as a light-driven proton pump. In this study, we crystallized aR2 into a hexagonal crystals belonging to P321 and P6₃. These crystals were grown from a mixture of claret membrane and a small amount of nonylglucoside in the presence of ammonium sulfate at pH 7.5. The diffraction data indicated that the P321 crystal is made up of stacked membranes, in each of which the trimeric aR2-lipid complexes are arranged on a honeycomb lattice. A similar membranous structure is found in the P6₃ crystals, but orientations of four membranes contained in the unit cell differ in such a way that the aR2 trimers are aligned on twelve-screw axes. Bacterioruberin binds to the same open space in the crevice between adjacent monomers within the trimeric structure. The principal axis of this chromophore is tilted by 23° from the membrane normal, while the retinal chromophore is located in the center of the protein with a tilt angle of 70°. This implies that the binding site of bacterioruberin is not optimized for an efficient energy transfer from bacterioruberin to retinal. The physiological function of bacterioruberin seems to reinforce the trimeric structure, in which the proton release channel takes a more tight conformation than observed in the monomeric form.