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Structural mechanics of bacterial transcriptional activators for sigma54-polymerase

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Most genes in bacteria are transcript by sigma70-polymerase, which can bind and initate transcription without any requirement for other proteins or energy. However in many bacteria sets of particular genes, including some that are vital for metabolic process and some that are important for virulence, use sigma54-polymerase instead, acting as part of signalling pathways. While sigma54-pol can bind at the appropriate promoters, it cannot initiate transcription without the action of a 'transcriptional activator'. In general the activators have three domains, one receiving a cellular signal and regulating the activity, one targetting the protein to the appropriate genes, and one that controls oligomerization and is an ATPase that drives a conformational change that leads to opening at the promoter. We have determined structures of different versions of the three activator domains to understand how regulation occurs, the nature of the inactive and active states of the ATPase, and how these proteins bind to DNA. Surprisingly for three homologous proteins we have found that there are three different regulatory mechanisms. We have also initiated studies of the sigma54 subunit of polymerase, and determined how it binds to target DNA. These results will be described to give an overall view of the mechanics of this class of transcriptional regulatory proteins.

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Structure of the yeast polarity protein Sro7 reveals a SNARE regulatory mechanism

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Polarized exocytosis requires coordination between the actin cytoskeleton and the exocytic machinery responsible for fusion of secretory vesicles at specific sites on the plasma membrane. Fusion requires formation of a complex between a vesicle-bound R-SNARE and plasma membrane Qa, Qb, and Qc SNARE proteins. Proteins in the lethal giant larvae (LGL) family, including LGL and tomosyn in metazoans and Sro7 in yeast, interact with Q-SNAREs and are emerging as key regulators of polarized exocytosis. The crystal structure of Sro7 reveals two seven-bladed WD40 b-propellers followed by a 60-residue "tail" that is bound to the surface of the N-terminal propeller. Deletion of the Sro7 tail enables binding to the Qbc SNARE region of Sec9, and this interaction inhibits SNARE complex assembly. The N-terminal domain of Sec9 provides a second, high-affinity Sro7 interaction that is unaffected by the tail. The results suggest that Sro7 acts as an allosteric regulator of exocytosis through interactions with factors that control the tail.

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Structural and functional insights into dom34, a key component of no-go mRNA decay

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In Saccharomyces cerevisiae, the two proteins Dom34 and Hbs1 play key roles in a newly identified mRNA-surveillance pathway called no-go decay, by which mRNAs with translational stalls are endonucleolytically cleaved and subsequently degraded. However, the identity of the endoribonuclease is unknown. Homologs of the yeast Dom34, called Pelota, are broadly conserved in eukaryotes and archaea. Dom34/Pelota is related to the translation termination factor eRF1. To gain insights into the structure and function of Dom34/Pelota, we have determined the crystal structure of a representative member of the Dom34/Pelota family and investigated the ribonuclease activity of Dom34. Dom34/Pelota consists of three domains of similar sizes, with domain 1 being structurally dissimilar from domain 1 of eRF1 but resembling the RNA-binding Sm fold. The yeast Dom34 shows an endoribonuclease activity with RNA substrates containing a stem-loop, suggesting that it could be responsible for the endonucleolytic cleavage of the stalled mRNA in no-go decay.

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Structural basis of activation of replication initiation factor, RepE

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DNA replication initiator protein RepE (251 residues; 29kDa) of mini-F plasmid strictly regulates the plasmid replication by use of its bifunctional roles correlated with the two molecular association forms, monomer and dimer. The RepE monomers are replication initiators bound to the iteron DNA located in the replication origin, whereas the dimer is an autogenous transcriptional repressor bound to the repE promoter/operator region. RepE normally forms a stable dimer in solution. Therefore, in order to function as intrinsic initiators, RepE dimers are to dissociate into monomers. It is proposed that this conversion process is mediated by actions of the DnaK molecular chaperone system. However, structural mechanism of the oligomeric switching is unclear. The crystal structure of monomeric RepE in complex with the iteron DNA indicated that the protein molecule consists of two domains, each containing a winged helix-turn-helix motif. The structure of the RepE monomer implies a crucial requirement of a drastic conformational change for the oligomeric conversion of RepE. In fact, the crystal structure of the dimerization domain of a homologous protein (RepA) adopts a different conformation from that of the RepE monomer. The structure of the full-length RepE dimer bound to the repE operator DNA will provide an insight into molecular association mechanism of this protein with the aid of the DnaK chaperones. Structural mechanism for activation of replication initiation activity will be discussed on the basis of the crystal structures of the RepE protein in several molecular association states.

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Structural understanding of the bacterial envelop stress response

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The bacterial envelope stress response senses stress signals in the extracytoplasmic compartment, and activates sigmaE-dependent transcription by degrading its antisigma factor RseA. RseB, a binding partner of RseA, plays a pivotal role in regulating this response, but its molecular mechanism is not understood. We therefore determined the crystal structure of Escherichia coli RseB at a resolution of 2.4 Å. RseB is composed of two domains linked by a flexible linker and forms a loosely packed dimer with two grooves on each side. This structural feature is confirmed by the small angle scattering in solution. Analysis of the binding of various RseA mutants to RseB allowed us to identify the major RseB binding motif in RseA. These data, coupled with analysis of small angle scattering of the RseA/RseB complex in solution, leads us to propose that two RseAs bind to the grooves of the dimeric RseB via conserved residues. The implications for modulating proteolytic cleavage of RseA are discussed.

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p53 and the like: natively unstructured proteins

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A growing number of proteins are found to exist in a natively unstructured or intrinsically unfolded state, i. e., without a tertiary structure, but are functional [1]. Bioinformatics tools predict that even as much as 50% of the whole protein kingdom could be such proteins. Discovery of natively unstructured proteins (NUPs) is challenging the long-standing dogma of structural biology that every protein must have a unique and well-defined three-dimensional structure to be biologically active. Absence of a unique globular structure in NUPs defies structure "determination" by x-ray crystallography. However, structural states of NUPs can be efficiently and quantitatively "characterized" by multidimensional NMR spectroscopy. Transcriptional activators constitute an important subfamily of NUPs. The transactivation domain of p53 is one of the first NUPs whose unstructured nature has been investigated in detail [2-4]. This lecture will present on-going efforts in my laboratory to analyze structural features of NUPs and their relevance to function, in particular, of a pre-molten-globule-like NUPs. 1. Dyson & Wright (2005) Intrinsically unstructured proteins and their functions. *Nat. Rev. Mol. Cell. Biol.* **6**, 197. 2. Lee et al., (2000) Local structural elements in the mostly unstructured transcriptional activation domain of human p53. *J. Biol. Chem.* **275**, 29426. 3. Dawson et al., (2003) The N-terminal domain of **p53** is natively unfolded. *J. Mol. Biol.* **332**, 1131. 4. Chi et al., (2005) Structural details on mdm2-p53 interaction. *J. Biol. Chem.* **280**, 38795