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From Structure to function: Structure of the Gre-factor reveals its binding site on RNA polymerase

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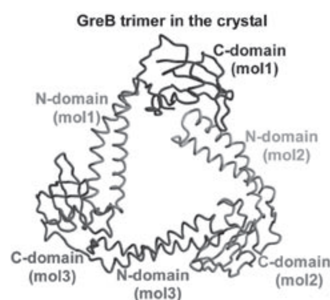
Bacterial Gre transcript cleavage factors (GreA/GreB) stimulate the intrinsic endonucleolytic activity of RNA polymerase (RNAP) to rescue stalled transcription complexes. They bind to RNAP and extend their coiled-coil (CC) domains to the catalytic centre through the secondary channel. Three existing models for the Gre/RNAP complex postulate congruent mechanisms of Gre-assisted catalysis, while offering conflicting views of the Gre/RNAP interactions. We have detremined the GreB structure of Escherichia coli at 2.6Å resolution. The GreB monomers form a triangle with the tip of the amino-terminal CC of one molecule trapped within the hydrophobic cavity of the carboxy-terminal domain of a second molecule. This arrangement suggests an analogous model for recruitment to RNAP. Indeed, the beta' subunit CC located at the rim of the secondary channel has conserved hydrophobic residues at its tip. We show that substitutions of these residues and those in the GreB C-terminal domain cavity confer defects in GreB activity and binding to RNAP, and present a plausible model for the RNAP/GreB complex.



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Keywords: transcription, Gre-factors, RNA polymerase

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Nature of the TRAP:Anti-TRAP complex revealed by symmetry remodeling

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Tryptophan biosynthesis is subject to exquisite control in species of Bacillus, and has become one of the best studied model systems in gene regulation. The protein TRAP (trp RNA-Binding Attenuation Protein) forms a ring-shaped 11-mer, which binds cognate RNA in the presence of tryptophan to suppress expression of the trp operon. TRAP is itself regulated by the protein Anti-TRAP, which binds to TRAP and prevents RNA binding. To date the nature of this interaction has proved elusive. Here we describe mass spectrometry and analytical centrifugation studies of the complex, and two crystal structures of the TRAP:Anti-TRAP complex. These crystal structures, both refined to 3.2 Å resolution, show that Anti-TRAP binds to TRAP as a trimer, sterically blocking RNA binding. Mass spectrometry shows that TRAP forms a small population of a 12mer form in solution. Crystallization with Anti-TRAP selectively pulls the 12mer TRAP form out of solution, so the crystal structure of wild-type TRAP:Anti-TRAP complex reflects a minor species from a mixed population, but with the same interactions between the two proteins.

Keywords: protein complex, transcription regulation, X-ray crystallography

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Vps9 assisted guanine nucleotide exchange intermediates of Rab5

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Rab small GTPases regulate vesicular transport in eukaryotes. Arabidopsis orthologs of mammalian Rab5 including ARA6, ARA7 and RHA1 control early endosomal fusion, and are specifically activated by an Arabidopsis guanine nucleotide exchange factor (GEF) for the Rab5 orthologs, VPS9a, which contains a Vps9 domain. Here we present crystal structures of complexes between ARA7 and the N-terminal catalytic domain of VPS9a in the nucleotide-free form and in complex with guanosine diphosphate, GDP or its analogue GDPNH2, which demonstrate that VPS9a promotes nucleotide exchange by wedging into the nucleotide binding site of ARA7 to move the two switch regions apart and to create a GDP specific recognition site. VPS9a inhibits coordination of magnesium ion to the beta-phosphate of GDP, thus making the GDP binding less stable.VPS9a Tyr225 pushes the N-terminal end of beta2-strand away and forms tight van der Waals contacts with three residues of ARA7, and as a result, moves Switch I out to the solvent. An invariant aspartate residue, VPS9a Asp185 keeps a critical contact with a lysine residue, Lys23, in the conserved P-loop of ARA7 during the release of GDP. Furthermore, the structures of the ARA7/VPS9a complex with GDPNH2 and the D185N mutant of VPS9a in complex with GDP form ARA7 indicate that the side-chain COO^- of VPS9a Asp185 guides NH^{3+} of Lys23 to regulate the interaction with the beta-phosphate oxygen atoms of GDP, thus affecting the GDP recognition and its release. The transient GDP recognition by VPS9a rationalizes a directional nucleotide exchange from GDP to GTP.

Keywords: G proteins, membrane trafficking, catalysis structure of intermediates

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Crystal structure of yeast Sec2p, the guanine nucleotide exchange factor for Sec4p

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Vesicular traffic during exocytosis is regulated by Rab GTPase, Sec4p in yeast. Sec2p is a guanine nucleotide exchange factor (GEF) for Sec4p, and the N-terminal 160 residues of Sec2p are sufficient for the GEF activity. Since this region of Sec2p shows no sequence similarity to any other GEFs with known structures, the GEF mechanism by Sec2p has remained unknown. To elucidate this nucleotide exchange mechanism by X-ray crystallography, we crystallized three constructs of the native Sec2p (Sec21-160p, Sec218-160p, and Sec231-160p) and three constructs of the selenomethionine (SeMet)-labeled Sec2p [Sec231-160p, Sec231-160p (M115L), and Sec231-160p (M115L, K121M, T142M)]. These six crystals diffracted to 8.8, 4.8, 2.6, 4.0, 3.3, and 3.0 Å resolutions, respectively. The data set of the SeMet-labeled Sec231-160p (M115L, K121M, T142M) crystal was processed for SAD phasing, producing an interpretable map after density improvement. The atomic model of the Sec2p GEF domain was refined to an Rfree value of 28.9%. Unexpectedly, the Sec2p GEF domain consists of a homodimeric, parallel coiled coil that extends over 180 Å. Pull-down and guanine nucleotide exchange assay using a series of deletion and point mutants of Sec2p unveiled the catalytic residues for its GEF activity and the Sec4p binding site.

Keywords: vesicle membrane fusion, GTP-binding proteins, membrane trafficking

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Structure and inhibition of Arf GTPases

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Transport of proteins and membranes sustains all aspects of cellular life. It is therefore associated to major cellular processes such as signalling, morphology and division and it is also sensitive to subversion by pathogens. Small GTP-binding proteins (GTPases) of Arf families are major player in cellular traffic, where they tag and organize membranes for specific trafficking events. They are activated by a family of guanine nucleotide exchange factors (GEFs) that carry a catalytic domain (the Sec7 domain), which stimulates the exchange of the tightly bound GDP nucleotide for GTP. Structural studies have elucidated the exchange mechanism of the Sec7 domain, yet its exquisite ability to discriminate between closely related Arf isoforms remains unexplained. Combining X-ray crystallography, NMR and the use of small molecular weight inhibitors, we identify structural dynamics as a previously overlooked aspect of Arf GTPases functions.

Keywords: GTPase, guanine nucleotide exchange factor, traffic

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Atomic model of Rab27a:Exophilin4/Slp2-a complex: Structural studies on vesicular transport

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Cycling between active and inactive conformations, Rab small GTPases coordinate vesicular trafficking within eukaryotic cells by collaborating with a set of effectors. Rab27a/b isoforms regulate numerous exocytotic pathways, and their dysfunction causes human immunodeficiency known as Griscelli syndrome. Exophilin4/Slp2-a localizes on the phosphatidylserine-enriched plasma membrane of melanocytes and its N-terminal Rab27 binding domain (RBD) specifically recruits Rab27a on the surface of melanosomes prior to docking and fusion events. To clarify the characteristic features for the selective binding of Rab27 to eleven distinct effectors, high resolution structures of both inactive GDP-bound and active GppNHp-bound Rab27 in complex with RBD of Exophilin4/Slp2-a were determined. While drastically reordering its switch and interswitch elements under nucleotide exchange, Rab27 moulds a compatible surface for recognizing Exophilin4/Slp2-a, presenting some particularities at the interface of the Rab27 effectors structural motif (S/T)(G/L)xW(F/Y)2 on account of modulating effector affinity for Rab27. The observed structural complementation in the interacting surfaces of Rab27a and Exophilin4/Slp2-a sheds light on the disparities among Rab27 effectors and untangles the general mechanism for their recruitment.

Keywords: membrane trafficking, Rab GTPase, macromolecular assemblies

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Crystal structure of *E.coli* MacA reveals the assembly of the tripartite bacterial efflux pump

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Periplasmic membrane fusion proteins (MFPs), including MacA and AcrA, are an essential component of multidrug efflux pumps in Gram-negative bacteria. They play a crucial role in bridging the outer membrane porin TolC and two distinctive types of inner membrane transporters. MacA and AcrA form the MacA-MacB-TolC and AcrA-AcrB-TolC efflux pumps, respectively, in Escherichia coli. Although the crystal structures of two MFPs have been reported, the functional form and the mechanistic role of MFPs are only vaguely understood. Here, we show that MacA forms a funnel-like hexameric assembly with a central channel whose diameter is similar to that of TolC and a conical mouth that appears to accommodate the periplasmic end of MacA. In accordance with the results of biochemical experiments, we propose a structural model for how MFP induces the opening of the central channel of TolC in the periplasmic space of Gram-negative bacteria. Based on the complementing available structures and information, realistic models for the tripartite multidrug efflux