**P04.14.323**  
*Acta Cryst.* (2008). A64, C332

**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 determined 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.

Vassylyeva et al. (2007) EMBO Reports, 8, 1038-1043

**Keywords:** transcription, Gre-factors, RNA polymerase

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**P04.14.324**  
*Acta Cryst.* (2008). A64, C332

**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. Crystalization 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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**P04.14.325**  
*Acta Cryst.* (2008). A64, C332

**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 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

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**P04.14.326**  

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

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