MS12-P7 **Mechanismfor DNA recognition by transcriptional factor AdpAfrom*Streptomyces griseus*** Masaru Tanokura,[a] Ming Dong Yao,[a] Jun Ohtsuka,[a] Koji Nagata,[a] Ken-ichi Miyazono[a] and Yasuo Ohnishi[b] *Departments of [a]Applied Biological Chemistry and [b]Biotechnology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Tokyo 113-8657, Japan*
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In *Streptomyces griseus*, AdpA is the central transcriptional factor in the A-factor regulatory cascade and activates approximate thousands of genes required for both secondary metabolism and morphological differentiation, leading to onset of streptomycin biosynthesis as well as aerial mycelium formation and sporulation [1]. AdpA consists of two domains—the dimerization domain at its N-terminal portion and the DNA-binding domain at its C-terminal portion—and belongs to the AraC/XylS family of transcriptional regulators. The DNA-binding domain of AdpA is characterized by two helix-turn-helix DNA-binding motifs [2] and shows a low nucleotide sequence specificity [3]. To reveal the molecular basis of the low nucleotide sequence specificity, we have determined the crystal structure of the complex of DNA-binding domain of AdpA (AdpA-DBD) and a 14-mer duplex DNA with two-nucleotide overhangs at 5'-ends at 2.9-Å resolution.The crsytal belonged to the space group $C222_1$, with unit cell parameters a = 77.0 Å, b = 101.3 Å, and c = 101.5 Å, and contained one complex ofAdpA-DBD and dsDNA in an asymmetric unit. The crystal structure was solved by molecular replacement. The model was refined to R_{factor}/R_{free} values of 19.7/25.2%. AdpA-DBD comprises two helix-turn-helix (HTH) motifs linked by a long α-helix. The N-terminal HTH motif engages the major groove of the binding site, whereas the C-terminal HTH motif only binds to phosphate groups and half-inserts into adjacent major groove of the dsDNA. The linker helix is also involved in interactions with the DNA and imposes the orientation and distance restraints on the two HTH motifs for proper binding. These interactions stabilize the complex of AdpA-DBD and dsDNA and reflect the sequence specificity of AdpA-DBD. By comparing the sequence specificity and the crystal structure of AdpA-DBD with those ofother AraC/XylS family members, MarA and Rob.we discovered that AdpA-DBD possesses the lowest DNA-binding specificity, which results in AdpA directly controls many more genes than these global transcriptional regulators for bacterial differentiation. To the best of our knowledge, the AdpAregulon seems to be the largest one in bacteria.

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MS12-P8 **The relevance of cyanobacterial Tic22 to plant and malarial protein transport.** Ivo Tews,[ab] Patrick Koenig,[b] Irmgard Sinning,[b] Johanna Tripp,[c] Oliver Mirus,[c] Enrico Schleiff,[c] *[a]University of Southampton, Centre for Biological Sciences, Southampton SO17 1BJ, UK, [b]Heidelberg University Biochemistry Center, INF328, 69120 Heidelberg, Germany, [c]Goethe University Frankfurt, Department of Biosciences, 60438 Frankfurt, Germany*
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Prokaryotic and eukaryotic cell membranes contain specific transporters for proteins to mediate transfer across and insertion into bio-membranes [1]. One can compare the transporters found in organelles such as mitochondria or plastids with the ones found in bacteria. For instance, membrane-spanning beta-barrel pore subunits are present in mitochondria (SAM50 and Tom40 [2]), in chloroplasts (Toc75 [3]), and in bacteria (Omp85 [4]). An interesting problem exists with respect to the directionality of transport, which must have changed during evolution if all these proteins should have common ancestry [5]. We had previously addressed this with the study of the so-called POTRA domains (POlypeptide-TRansport-Associated) of a cyanobacterial Omp85 protein [6], and have now extended these studies to the cyanobacterial protein Tic22. The crystallographic 3D structure has a "butterfly" shape revealing a repeat likely caused by gene duplication [7]. Four helices point orthogonal at each other, adding up their dipole moments in a central cavity. The surface of the structure is dotted with hydrophobic pockets in which we identified bound solvent molecules. While the functional significance of these features is not yet clear, it is likely that they represent binding sites for protein substrates, and Tic22 is thus assigned a chaperone function. We demonstrate that Tic22 is present in the cyanobaterial periplasm as well as in thylakoids, and it can be functionally replaced by knock-in of a plant orthologue. The structural clues together with the functional data suggest that Tic22 can have a function in both, protein import or protein insertion, depending on the organism where it is found. The protein is conserved in bacteria, plants, and unicellular organisms such as the plastid containing apicomplexa with the important human pathogen *Plasmodium*, the causative agent of malaria. It therefore links back these protein transporters to a common ancestry.

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