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The Structure of Ce-FAR-7 from *C. Elegans* Indicates How This Family of Nematode Fatty Acid and Retinoid Binding Proteins Bind Their Cargo. Rositsa Jordanova[a], Matthew R. Groves[a], Elena Kostova[a], Christian Woltersdorf[b], Eva Liebau[b], Paul A. Tucker[a]. [a]*European Molecular Biology Laboratory, Hamburg Outstation, Hamburg, Germany.* [b]*Institute of Animal Physiology, University of Muenster, Muenster, Germany.*
E-mail: jordanova@embl-hamburg.de

Parasitic nematodes cause serious diseases in humans and animals, economically impacting agricultural industries. Chemoprophylaxis offers short-term benefits, but due to rapid development of drug resistance in parasites there is a pressing need for novel treatments of nematode infections. Lipid binding proteins (LBPs) play an important role in parasitic nematode's limited lipid metabolism. Several structurally novel families of LBPs in nematodes have been described, including the fatty acid and retinoid binding protein family (FAR). In *C. elegans*, used as a model for studying parasitic nematodes, eight Ce-FAR proteins have been described. Here we report the first high resolution structure of a FAR protein – the 1.8 Å crystal structure of Ce-FAR-7. Ce-FAR-7 has nine helices, packed into a novel fold containing two hydrophobic binding pockets. Pocket P1 can accommodate fatty acids with different length of the aliphatic chain, whereas P2 is more suitable for bulkier ligands like retinoids and other signaling lipids. Our results suggest that retinol binding can be upregulated by casein kinase 2, via phosphorylation of Thr26 positioned at the bottom of the pocket P2. GFP localization in vivo shows, that Ce-FAR-7 is intracellular and present in all development stages of the nematode. It is mainly localized in the excretory cell and head hypodermis region of the worm. However, during starvation its localization changes and Ce-FAR-7 is expressed in the body hypodermis. In conclusion, our study provides the basic structural and functional information for investigation of inhibitors of lipid binding by FAR proteins, which could result in new generation of anti-nematode drugs.

Keywords: fatty acid and retinoid binding; nematode metabolism; phosphorylation

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Structural and Functional Analysis of PhnP from Carbon-Phosphorous Lyase Pathway. Kateryna Podzelinska[a], Shu-Mei He[b], Matthew Wathier[b], Alexander Yakunin[c], Michael Proudfoot[c], Bjarne Hove-Jensen[d], David L. Zechel[b], Zongchao Jia[a]. *Departments of* [a]*Biochemistry and* [b]*Chemistry, Queen's University, Kingston, Canada, the* [c]*Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada, and the* [d]*Department of Biology, University of Copenhagen, Copenhagen, Denmark.*
E-mail: 9kp8@queensu.ca

Free phosphate availability in nature can be a limiting factor for bacterial growth. During phosphate limitation, bacteria exhibit many hundred fold upregulation of a C-P lyase pathway to obtain phosphate from phosphate esters and organophosphonates. The C-P lyase operon consists of 14 genes (phnCDEFGHIJKLMNOP). Based on mutational and sequence analysis, gene products PhnC to PhnE are thought to be involved in transport of substrates, PhnF is a transcription repressor protein, PhnO is an aminoalkylphosphonate N-acetyltransferase, PhnG to PhnM are enzymes involved in C-P bond cleavage, and PhnN is a ribose 1,5-bisphosphokinase. Mutational studies suggest that PhnP is required in the presence of a functional phnN product for phosphonate utilization. In order to determine the role of PhnP in the C-P lyase pathway, structural studies have been undertaken. The three-dimensional crystal structure of PhnP was determined at 1.4 Å resolution and exhibits significant similarity to Zn-dependent ribonucleases. Phosphodiesterase substrate screen has identified 2'3'-cyclic nucleotides as substrates for PhnP, and cellulose TLC showed that 3'- nucleotides are the products. Interestingly, PhnP contains both Zn and Mn as determined by IPC-MS analysis. Kinetic studies revealed that PhnP enzymatic activity is Mn- but not Zn-dependent. Mutagenesis was used to elucidate the roles of active site residues, and the enzymatic mechanism was proposed. (Supported by NSECR and CIHR)

Keywords: phosphodiesterase; C-P lyase; organophosphonate metabolism

FA1-MS04-P03

Crystal Structure of the Full-length Sorbitol Operon Regulator SorC from *Klebsiella Pneumoniae*: Structural Evidence for a Novel Transcriptional Regulation Mechanism. Daniele de Sanctis[a,b], Colin E. McVey[b] , Francisco J. Enguita[c], Maria Armenia Carrondo[b]. [a]*Structural Biology Group, European Synchrotron Radiation Facility, Grenoble, France.* [b]*Instituto de Tecnologia Química e Biológica, Oeiras, Portugal.* [c]*Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Portugal.*
E-mail: daniele.de_sanctis@esrf.fr

SorC transcriptional regulators are common regulators in prokaryotes. Here we report the first crystal structure of a full-length SorC, the sorbitol operon regulator SorC from Klebsiella pneumoniae, the prototype of its family. SorC was found to be a homotetramer (which seems to be the biologically active form) that is able to recognize its DNA operator. The tetramer can be regarded as a dimer of dimers, with each dimer being composed of two identical subunits in different conformations. The DNA-binding domains divergently protrude from the core of the tetramer, suggesting that SorC may bind its operator in two distinct regions. The sugar-binding domain presents the same fold identified in members of the SorC family that shows some features identified as specific for sugar recognition. An in

silico analysis shows that the localization of the putative sugar-binding site is close to the dimeric interfaces. This supports the proposal of a new mechanism of transcriptional regulation that is in complete agreement with functional studies recently reported on a protein belonging to the same family.

Keywords: SorC; sorbitol operon regulator; X-ray crystallography; transcriptional regulation

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Crystallographic Analysis of the PAS Domain of RsbP, a Stress-Response Phosphatase in *Bacillus Subtilis.*

Masatomo Makino[a], Takashi Kumasaka[a]. [a]*JASRI/SPring-8, Japan.*
E-mail: makino@spring8.or.jp

The bacterium *Bacillus subtilis* exploits a signaling cascade to respond to deleterious effects of stresses or sudden changes in the environment. Through the cascade, stress information converges on and is transmitted to the general stress response transcription factor σ^B, leading to the expression of more than 200 general stress genes to exclude stresses or to repair the damage. The activity of σ^B in forming the complete transcriptional complex is governed by the signaling cascade with two distinct branches, which are composed of a set of signal transduction proteins termed *r*egulators of *s*igma *B* (Rsb). One branch is specific for energy stresses, such as carbon, phosphate, or oxygen limitation, and the other is specific for environmental stresses, such as acid, ethanol, heat, or salt shock. Each branch terminates with two serine phosphatase RsbP and RsbU. Both of them dephosphorylate the common downstream factor, RsbV. Depending on the phosphorylation state of RsbV, RsbW regulates σ^B activity *via* a partner-switching mechanism. RsbP is an essential protein to sense energy limitation in conjunction with the α/β hydrolase RsbQ that is co-expressed with RsbP from the *rsbQP* operon. The amino acid sequence of RsbP indicates that it is comprised of three domains: Per-Arnt-Sim (PAS) domain, coiled-coil domain and PPM/PP2C Ser/Thr phosphatase domain. Since PAS domains are structural modules that can be found in proteins in all of life and are involved in many signaling proteins where they are used as a signal sensor domain, RsbP-PAS is expected to sense energy stress information. We have crystallized the recombinant RsbP-PAS using 40% PEG400 as a precipitant. The crystals belong to space group $P2_1$, with unit cell parameters of *a*=55.2, *b*=71.7, *c*=60.2 Å, β=92.1°. The X-ray crystal structure of RsbP-PAS was determined by the SeMet SAD phasing at a resolution of 1.7 Å. RsbP-PAS maintains a typical PAS fold with 5 α-helices and 5 β-strands, and forms a homodimer through mainly hydrophobic interactions between the two monomers. Two small and highly hydrophobic cavities are located in the protein core region, which closely match the ligand/co-factor binding sites found in other PAS domains. These results suggest that RsbP senses some small hydrophobic compounds transmitted from RsbQ in the protein core as stress signals. Slight structural rearrangements around the cavities are predicted upon ligand binding, which may initiate a switch in regulating RsbP's phosphatase activity.

Keywords: signal transduction; structure-function relationships; macromolecular crystallography

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Structure of a Secondary Vitamin D_3 Binding Site of Milk β-Lactoglobulin for Vitamin D Transport.

Chun-Jung Chen[a], Ming-Chi Yang[b], Hong-Hsiang Guan[a], Jinn-Moon Yang[b], Wen-Liang Chen[b], Simon J. T. Mao[b]. [a]*Life Science Group, Research Division, National Synchrotron Radiation Research Center.* [b]*Department of Biological Science and Technology, National Chiao Tung University, Hsinchu, Taiwan.*
E-mail: cjchen@nsrrc.org.tw

β-lactoglobulin (LG), one of the most investigated proteins, is a major bovine milk protein with a predominantly β structure. The structural function of the only α-helix with three turns at the C-terminus is unknown. Vitamin D_3 binds to the central calyx formed by the β-strands. Whether there are two vitamin D binding-sites in each LG molecule has been a subject of controversy. Here, we report a second vitamin D_3 binding site identified by synchrotron X-ray diffraction (at 2.4 Å resolution) and fluorescence spectral analyses. In the central calyx binding mode, the aliphatic tail of vitamin D_3 clearly inserts into the binding cavity, where the 3-OH group of vitamin D_3 binds externally through interacts with the carbonyl of Lys60 forming a hydrogen bond. The second binding site, however, is near the surface at the C-terminus (residues 136-149) containing part of an α-helix and a β-strand I. A remarkable feature of the second exosite is that it combines an amphipathic α-helix providing non-polar residues (Phe136, Ala139, and Leu140) and a β-strand providing a non-polar (Ile147) and a buried polar residue (Arg148). They are linked by a hydrophobic γ-turn loop (Ala142, Leu143, Pro144, and Met145). Thus, the binding pocket furnishes strong hydrophobic force to stabilize vitamin D_3 binding. Using site-directed mutagenesis, we showed that the γ-turn loop play a crucial role in the binding. Using mice as an animal model, we demonstrated that LG is a major fraction of milk proteins responsible for uptake of vitamin D. Most interestingly, dosing mice with LG supplemented with vitamin D_3 revealed that native LG containing two binding sites gave a saturated concentration of plasma 25-hydroxyvitamin D at a dose ratio 2:1 (D_3:LG), whereas heated LG containing one exosite (lacking a central calyx) gave a ratio 1:1. This finding provides a new insight into the interactions between vitamin D_3 and LG, in which the exosite may provide another route for the transport of vitamin D_3 in vitamin D_3 fortified dairy products. LG has a functional advantage in the transport of vitamin D, indicating that supplementing vitamin D in milk effectively enhances its uptake.

[1] Yang, M.-C., Guan, H.-H., Liu, M.-Y., Lin, Y.-H., Yang, J.-M., Chen, W.-L., Chen, C.-J., Mao, S. J. T., *Proteins*, **2008**, 71, 1197. [2] Yang, M.-C., Guan, H.-H., Yang, J.-M., Ko, C.-N., Liu, M.-Y., Lin, Y.-H., Huang, Y.-C., Chen, C.-J., Mao, S. J. T., *Crystal Growth & Design*, **2008**, 8, 4268. [3] Yang, M. C., Chen, N. C., Chen, C.-J., Wu, C.-Y., Mao, S. J. T., *FEBS J.*, 276, 2251.

Keywords: vitamin D; β-Lactoglobulin; transport