KAIST, Chemistry, 373-1 Kusong-dong, Yusong-gu, Daejeon, N/A, 305-701, Korea (S), E-mail：jieoh.lee@kaist.ac.kr

Toll-like Receptors (TLRs) are central to vertebrate innate immune responses. To facilitate soluble expression and crystallization of human TLRs with bound ligands, we have developed a novel technique that we term the Hybrid LRR Technique. The hagfish VLR proteins were chosen as the fusion partners and connected to human TLRs at the conserved LxxLxLxxN regions. The hybrid LRR technique neither interrupts function of TLR nor causes substantial structural changes. TLR4 and MD-2 form a heterodimer that recognizes LPS from Gram negative bacteria. TLR2 in association with TLR1 or TLR6 responses to microbial lipoproteins and lipopeptides. The crystal structures reveal that TLR1, 2 and 4 are atypical members of the LRR family and are composed of N-terminal, central and C-terminal domains. The beta sheet of the central domain shows unusually small radii and large twist angles. MD-2 binds to the concave surface of the N-terminal and central domains of TLR4. The interaction with Eritoran, a candidate anti-sepsis drug, is mediated by a hydrophobic internal pocket in MD-2. Binding of the tri-acylated lipopeptide, Pam3CSK4, induced the formation of an m shaped heterodimer of the TLR1 and TLR2 ectodomains whereas binding of the di-acylated lipopeptide, Pam2CSK4 did not. The three lipid chains of Pam3CSK4 mediate the heterodimerization of the receptor; the two ester-bound lipid chains are inserted into a pocket in TLR2, while the amide-bound lipid chain is inserted into a hydrophobic channel in TLR1. An extensive hydrogen bonding network, as well as hydrophobic interactions, between TLR1 and TLR2 further stabilize the heterodimer. We propose that formation of the TLR dimer brings the intracellular TIR domains close to each other to promote dimerization and initiate signaling.

Keywords: protein structure, TLR, LRR

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Crystal structure of the sodium pump at 3.5 Å

Jens P Morth[1], Bjoern P Pedersen[1], Mads S Toustrup-Jensen[2], Thomas LM Soerensen[1,3], Janne Pedersen[2], Jens-Peter Andersen[2], Bente Vilsen[2], Poul Nissen[1]

[1]Aarhus University, Department of Molecular Biology, Gustav Wieds Vej 10C, Aarhus, Denmark, 8000, Denmark, [2]Department of Physiology, Institute of Physiology and Biophysics, University of Aarhus , Ole Worms Alle, DK-8000 Aarhus C, Denmark, [3]Diamond Light Source Ltd, Diamond House, Chilton, Didcot, Oxfordshire, OX11 0DE, UK, E-mail：jpm@mb.au.dk

The Na^+,K^+-ATPase, the sodium-potassium pump, was first described in 1957 by Jens C. Skou (1) - a discovery for which he was awarded the Nobel prize in Chemistry in 1997. The Na^+,K^+-ATPase belongs to the P-type ATPase family, and via formation and break-down of phosphoenzyme intermediates it derives the energy from ATP hydrolysis to pump Na^+ out of the cell and K^+ into the cell, thereby energizing the plasma membrane with steep electrochemical gradients for these key cations. The Na^+,K^+-ATPase is a hetero-trimeric complex composed of an α, β and γ chain that all contain transmembrane segments. A complete native dataset was obtained at 3.5 Å resolution on the X06SA beam line at the Swiss Light Source (SLS). The brilliant light source present at SLS was necessary to obtain useful data from these very weakly diffracting crystals. A low resolution molecular replacement solution allowed us to identify two heavy-atom derivatives by difference-Fourier analysis forming the basis for MIRAS phasing at 7 Å resolution. The crystal form has 75% solvent and contains two-fold NCS. Careful density modification with NCS and inter-crystal averaging was applied and extended the MIRAS phases to 3.5 Å resolution thus allowing for model building and refinement of the structure. This the first structure of the Na^+,K^+-ATPase in the K^+/Rb-bound form contains a nearly complete model of the α -subunit and shows the location of the transmembrane helices of the beta and gamma subunits associated with alpha. Two strong peaks in the anomalous difference Fourier map pinpoint the position of two occluded Rb^+ sites (K^+)(2).

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Structural studies of pre-mRNA 3'-end processing

Liang Tong, Song Xiang, Corey R Mandel, Yun Bai, James L Manley

Columbia University, Biological Sciences, 1212 Amsterdam Avenue, New York, NY, 10027, USA, E-mail：ltong@columbia.edu

Most eukaryotic messenger RNA precursors (pre-mRNAs) must undergo extensive maturational processing, including 3'-end cleavage and polyadenylation. Despite the characterization of a large number of proteins that are required for the cleavage reaction, the identity of the endoribonuclease is not known. In addition, very little structural information is available for the proteins involved in 3'-end processing, which include the cleavage and polyadenylation specificity factor (CPSF), cleavage stimulation factor (CstF), cleavage factors I and II (CFI and CFII), and poly(A) polymerase. This 3'-end processing machinery also plays an important role in transcriptional termination by RNA polymerase II. We have recently determined the crystal structures of the 73 kD and 100 kD subunits of CPSF (1), as well as the 77 kD subunit of CstF (2). Our studies provide direct structural and biochemical evidence that CPSF-73 is the endoribonuclease for the cleavage reaction and reveal a dimeric association of the CstF-77 subunit that may be crucial for pre-mRNA 3'-end processing. Our latest results on other factors in this machinery will also be presented. Crystallization of CPSF-100 and CstF-77 required in situ proteolysis (3,4), and this attractive technique of crystallization will be described as well. Supported in part by a grant from the NIH.

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