the transmembrane segments harboring the two Ca^{2+} transport sites. Previous work has identified the mechanism for phosphorylation of the enzyme coupled to occlusion of Ca^{2+} ions in the membrane, and also the dephosphorylation step coupled to counter-cation occlusion has been described. The missing link of the reaction cycle has been the E2P'ground state' where the Ca^{2+} ions after translocation across the membrane are exchanged for protons via a'long sought' luminal opening. Here we present functional studies and a new crystal structure of Ca^{2+}-ATPase in complex with beryllium fluoride representing the phosphorylated E2P form with full exposure of the luminal exit pathway. The exit path has developed as a trilobed opening between the transmembrane segments M1 through M6 which are found in all P-type pumps and it exposes the Ca^{2+} transport sites to the luminal space. The conformational change is actuated by a rotation of the A-domain bringing the dephosphorylation TGES motif in close apposition to phosphorylation site in recognition of phosphorylation. The release of Ca^{2+} ion is promoted by movement of the M4 helix, exposing the Ca^{2+} coordinating directly towards the lumen

Keywords: Ca^{2+}-ATPase, luminal opening, proton/ion exchange

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Recognition and catalysis of ribosomal protein L11 by the protein trimethyltransferase PrmA

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Bacterial ribosomal protein L11 is post-translationally trimethylated at multiple residues by a dedicated methyltransferase, PrmA. Here, we describe multiple apoenzyme structures and enzyme-substrate complexes of PrmA from the extreme thermophile T. thermophilus. We have determined two apo-PrmA structures at 1.59 and 2.3 Å and two structures with bound cofactor AdoMet at 1.75 and 2.9 Å resolution. A PrmA-L11 enzyme-substrate complex structure at 2.4 Å resolution illustrates the highly specific interaction of the PrmA N-terminal domain with its substrate and places the L11 ε -amino group of Lys39 in the active site in a pre-catalytic state. Two other complex structures at 2.56 and 1.75 Å resolution show the di- and trimethylated α-amino group of Met1 and AdoHcy in the intermediate and post-catalytic states. In addition, we have determined the structures of two active site mutants H104A and T106Y complexed with L11 at 2.1 and 2.35 Å resolution. A structure of the truncated PrmA substrate recognition domain complexed with the L11 N-terminal domain at 1.37 Å suggests that a single recognition mode is sufficient to place all substrate side chains in the active site. All structures of PrmA exhibit distinct relative positions of the substrate recognition and catalytic domains, revealing how the remarkable plasticity of PrmA can position the L11 substrate for multiple, consecutive side-chain methylation reactions. The presence of a unique flexible loop in the cofactor-binding site suggests how exchange of AdoMet with the reaction product AdoHcy can occur without necessitating the dissociation of PrmA from L11. Finally, the mode of interaction of PrmA with L11 explains its preference for L11 as substrate before its assembly into the 50S ribosomal subunit.

Keywords: post-translational modification, methylation, multi-specificity

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The structural analysis of BUB1 and BUBR1 reveals their role in the mitotic checkpoint

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Premature separation of sister chromatids results in the loss or gain of chromosomes in daughter cells. This condition is a prevalent form of genetic instability observed in various classes of human cancer. BUB1 and BUBR1 are central components of the mitotic checkpoint control, the regulatory mechanism of the cell cycle that ensures the even distribution of chromosomes during the transition from metaphase to anaphase and prevents dividing cells from such disastrous scenario. BUB1 and BUBR1 are multidomain proteins consisting of a conserved N-terminal region that is essential for efficient mitotic checkpoint function, an intermediate disorder region that contains the binding site of the upstream regulator BUB3, and a catalytic C-terminal domain of serine/threonine kinase activity. We present the high-resolution crystal structures of the N-terminal region of BUB1 and BUBR1. The two domains are organised as a divergent tandem arrangement of alpha-helices and show conserved residues that are important in determining the relative orientation between neighbour alpha-helices and, consequently, of the overall shape of the protein. The projection of the degree of sequence conservation onto the surface of BUB1 and BUBR1 reveals characteristic features, including an alternating pattern of loop conservation throughout most of the sequence. We mapped onto the structure the various mutations that have been identified and associated with aneuploidy and cancer progression and provide a structure-based explanation of how these mutants lead to the impairment of mitotic checkpoint activity. The crystal structures contribute to the understanding of how genetic alterations of this domain impair the functions of these proteins, resulting in a defective mitotic checkpoint response.

Keywords: BUB1/BUBR1, mitotic checkpoint, cancer

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Structure of a Swi2/Snf2 protein (RapA) and mechanism of RNAP recycling during transcription

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Members of the Swi2/Snf2 family mediate mobilization of various nucleic acid-protein complexes to facilitate gene expression. Bacterial RapA is an RNA polymerase (RNAP)-associated Swi2/Snf2 enzyme with ATPase activity. It stimulates RNAP recycling during transcription. Here, we report the first structure of RapA, which is also the first full-length structure for the entire Swi2/Snf2 family. Our structure and in vitro competition experiment establish that RapA and σ70 share a common binding site on RNAP and that σ70 exhibits higher affinity for the core RNAP (CORE). The structure also indicates that RapA may facilitate the release of sequestered RNAP from DNA template, which is demonstrated by our kinetic template-switching assay, showing the recycling of RNAP in vitro