MS6-O4 Crystal structure of the 239 kDa nuclear export complex CRM1 - RanGTP - Snurportin1 - Nup214 - MBP

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In eukaryotic cells nucleocytoplasmic transport of macromolecules is a sophisticated active process. It proceeds through nuclear pore complexes (NPCs) mediated by soluble nuclear transport receptors of the karyopherin-β superfamily termed importins and exportins. During passage through the NPC, importins and exportins transiently interact with the intrinsically disordered phenylalanine-glycine (FG-) repeat-domains of the nucleoporin proteins (Nups). These transient interactions with FG-Nups are necessary to overcome the nuclear envelope barrier and thus are crucial for all nuclear transport events. CRM1 is the major and most versatile nuclear exportin. Several crystals structures of different functional CRM1 complexes have been determined, revealing the structural basis for the cooperativity of cargo and RanGTP binding [1-3]. However, detailed structural insight into the interaction of CRM1 with the nuclear pore complex has remained an enigma.

Here, we present the crystal structure of a nuclear export complex comprising CRM1, the cargo Snurportin1 and the GTPase Ran, and a 117 amino acid FG-repeat containing fragment of Nup214 fused to MBP [4]. Optimization of protein constructs, seeding and the development of a sophisticated protocol including successive PEG-mediated crystal dehydration as well as additional post-mounting steps were pivotal to obtain well diffracting crystals and to solve the crystal structure [5]. The crystal structure, which was refined at 2.85 Å resolution, shows eight binding sites on CRM1 for Nup214 FG-motifs, with its intervening sequences loosely attached to the transport receptor. Interestingly, Nup214 binds to N- and C-terminal regions of CRM1, thereby clamping CRM1 in a closed conformation and stabilizing the export complex. The role of these hydrophobic pockets for the recognition of FG-motifs was analysed by means of biochemical and cell-based assays.

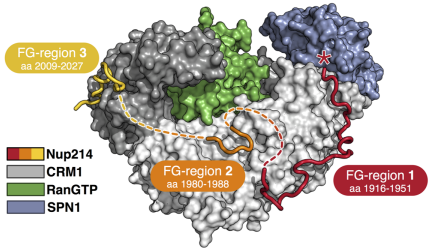

Figure 1. Structure of the quarternary export complex CRM1-RanGTP-Snurportin1-Nup214 reveals 3 binding regions for FG-repeats. The MBP fused to the N-terminus of the Nup214 fragment is not shown.

Keywords: Nucleocytoplasmic transport, crystal dehydration

References

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