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An allosteric mechanism to displace nuclear export cargo from CRM1 and RanGTP by RanBP1

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Introduction

The karyopherin CRM1 mediates nuclear export of proteins and ribonucleoproteins bearing a leucine-rich nuclear export signal (NES). CRM1 is a ring-shaped molecule constructed from 21 tandem HEAT repeats. Leu-rich NES at N-terminus of Spn1 that adopts a combined α-helical-extended conformation binds to a hydrophobic cleft on the convex surface of CRM1, between helices 11A and 12A [1]. Structural studies of CRM1 so far revealed only the NES-cargo-bound conformation of CRM1 and so the key question of how cargo is released in the cytoplasm has not been addressed unequivocally. Here we determined a crystal structure of CRM1:RanBP1:RanGTP complex at 2.0-Å resolution. The structure highlighted an inactive conformation of CRM1 (i.e. a conformation that is unable to bind NES-cargo) and revealed a remarkably efficient mechanism by which the long intra-HEAT9 loop of CRM1 functions as an allosteric autoinhibitor to control NES-cargo loading and unloading.

Methods

Crystals of yeast CRM1:RanBP1:RanGTP complex were grown at 20°C from 6.2 mg/ml protein by hanging drop vapor diffusion. The crystal had $P4_32_12$ symmetry with one complex in the asymmetric unit. The structure was solved by molecular replacement using MOLREP. Iterative cycles of modelbuilding using COOT and refinement using REFMAC5 yielded a final model with R_{free} 22.1 % (R_{cryst} 17.5 %).

Results and Discussion

The structure of CRM1:RanBP1:RanGTP complex suggested that a long hairpin loop connecting the A- and B-helices of HEAT repeat 9 (hereafter referred to as HEAT9 loop, Figure 1) is important in the long-range communication between RanBP1 and the NES-binding site. The binding of RanBP1 would displace HEAT9 loop because the acidic C-terminal residues of Ran would clash with HEAT9 loop [2]. Unexpectedly, the structure of the CRM1:RanBP1:RanGTP complex showed that the displaced HEAT9 loop in turn binds to the inner surface of HEAT repeats 11 and 12, immediately adjacent to the NES-binding site, and the way HEAT9 loop interacted with HEAT repeats 11 and 12 provided structural insight into NES release mechanism (Figure 1). The highly conserved hydrophobic residues (Val441, Leu442, Val443 and Ile451 in yeast CRM1) of the HEAT9 loop are involved in this interaction and, although the hydrophobic side chains of the corresponding residues are mostly exposed to solvent in CRM1:Spn1:RanGTP complex, these residues pack intimately against the nonpolar patch on the CRM1 inner surface in CRM1:RanBP1:RanGTP complex. The intimate packing of hydrophobic side chains at the interface is probably optimized by rotations of the side chains of Met594 (on 12B helix) and Met556 (on 11B helix) towards HEAT9 loop, and movement of Phe583 (on 12A helix) towards HEAT11, concomitant with rotation of 12B helix and movement of 12A helix closer to 11A helix. As a consequence, HEAT repeats 11 and 12 pack intimately against each other, and there is no room to accommodate hydrophobic side chains of Leu-rich NES between the outer helices 11A and 12A in CRM1:RanBP1:RanGTP complex (Figure 1).

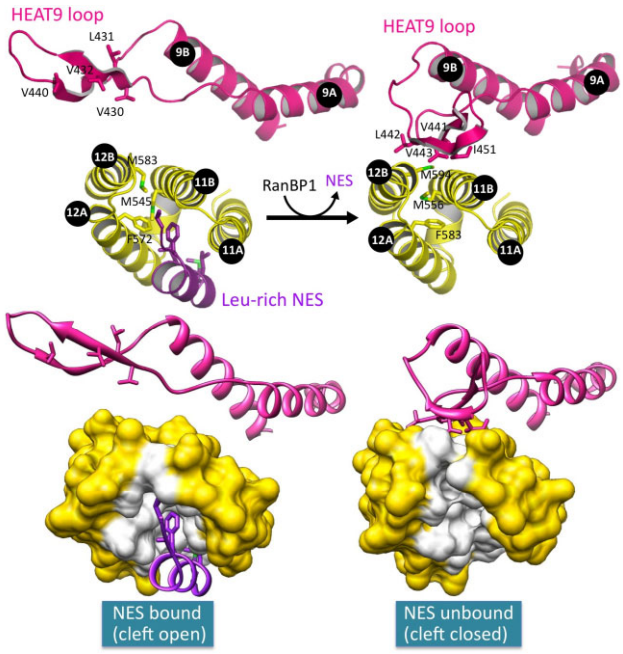

Figure 1 Structural rearrangements of the NES-binding cleft associated with RanBP1 binding and NES release. Left, CRM1:NES:RanGTP complex [1]; right, CRM1:RanBP1:RanGTP complex [2]. HEAT9, magenta; HEAT11 and 12, yellow; Leu-rich NES, purple.

References

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