Structural Analysis of the Complex between Rab27a and Exophilin4/Slp2-a

The Rab27 subfamily of Rab GTPases regulates the exocytosis of secretory granules in a cell-type dependent manner, by cooperating with multiple organelle-specific effectors. Among 19 Rab27-interacting proteins characterized so far, Exophilin4/Slp2-a mediates the peripheral localization of melanosome and glucagon granules by bridging Rab27a to plasma membrane phospholipids. The crystal structure of Rab27a in complex with Slp2-a solved at 1.8 Å resolution reveals unique features of Slp2-a in contact with Rab27a, providing new insights into the molecular basis for Rab27a/b recruitment by their effectors.

The integrity of eukaryotic cells strongly depends on the tight regulation of membrane trafficking, in particular through the combined actions of the Ras super-family of small GTPases. Members of the Rab family, in particular, act as essential regulators of vesicular transport events through a GTP-bound dependent recruitment of various effectors, thus providing key elements for the regulation of vesicular-trafficking machinery. Rab27a and Rab27b isoforms, together with their associated effectors, have been implicated in human diseases. In order to understand more deeply the differences between Rab27 and other Rabs, and to unravel the molecular basis behind its specific recruitment of effectors, we solved the structure of the complex Rab27a with Slp2-a.

Both Rab27a and the Rab27-binding domain (RBD27) of Slp2-a were independently expressed in bacteria as recombinant proteins, and then purified as a monodisperse soluble sample. Initial crystals of the complex were obtained using the PXS crystallization robot [1], and further refined manually. The crystals belonged to the space group $P2_12_12_1$, with unit cells a = 53.51, b = 77.78, and c = 115.01 Å, respectively [2]. A full data set was collected at BL-5A, at a resolution of 1.80 Å. The structure of the complex was solved by molecular replacement using a chimeric structure as a search model that consisted of the coordinates of a single GDP-bound Rab27b molecule [3] in which the switch regions were substituted with those from the GTP-bound Rab3a (PDB coordinates 2if0 and 1zbd, respectively) [2]. The structure was refined, analyzed and compared with other GTPase:effector complexes using programs of the *CNS* and *CCP4* suites [4].

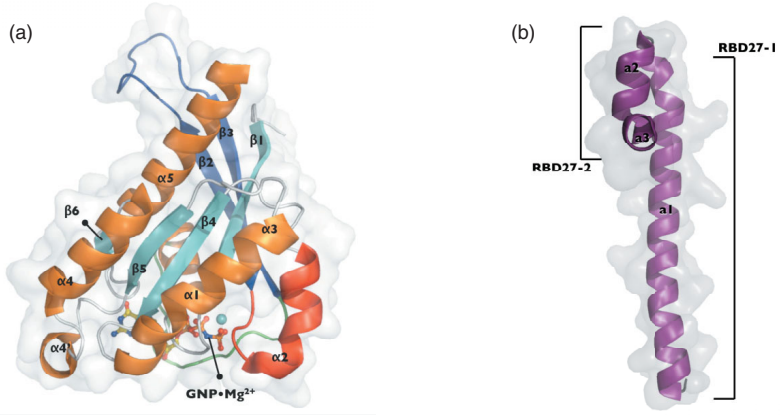

Figure 1
Overall structure. Ribbon diagram representation of Rab27a (a) and Slp2-a (b), together with molecular surfaces shadowed in the background. The switch elements (switch I, interswitch, and switch II) of Rab27a are highlighted in green, blue, and red, respectively. The GTP-analogue (*GNP*) bound to one Mg ion is represented as a ball-and-stick model.

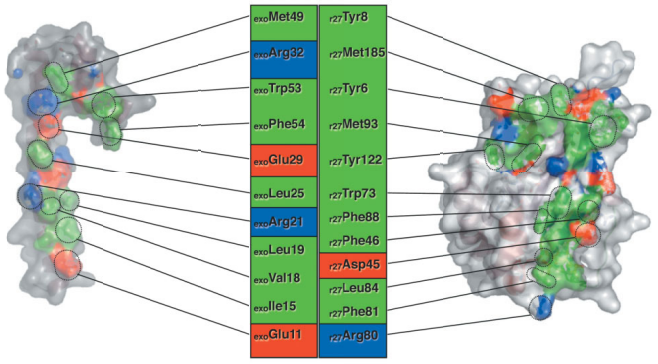

Figure 2
Features of the complex formation. Interacting molecular surfaces of Slp2-a (left) and Rab27a (right). Hydrophobic, basic, and acidic residues responsible for complex formation are colored in green, blue, and red, respectively.

The structure of Rab27a in its GTP-bound form exhibits a typical monomeric small GTPase globular fold [Fig. 1(a)]. The Slp2-a RBD27 structure consists of a long N-terminal α helix (RBD27-1) followed by shorter C-terminal α helices (RBD27-2) in a hook-like arrangement [Fig. 1(b)]. In the complex structure, Rab27a and Slp2-a share an extensive hydrophobic interface, encircled by electrostatic interactions via charged residues highly conserved within RBD27-containing effectors (Fig. 2). More precisely, the effector packs against the switch and interswitch elements of Rab27a, notably with the RBD27-1 binding interface of the GTPase centered at an invariant hydrophobic triad of residues. The specific affinity towards Rab27a is modulated at the RBD27-2 interface, with a tight surface complementation between the effector WFY motif and the Rab27a Tyr122 residue. The observed structure complementation between the interacting surfaces of Rab27a and Slp2-a sheds light on the disparities among the Rab27 effectors and outlines a general mechanism for their recruitment.

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