# Structural Basis for the Exclusive Specificity of Slac2-a/Melanophilin for the Rab27 GTPases

Melanin, a pigment found in hair and skin, plays important roles in protecting the human body against radiation damage, but leaves unfavorable dark pigments in the skin. Melanin is made by skin color cells known as melanocytes and is stored in intercellular vesicles called melanosomes. Melanosomes mature near the nucleus, transfer along microtubules and actin filaments to the cellular membranes, distribute to skin cells called keratinocytes, and then pigment skin and hair.

Rab27A, a member of the monomeric Ras-like GTPase superfamily, is a key player in melanosome transport in skin melanocytes, through interactions with two specific effector molecules, Slac2-a/melanophilin and Slp2-a/Exophilin4. In melanocytes, Rab27A forms a transport complex with Slac2-a and myosin Va (an actin-based motor). The resulting tripartite protein complex regulates melanosome transfer from microtubules to actin filaments as well as subsequent actin-based melanosome transport. After actin-based melanosome transport, Rab27A interacts with Slp2-a, another Rab27-specific effector, and promotes the anchoring of melanosomes to the plasma membrane. The former interaction seems to be more crucial for melanosome transport, because mutations of Rab27A or Slac2-a cause human Griscelli syndrome, which is characterized by pigment dilution in hair (i.e., defect in melanosome transport in melanocytes) as well as immunodeficiency.

More than 60 Rab isoforms have been reported in humans, and they appear to be present on distinct organelle membranes, where they regulate certain types (or distinct steps) of membrane trafficking. Rab27B, a closely related isoform of Rab27A (72% sequence identity), also binds all of the known effectors of Rab27A including Slac2-a and Slp2-a. Thus far, nothing is known about the structural basis of the Rab27•Slac2-a interaction, although this information is critical for the molecular diagnosis of human Griscelli syndrome as well as for understanding the mechanism by which Slac2-a specifically recognizes Rab27A/B, but not the other Rab proteins.

Rab, like other G proteins, functions as a molecular switch by cycling between two nucleotide-bound states, a GDP-bound inactive state and a GTP-bound active state. The GTP-bound active form of Rab27A interacts with its specific effector molecules, such as Slac2-a and Slp2-a, and the resultant Rab27A•Slac2-a and Rab27A•Slp2-a complexes promote melanosome transport. Because active Rab27 proteins are notoriously difficult to crystallize, we generated a GTPase-deficient mutant of Rab27B, and succeeded in crystallizing the active Rab27B complexed with the effector domain of Slac2-a and solved its crystal structure [1]. The structure analysis was carried out using beamline **BL41XU**.

The structure of the Slac2-a effector domain, in complex with Rab27B, comprises a coiled-coil and a zinc-binding subdomain (Fig. 1). The main interface of Slac2-a with Rab27B is formed by the coiled-coil. In the Rab27B side, the switch, interswitch and Rab complementarity-determining regions (RabCDR) are involved in the interaction. In the Rab27B•Slac2-a interface, we found many hydrophobic interactions and several electrostatic interactions. Most of the Slac2-a-interacting residues of Rab27B are conserved in Rab27A. These include the Rab27A residue Trp73, whose mutation to Gly is found in type 2 Griscelli syndrome patients (Fig. 2). The Slac2-a residue Arg35, whose mutation to Trp is found in type 3 Griscelli syndrome patients, is also involved in the Rab27B

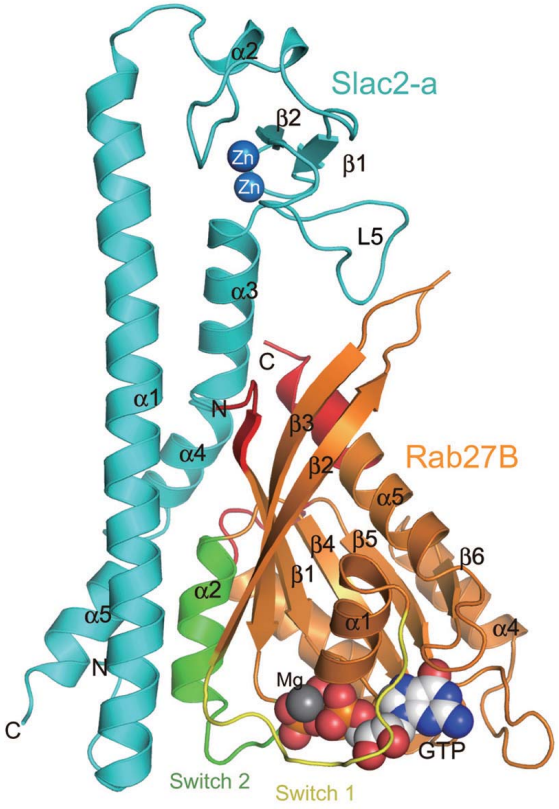


Fig. 1. Crystal structure of the Rab27B•Slac2-a complex.

interaction. Therefore, we suggest that Griscelli syndrome would be caused by the disruption of the complex formation between Rab27A and Slac2-a.

Rabphilin, a Rab effector, binds several subfamilies, including Rab3 and Rab27, while Slac2-a exclusively binds the Rab27 subfamily (Rab27A and Rab27B). Precise comparison of the Rab27B•Slac2-a complex with the previously reported Rab3A•rabphilin complex [2] revealed several intermolecular hydrogen bonds that are specific between Rab27B and Slac2-a. Rab27A mutations that disrupt any of these specific hydrogen bonds with Slac2-a resulted in the dramatic reduction of Slac2-a binding activity (Fig. 3(a)). Furthermore, we found that transplantation of only four Rab27-specific residues into Rab3A was sufficient to make the Rab3A mutant associate with Slac2-a (Fig. 3(b)).

The present Rab27B•Slac2-a complex structure may be used as a basis for the development of drugs for the treatment of rare, lethal Griscelli syndrome for which there is no effective treatment. It is also hoped that the regulation of melanosome transport in skin melanocytes will enable the development of cosmetics that are effective against skin pigmentation.

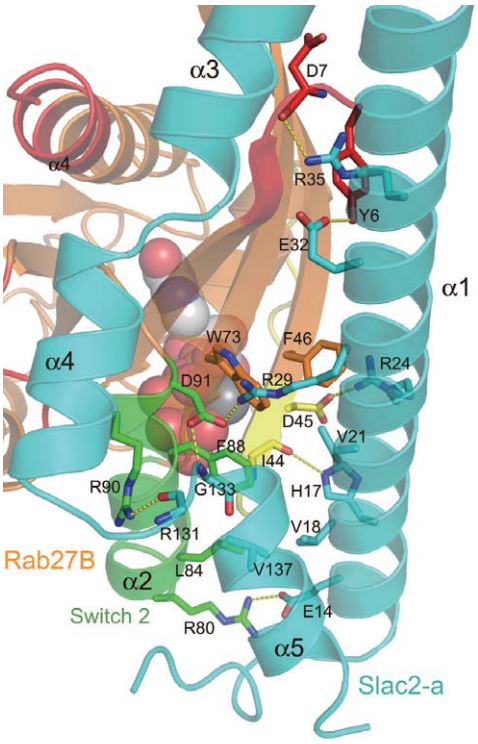


Fig. 2. Rab27B•Slac2-a interfaces, which can explain the structural basis of the human diseases caused by mutations in Rab27A and Slac2-a.

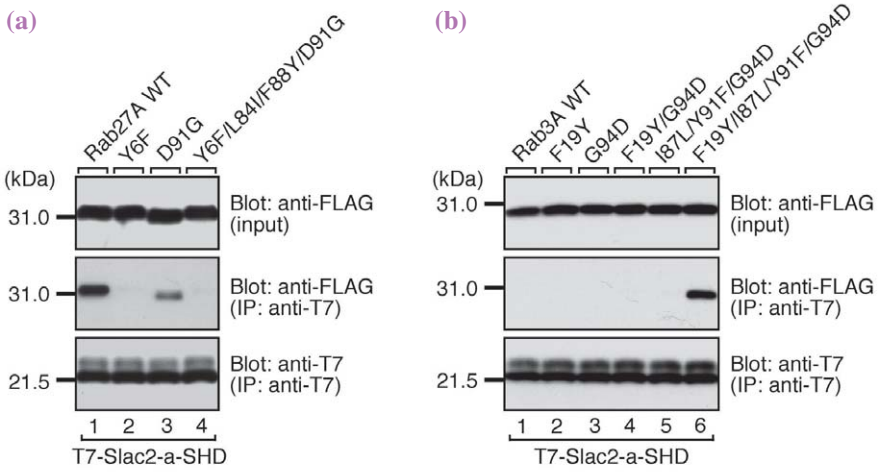


Fig. 3. Mutational analyses of Rab27A **(a)** and Rab3A **(b)** showing the requirement of specific hydrogen bonds for the Rab27A•Slac2-a interaction.

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**References**

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[2] C. Östermeier and A.T. Brunger: Cell **96** (1999) 363.