Synopsis of Original Research Paper

Elucidation of the Degradation Mechanism of Melanosomal Protein Slac2-a.

Mitsunori Fukuda

Fukuda Initiative Research Unit, RIKEN

Slac2-a/melanophilin was recently identified as the "missing link" between the small GTPase Rab27A and the actin-based motor protein myosin Va. Formation of a tripartite protein complex by three molecules is essential for melanosome transfer from microtubules to actin filaments and subsequent actin-based melanosome transport in melanocytes. However, the regulatory mechanisms of the disassembly of the complex after actin-based melanosome transport had never been elucidated. In this study, we discovered that Slac2-a and a closely related isoform, Slac2-c, contain multiple PEST-like sequences (potential signals for rapid protein degradation) in the myosin Va- and actin-binding domains at the C terminus. We found that the C-terminal domain of Slac2-a/c is highly sensitive to low concentrations of proteases, such as trypsin and calpain, *in vitro*, whereas the N-terminal Rab27A-binding domain is highly resistant to these proteases. We further found that endogenous calpains selectively cleave Slac2-a, but not Rab27A or myosin Va, in melanocytes. A mutant Slac2-a lacking one of the PEST-like sequences located at the interface between the myosin Va- and actin-binding domains (ΔPEST; amino acids 399-405) is more stable than the wild-type protein, both *in vitro* and in melanocytes. Expression of the mutant Slac2-a-ΔPEST with an N-terminal green fluorescence protein tag often induced perinuclear aggregation of melanosomes (approximately 40% of the transfected cells) compared with the wild-type Slac2-a. Our findings suggest that protein degradation of Slac2-a is an essential process for proper melanosome distribution in melanocytes.