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STRUCTURAL CHARACTERISATION OF THE MELANOPHILIN-MYOSINVA COMPLEX

Principal Investigator:

Margarida ARCHER (Membrane Protein Crystallography Laboratory), Instituto Technologia Quinica Biológica, ITQB-UNL, Av. República, EAN, Oeiras, Portugal; archer@itqb.un.1.pt

Collaborators:

Miguel SEABRA, CEDOC, Faculdade de Ciências Médicas, FCM, Universidade Nova de Lisboa, 1169-056 Lisboa, Portugal and Molecular Medicine, National Heart and Lung Institute (NHLI), Imperial College London, London SW7 2AZ, UK

Intracellular transport in eukaryotic cells is regulated by actin and microtubule (MT)-based cytoskeletal networks and motor proteins of the myosin, dynein and kinesin families. Co-operative interaction between these transport and motor systems is crucial for the correct localisation and transport of membrane bound organelles such as melanosomes in melanocytes, Weibel-Palade bodies in endothelial cells, ER vesicles in neurons and secretory granules in pancreatic β-cells and immune cells amongst others. Rab proteins, a family of membrane associated small GTPases, are key regulators of organelle movement on actin tracks due to their ability to specifically recruit actin-based Myosin motors to the membrane of organelles upon which they reside. For example, Rab27a and MyosinVa (MyoVa) interact via Melanophilin (Mlph) to regulate melanosome motility in melanocytes, whilst Rab27a and MyosinVIIa interact via Myrip to regulate movement of melanosomes within the apical processes of Retinal Pigment Epithelial cells. Other examples include Rab11 and MyosinVb on recycling endosomes through a linker protein termed FIP2, and Rab8 and MyosinVI through a linker protein termed optineurin on the Golgi apparatus. Rab proteins recruiting Myosin motors to the membrane of organelles via an adaptor protein is a growing paradigm in the crucial process of switching membrane bound organelles from MT to actin-based transport.

The focus of the proposed work is the Mlph-MyosinVa complex. The group headed by Miguel Seabra has shown that in dermal melanocytes Mlph and MyoVa together with Rab27a regulates transport of melanosomes from their site of synthesis in the perinuclear region of the cell to the cell periphery where they can be transferred into adjacent keratinocytes. This process forms the basis of skin pigmentation and protection against UV damage. After synthesis melanosomes undergo fast bi-directinal movement along MT.

Rab27a, in its active form recruits Mlph which in turn recruits MyoVa to the membrane of melanosomes allowing tethering of the melanosome to the actin cytoskeleton at the periphery of the cell. Using a cell culture melanosome transport assay in Mlph knockout cells coupled with *in vitro* binding assays the functional domains of both Mlph and MyoVa required for recruitment of MyoVa to melanosomal membranes and efficient melanosome transport in cells have been defined. This analysis revealed a complex mechanism of interaction between Mlph and MyoVa with three defined regions in Mlph, including a putative coiled-coil region, involved in the interaction with the C-terminal cargo binding tail of MyoVa.

The aim of the proposed work is to characterize the structure of Mlph, the cargo binding tail of MyoVa and the Mlph-MyoVa cargo binding tail in complex. The student will express and purify recombinant Mlph and MyoVa cargo binding tail from either prokaryotic or eukaryotic expression systems using previously generated constructs. Informed by the functional characterization of Mlph and MyoVa described above the student will also generate truncation and point mutant variants of both Mlph and MyoVa and devise strategies to form and purify the Mlph-MyoVa complex *in vitro*. Crystallisation of the purified individual proteins and complex will be analysed in a number of screens and conditions. Sample quality will be continuously improved to obtain high quality crystals. X-ray diffraction data will be measured using our in-house system or at synchrotron sources to allow three-dimensional structure determination. Predictions obtained from the solved structure will then be tested in the cell based melanosome transport assay in the laboratory of Miguel Seabra.

Suggested Reading:

1. Seabra, M.C. and E. Coudrier, *Rab GTPases and myosin motors in organelle motility.* Traffic, 2004. **5**(6): p. 393-9.

2. Hume, A.N., et al., *Rab27a and MyoVa are the primary Mlph interactors regulating melanosome transport in melanocytes.* J Cell Sci, 2007. **120**(Pt 17): p. 3111-22.

3. Hume, A.N., et al., *A coiled-coil domain of melanophilin is essential for Myosin Va recruitment and melanosome transport in melanocytes.* Mol Biol Cell, 2006. **17**(11): p. 4720-35.

4. Hammer, J.A., 3rd and X.S. Wu, *Rabs grab motors: defining the connections between Rab GTPases and motor proteins.* Curr Opin Cell Biol, 2002. **14**(1): p. 69-75.

5. Geething N.C., *Identification of a minimal myosin Va binding site within an intrinsically unstructured domain of melanophilin* J. Biol. Chem. 2007. **282**(29) p. 21518-28.