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IN CELLULAR DEVELOPMENT AND DISEASE

P3

Ca^{2+}-dependent exocytosis of synaptic vesicles, chromaffin granules and lytic granules (Jens Rettig)

The Ca^{2+}-dependent exocytosis of vesicles containing intracellular cargo is one of the most important mechanisms in our body. Before these vesicles can fuse with the plasma membrane to release neurotransmitters, hormones and enzymes, they must undergo several maturation steps. Upon arrival at the plasma membrane vesicles are anchored by the physical interaction of vesicle proteins and integral membrane proteins. Following this docking process, vesicles must undergo a further maturation step, the priming process, in order to achieve fusion competence. In the proposed graduate research school we will focus on the proteins that mediate the docking of lytic granules at the immunological synapse (IS) formed between cytotoxic T cells and dendritic cells. Upon target cell recognition lytic granules are being transported to the IS where they fuse with the plasma membrane in order to release cytotoxic substances which kill the target cell. Mechanistically it must be secured that upon arrival at the IS the lytic granules are restrained from going back into the cytosol, most likely by physical interaction of granule and plasma membrane proteins. We have several candidate proteins for this function, with Munc18-2 being the most promising one. It has recently been shown that familial hemophagocytic lymphohistiocytosis type 5 (FHL5) is linked to mutations in the Munc18-2 gene. FHL is a rare, recessive immune disease that is lethal already at early age. We will express wild-type and mutant Munc18-2 isoforms coupled to XFP in human T cells and will investigate their localization upon IS formation. For this purpose we will take advantage of three high-resolution microscopy techniques that are established in our lab. Total internal reflection fluorescence (TIRF) microscopy allows the quantitative analysis of molecule or organelle movement at the cell/surface interface. Since lytic granules will be labeled simultaneously with Perforin-TFP, we can follow the arrival and fusion of lytic granules in real time and correlate their behaviour to the presence of wild-type and mutant Munc18-2, respectively. Structured illumination microscopy (SIM) and photo-activated localization microscopy (PALM) enables us to increase the resolution of fluorescence microscopy to 100 nm and 40 nm, respectively. This increase in resolution is particularly useful to investigate whether Munc18-2 is colocalizing with potential interaction partners, e.g. on intracellular organelles. We will pay particular attention to SNARE proteins of the Qa-class, since it has been shown in neurons that Munc18 is required to transport the Qa-SNARE syntaxin1a to the active zone of synapses. The imaging experiments will be complemented with biochemical analyses (e.g. immunoprecipitation) to identify and verify potential interaction partners of Munc18-2. In addition, we hope to identify further molecules involved in the docking reaction and understand their interplay to mediate this essential granule maturation step.