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Munc13-4 serves a conserved function in maturation of secretory lysosomes in immune cells, while tethering at the plasma membrane requires munc13-4 - rab27a complex

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Cytotoxic lymphocytes are critical in the immune response against virus infections. The cytotoxic function is exerted through recognition of target cells, followed by regulated exocytosis of effector molecules from lytic storage granules-secretory lysosomes. We showed before that the multidomain protein munc13-4 is a rab27a effector, two proteins known to be essential for a late step in degranulation. Mutants in rab27a cause Griscelli Syndrome type 2, while loss of munc13-4 causes Familial Hemophagocytic Lymphohistiocytosis type 3, a related auto-immune disorder. Munc13-4 also may have an upstream function in assembly of a exocytic endosomal precursor in maturation of secretory lysosomes. In spite of this progress, the molecular principles underlying directed membrane trafficking in secretory lysosome degranulation are not understood, and a requirement for a complex between munc13-4 and rab27a in the lytic degranulation pathway is unclear.

Through the use of truncations and point mutants in binding assays, we identified a unique patch in munc13-4 that is essential for rab27a binding and not conserved in other rab27a effectors or munc13 proteins. Complementation experiments with these munc13-4 point mutants (in the absence of endogenous munc13-4) showed that the role of munc13-4 in granule maturation is conserved in haematopoietic cells, and importantly, does not rely on rab27. In striking contrast, the same munc13-4 point mutants failed to rescue degranulation in the absence of endogenous munc13-4. Further analysis using TIRF imaging showed that the rab27a-munc13-4 complex is necessary for docking of secretory lysosomes with the plasma membrane.

Our results uncover a generic mechanism of secretory lysosome maturation involving the merger of recycling endosomes with late endosomes in a munc13-4 dependent, rab27a independent manner. The subsequent interaction with rab27a is then required for tethering of mature secretory lysosomes at the immune synapse, leading to fusion and release of lytic content.

Keywords: cytotoxic lymphocyte, degranulation, rab27 - munc13-4, tethering complex