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Single-vesicle analysis of SNARE-mediated membrane fusion involved in autophagy

（单分子研究自噬过程中的膜融合）

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Abstract

Membrane fusion is a fundamental cellular process by which two initially distinct lipid bilayers merge their hydrophobic cores, resulting in one interconnected structure. The highly conserved SNARE protein family mediates membrane fusion in eukaryotic cells. Over the last decade, an in vitro ensemble lipid mixing assay using has been commonly used for studying SNARE-mediated membrane fusion. V- and t-SNARE proteins are reconstituted into two distinct populations of vesicles where these two populations are typically labeled with Förster resonance energy transfer (FRET) donor and acceptor fluorophore-labeled-lipid molecules. Upon fusion, lipid mixing results in an increase in FRET, and, so, the degree of lipid mixing can be quantified from this increase. There are inherent limitations with this lipid mixing assay since it cannot distinguish different stages of fusion such as docking, hemifusion, and full fusion (i.e., pore opening). In order to overcome this limitation, new methods have been developed for observing either lipid or content mixing upon SNARE-mediated membrane fusion at the single-vesicle level, which differentiates between single-vesicle interaction, membrane lipid exchange, and complete fusion (i.e., pore formation). Our system demonstrates a minimal fusion system involving autophagy SNAREs, and reveals the important role of accessory proteins.

**** All are welcome ****