Cloud storage for endosomes

Teegan Dellibovi-Ragheb & Nihal Altan-Bonnet

Coordinated regulation of vesicle trafficking is critical for the proper functioning of a cell. The bulk of cellular transport vesicles are sequestered in a “perinuclear cloud”, with only a small fraction released to the cell periphery. Jongsma *et al* (2016) found that the ER-associated E3 ubiquitin ligase RNF26 is responsible for establishing and maintaining the architecture of the perinuclear cloud and that this spatiotemporal positioning is critical for effective regulation of the endocytic and exocytic systems.

See also: **MLM Jongsma *et al*** (June 2016)

In order to maintain intracellular homeostasis in a changing environment, cells must continually shuttle cargo between cellular compartments, take in nutrients, and respond to signals from the extracellular space. These complex tasks are accomplished by the coordinated activities of the endocytic and exocytic systems. Endosomes derived from the plasma membrane can either recycle their cargo back to the cell surface or undergo maturation into late endosomes/multi-vesicular bodies (MVBs). MVBs then fuse either with lysosomes for degradation, or with the plasma membrane for the release of secretory exosomes. In the exocytic system, newly synthesized proteins are trafficked from the endoplasmic reticulum (ER) to the Golgi apparatus and the trans-Golgi network (TGN) from which they are disseminated in secretory vesicles to their final destinations. There is significant cross talk between the endocytic and exocytic systems, and deregulation of either of these systems causes a wide range of human diseases, including cellular transformation, defects in the immune response, and neuronal disorders (Puertollano, 2006).

Vesicles of the endo- and exocytic pathways have previously been observed to cluster in the perinuclear region of the cell (Wasmeier *et al*, 2008; Reed *et al*, 2013) (Fig 1A). The vesicles associated with this “perinuclear cloud” are diverse and include early and late endosomes, lysosomes, and the vesicles of the TGN. Vesicles within the perinuclear cloud exhibit limited mobility, while the small fraction released from this region are dynamic and undergo fast bidirectional transport along microtubules to the cell periphery via dynein and kinesin motors. How the architecture of the perinuclear cloud is established, and what role it plays in regulating the endosomal network, has remained unexplored until now.

Jongsma *et al* (2016), in a study published recently in *Cell*, have discovered a key regulator of the positioning of the entire endosomal system. In a series of elegant experiments, they reveal that the ER-associated E3 ubiquitin ligase RNF26 (RING finger protein 26) is responsible for maintaining the architecture of the perinuclear cloud. If RNF26 is depleted from cells, vesicles are no longer sequestered in the perinuclear cloud and instead spread out into the cell periphery. Moreover, the maturation of endosomes generated through either fluid-phase or ligand-mediated endocytosis and the distribution of post-Golgi vesicles were all disrupted when RNF26 is silenced.

Both the ER localization and the catalytic activity of RNF26 were found to be required for its function in endosomal positioning. RNF26 localizes to a subdomain of the ER proximal to the nucleus, which provides the geographical information necessary for endosomal organization. RNF26 recruits and ubiquitinates the ubiquitin scaffold p62/SQSTM1 (Sequestosome 1) (Fig 1B). This provides a docking platform for

vesicle-associated adaptor molecules that contain ubiquitin-binding domains (UBDs) and tethers these vesicles to the perinuclear region of the ER. Vesicles are released through the action of the deubiquitinating enzyme USP15. It remains to be determined how USP15 is recruited to specific vesicle subpopulations for release.

The authors identified three adaptor proteins that interact with the RNF26/SQSTM1 complex: TOLLIP, EPS15, and TAX1BP1. Importantly, these adaptors exhibit diverse vesicle specificity and participate in different signal transduction pathways, suggesting that this is a general mechanism. TOLLIP is a critical regulator of TLR-mediated innate immune responses; EPS15 facilitates GLUT4 (glucose transporter 4) recycling in response to insulin as well as EGFR turnover following ligand binding, and TAX1BP1 functions as an autophagy receptor and negative regulator of innate immune signaling. Strikingly, the authors show delayed degradation of ligand-bound EGF receptors when RNF26 is silenced, thus elucidating a novel role of the ER in regulating signaling of cell surface receptors. Given the abundance of UBD-containing adaptors in the endocytic system, additional proteins will likely be identified that participate in tethering vesicles to the perinuclear region.

Central to membrane trafficking is the large and diverse family of membrane-associated Rab GTPases. This protein network directs vesicle coating, vesicle trafficking via cytoskeletal motors, tethering vesicles to target membranes, and recruitment of SNARE proteins to mediate membrane fusion (Stenmark, 2009). Future work will be required to understand how Rab GTPase regulation intersects with vesicle organization mediated by the RNF26/SQSTM1 perinuclear cloud.

Figure 1. RNF26 establishes and maintains the architecture of the perinuclear cloud.

(A) Transport vesicles from both the endocytic and exocytic pathways cluster in a perinuclear cloud closely associated with the endoplasmic reticulum (ER). These vesicles include early endosomes, late endosomes/MVBs, lysosomes, and post-Golgi secretory vesicles. (B) The ER-associated E3 ubiquitin ligase RNF26 recruits and ubiquitinates the SQSTM1 scaffold protein. This provides a docking platform for vesicle-associated adaptor molecules and tethers these vesicles to the perinuclear region of the ER. Vesicles are released through the action of the deubiquitinating enzyme USP15.

The study by Jongsma *et al* (2016) also highlights the central role of the ER in maintaining cellular homeostasis. The ER is the only membrane compartment that reaches every region of the cytosol, making it uniquely suited to direct the spatiotemporal organization of the intracellular environment. The ER makes direct contact with numerous subcellular organelles including

the Golgi, plasma membrane, mitochondria, lipid droplets, and endosomes. These contact sites are important for processes including lipid exchange, calcium homeostasis, apoptosis, and organellar fission and fusion (Helle *et al*, 2013). ER-endosome contact sites were first identified in 2009 (Rocha *et al*, 2009). Since then, the molecular composition of a number of contact

sites has been characterized; however, the RNF26/SQSTM1 network is the first mechanism identified that can propagate global changes through the endosomal system and translate positional information from the ER to dynamic transport vesicles.

The work by Jongsma *et al* (2016) has far-reaching implications for inter-organellar cross talk and regulation. The perinuclear cloud has now emerged as a critical cellular hub for the control of endosomal maturation, the exchange of cargo between different arms of the vesicle trafficking network, the regulation of the timing of exocytosis, the determination of endosome fates (e.g. recycling versus lysosomal degradation), and for the communication between biosynthetic pathways, autophagy, and endocytosis, among many other potential functions.

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