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ER-endosome contact sites: specialised interfaces for orchestrating endosomal tubule fission?

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The endomembrane system enables the delegation of separate biological processes to spatially distinct organelles within eukaryotic cells. While it is imperative that the unique biochemical compositions of these compartments are maintained, inter-organellar communication provides a means to dynamically exchange proteins, lipids and ions, allowing eukaryotic cells to achieve a level of complexity that supersedes the sum of its parts. Recently, membrane contact sites (MCSs) have emerged as a novel mechanism for crosstalk between organelles, in addition to canonical processes of vesicular membrane trafficking. Most notably, tubules of the endoplasmic reticulum (ER) have been demonstrated to contact the plasma membrane, mitochondria and endosomes, amongst other organelles. ER MCSs tether the opposing organelle membrane within a <30 nm distance without the occurrence of membrane fusion. A range of functions of ER MCSs have thus far been established, including bi-directional lipid transfer, Ca^{2+} exchange, and fine-tuned control over organelle transport and positioning. A fascinating emerging concept is the potential role for tubular ER projections in the regulation of organelle fission. In a recent publication in *Cell*, Hoyer et al. investigated the role of ER-endosome MCSs in the regulation and timing of endosomal fission by developing a proximity-dependent labelling strategy with the biotin ligase enzyme BioID to identify novel ER proteins within the vicinity of dynamic endosomal tubules[1].

Endosomal recycling is an essential process whereby transmembrane proteins (termed 'cargoes') are recognised by their cytosolic sequence motifs and sorted away from a degradative fate as endosomes mature and fuse with lysosomes. This recognition is achieved by an array of evolutionarily conserved protein complexes, such as the retromer or retriever complexes, that integrate into higher-order coat structures[2]. These multi-protein assemblies corral cargo into a retrieval subdomain on the endosomal membrane and mediate the biogenesis of tubular structures that ultimately separate and traffic to an acceptor compartment such as the plasma membrane or *trans*-Golgi network (TGN). Despite the molecular details of this sequence-based cargo recognition and tubule formation becoming clearer, the final stages of tubule scission remain ambiguous. ER-endosome contact sites have been suggested to influence the process of endosomal sorting and trafficking by imposing a tight diffusion barrier on endosomal buds and defining the sites of tubule fission[1].

The WASH complex, which is the major activator of the branched actin-nucleating Arp2/3 complex on endosomes, localises to budding retrieval subdomains and plays an orchestrating role in the clustering of recycling complexes along the tubule[2]. By tagging BioID to the WASH

complex subunit FAM21, Hoyer et al. establish a system to biotinylate vicinal proteins to this transient subdomain. Mass spectrometric analysis of labelled proteins revealed the ER transmembrane protein TMCC1 as a proximal protein to FAM21, in addition to a previously established ER MCS protein, VAPA/B. When expressed as a GFP fusion protein, TMCC1 (and its paralogues TMCC2 and TMCC3) localised to discrete domains in the peripheral ER that colocalise with Rab7-positive budding endosomal tubules prior to fission. This represents a distinct distribution to other markers of ER-endosome MCSs, such as Protrudin which primarily contacts the vacuolar portion of the endosome, suggesting that the specific location of MCS formation is governed by precise protein-protein interactions that contribute towards its function. Moreover, silencing of TMCC1 induced an impaired endosomal fission phenotype, whereby FAM21-positive endosomal buds form as usual, but ultimately collapse back into the endosome following unsuccessful fission. Accordingly, TMCC1 knockdown resulted in dispersal of the prototypical endosome-to-TGN cargo CI-MPR, suggesting a defect in endosomal recycling[1]. These data are in agreement with the model of ER-endosome contact comprising a late step in endosomal fission.

On endosomes, Coronin 1C was also identified as a requisite for MCS formation at budding profiles. Coronin 1C is an actin-binding regulatory protein, that mediates the turnover of actin filaments through Arp2/3 disassembly. Like FAM21, Coronin 1C localises to budding Rab7-positive endosomal tubules. Depletion of Coronin 1C reduced the frequency of ER-endosome contacts and the efficiency of tubule fission, in a manner comparable to the phenotype observed upon TMCC1 knockdown[1]. Given the known function of Coronin 1C, this raises the intriguing possibility of actin dynamics playing an intimate role in the formation and/or stabilisation of ER-endosome MCSs.

With the identification of two novel proteins that are required for the formation and maintenance of ER-endosome MCSs, the molecular landscape of these dynamic events is becoming clearer (Figure 1). However, the precise functions of these proteins in this process remain ambiguous, and a biochemical model that describes the order of events leading from MCS formation to tubule fission remains to be established. Recently, a mechanism was proposed whereby SNX2, a component of the cargo-selective SNX-BAR complex that localises to endosomal tubules, directly contacts VAPA/B along with the endosomal lipid transfer protein OSBP[3]. Given that SNX2 localises to the same retrieval subdomains as FAM21 on endosomes, this pathway provides a potential clue as to how ER-endosome MCSs at these sites of fission may be initiated. A variety of candidate fission factors on endosomal tubules have been proposed, including dynamins, EHD proteins, and ESCRT-III components IST1 and CHMP1B, but without definitive mechanisms. Furthermore, a biophysical hypothesis posits that frictional force generated as BAR-coated tubules elongate, combined with opposing ‘pushing’ forces from branched actin polymerisation and a mechanical ‘pulling’ force from cytoskeletal motors, drives tubule scission (Figure 2)[4]. An intriguing recent study demonstrated that artificially-applied mechanical forces are sufficient to induce mitochondrial fission independently of ER MCSs, suggesting that the broad role of the ER in organelle fission may be to constrict the target membrane to a point by which curvature-sensing adaptors for fission machinery may assemble. Could it be possible that an analogous mechanism governs endosomal tubule fission? It is tempting to speculate that an initial

constriction induced by ER contact serves to recruit fission factors, or more generally imposes local tension that aids the process of friction-driven scission.

The functional consequences of ER-endosome MCSs on global cellular health are beginning to be determined. Recently, it was demonstrated that mutations in the ER-localised protein M1-spastin, that disrupt its ability to interact with IST1 on endosomes, also induces a perturbed endosomal tubule fission phenotype that leads to pronounced lysosomal defects[5]. The process of tubular endosomal budding and trafficking is responsible for the dynamic subcellular localisation of hundreds of cargo proteins[2]. For example, the β2-adrenergic receptor (β2AR) is a validated SNX27-retromer cargo for endosome-to-plasma membrane recycling that localises to FAM21-positive, actin-rich endosomal tubules. A direct readout of the functional importance of these ER-endosome MCSs may therefore be achieved by analysing the consequence of TMCC1 depletion on cell surface β2AR levels. Moreover, it will be interesting to investigate whether ER-endosome MCSs are required for all instances of endosomal tubule fission or solely in the fission of actin-rich, cargo retrieval tubules decorated with recycling complexes.

Mutations affecting endosomal cargo recognition and recycling have been linked to a range of diseases, most of which are neurological in their aetiology[2]. In the future, it will be interesting to investigate the impact of MCSs at the level of model organisms, and whether the effects of perturbing these contacts resemble the phenotypes observed following the disruption of endosomal recycling. As demonstrated in this most recent study, the continued partnership of spatiotemporally-resolved live imaging and protein labelling will likely provide the key to discovering additional candidates that function at this dynamic membrane interface in the future.

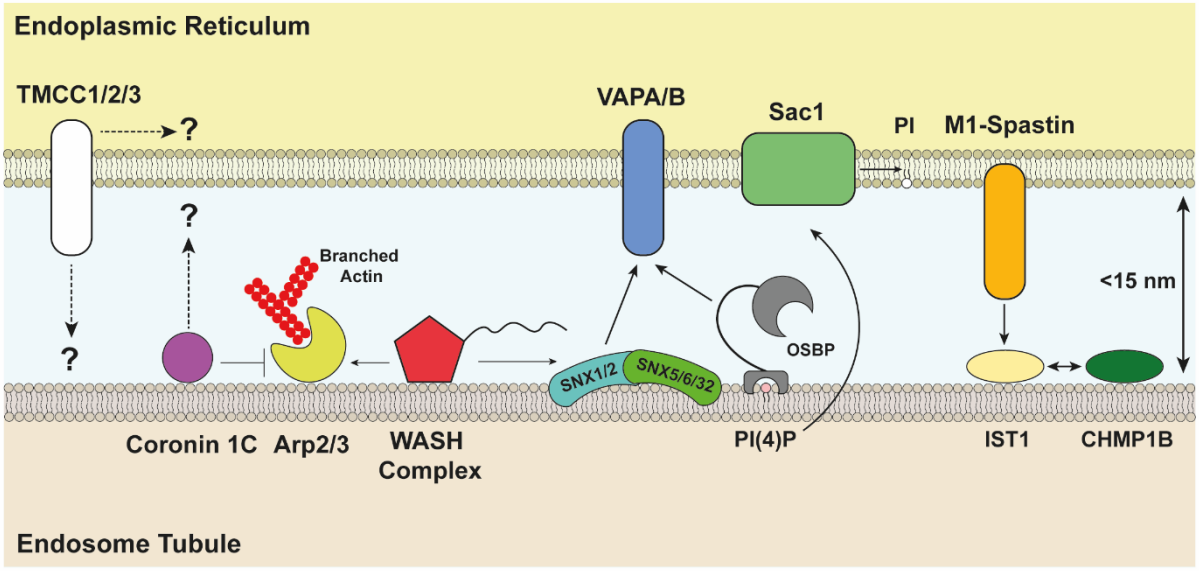

Figure 1: Mechanistic Details of Protein-Protein Interactions at the ER-Endosomal Tubule Interface. The specific process of tubule recognition may be achieved by a combination of the validated VAPA/B-SNX2 interaction, association of TMCC1/2/3 with a currently unknown partner, or further as-yet unidentified proteins. The role of Coronin 1C in the formation and stabilisation of MCSs is unknown, but may involve its actin-regulating activity. The interaction of M1-Spastin with IST1 has also been highlighted as a key interaction in the regulation of tubule fission. It should be noted that this is not an exhaustive representation of all proteins involved at ER-endosome MCSs, but rather those associated specifically with tubular subdomains. PI – phosphatidylinositol, PI(4)P – phosphatidylinositol-4-phosphate.

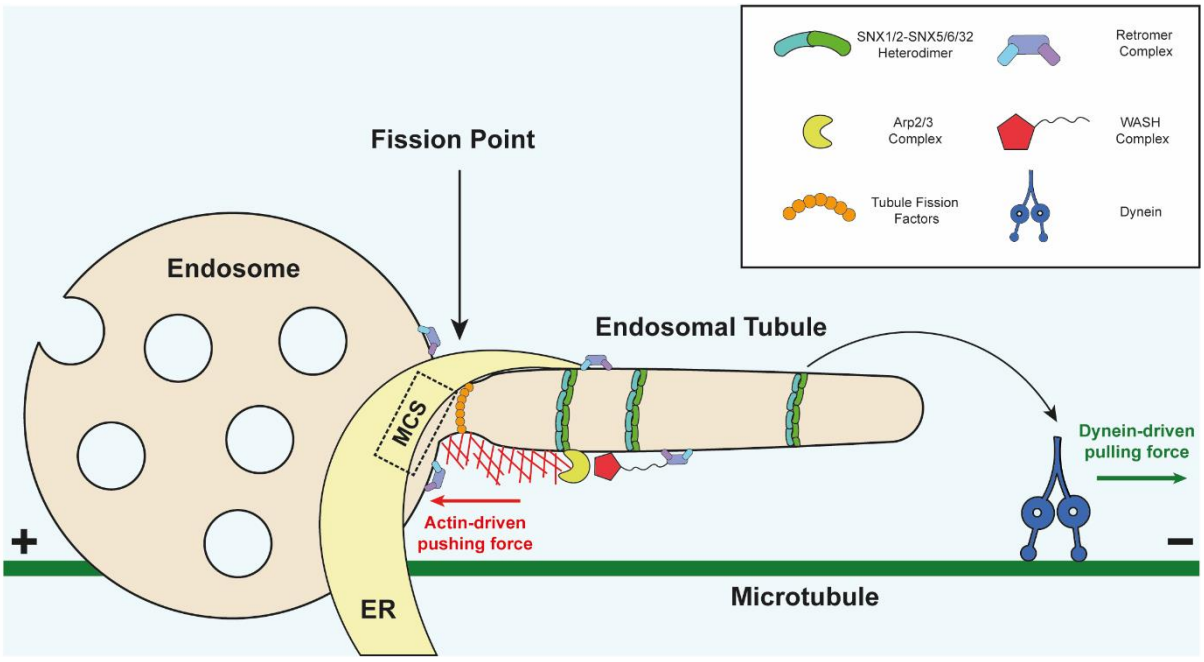

Figure 2: Overview of ER-Endosome Membrane Contacts at Endosomal Tubules. Following tubule biogenesis and cargo enrichment, which is independent of ER contact, an ER-endosome MCS is proposed to form as a late stage in the fission process. From a biophysical viewpoint, this contact may serve to constrict the tubule membrane, aiding the process of tubule fission. A 'pushing' force at the base of the tubule generated by branched actin polymerisation and an opposite 'pulling' force produced by dynein-dependent microtubule transport may also contribute to fission efficiency. It is also possible that constriction of the tubule membrane facilitates the recruitment of curvature-sensing fission factors to catalyse this process.

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References

(1) Hoyer, M. J., Chitwood, P. J., Ebmeier, C. C., Striepen, J. F., Qi, R. Z., Old, W. M., and Voeltz, G. K. (2018) A Novel Class of ER Membrane Proteins Regulates ER-Associated Endosome Fission. *Cell 175*, 254–265.e14.

(2) Cullen, P. J., and Steinberg, F. (2018) To degrade or not to degrade: mechanisms and significance of endocytic recycling. *Nature Reviews Molecular Cell Biology 19*, 679–696.

(3) Dong, R., Saheki, Y., Swarup, S., Lucast, L., Harper, J. W., and De Camilli, P. (2016) Endosome-ER Contacts Control Actin Nucleation and Retromer Function through VAP-Dependent Regulation of PI4P. *Cell 166*, 408–423.

(4) Simunovic, M., Manneville, J. B., Renard, H. F., Evergren, E., Raghunathan, K., Bhatia, D., Kenworthy, A. K., Voth, G. A., Prost, J., McMahon, H. T., Johannes, L., Bassereau, P., and Callan-Jones, A. (2017) Friction Mediates Scission of Tubular Membranes Scaffolded by BAR Proteins. *Cell 170*, 172–184.e11.

(5) Allison, R., Edgar, J. R., Pearson, G., Rizo, T., Newton, T., Günther, S., Berner, F., Hague, J., Connell, J. W., Winkler, J., Lippincott-Schwartz, J., Beetz, C., Winner, B., and Reid, E. (2017) Defects in ER-endosome contacts impact lysosome function in hereditary spastic paraplegia. *The Journal of cell biology 216*, 1337–1355.