Mini-Review

Endosomal recycling regulation during cytokinesis

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Abbreviations: RE, recycling endosome; MT, microtubule; MTOC, microtubule organizing center; C. elegans, Caenorhabditis elegans

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Successful cytokinesis is critical for cell proliferation and development. In animal cells, cytokinesis relies on temporally and spatially regulated membrane addition to the cleavage site. An important source for the new membrane is recycling endosomes. Yet how these endocytic vesicles are transported and regulated remains unclear. Several potential factors have been recently identified that regulate the trafficking of recycling endosomes during cytokinesis. Dynein and dynactin are required for the retrograde transport of recycling endosomes, while Kinesin-1 is responsible for endosome delivery to the furrow and midbody. Other regulators of recycling endosome trafficking have been identified, including RACK1, JIP3/4 and ECT2, which target recycling endosomes during the cell cycle. Here, we provide insights into the mechanisms controlling endosomal trafficking during cytokinesis.

In animal cells, cytokinesis is driven by constriction of the actomyosin contractile ring. Membrane addition is required for the increase in cell surface area and the drastic cell shape changes during cytokinesis.1-3 The requirement of membrane trafficking in cytokinesis has long been recognized as solely associated with plant cell division, as Golgi-derived vesicles form the phragmoplast structure in the center of the cell to build a new cell wall.4 However, numerous studies over the past decade have identified membrane trafficking components as key regulators of animal cytokinesis.5-9 As the cleavage furrow ingresses, lipids and transmembrane proteins are targeted to the ingressing furrow.10 During the final phase of cytokinesis, abscission, elaborate membrane targeting and fusion events within the midbody allow for proper daughter cell separation.11 Membrane trafficking is also necessary for local enrichment of particular signaling molecules at the cleavage furrow.12

New membrane within the cleavage furrow is thought to be derived from the Golgi compartment, namely the trans-Golgi network.13 Blocking Golgi function by Brefeldin A prevents vesicle accumulation at the furrow and inhibits the completion of cytokinesis.14,15 A recent study showed that Golgi-derived vesicles are targeted to the furrow where they fuse with the plasma membrane.16 However, Golgi is not the only source for the membrane addition during cytokinesis. Accumulating evidence suggests that endocytic recycling is important for the delivery of membrane to sites of division in several animal models, not unlike plants.17 During cellularization in Drosophila, recycling endosomes (REs) provide material for the growth of the lateral membrane.18 The Drosophila pericentrosomal proteins RAB11 and NUF (Nuclear fallout) localize to REs and are required for the dramatic remodeling of the cortical cytoskeleton as well as membrane addition.19 In mammalian cells, REs accumulate near the cleavage furrow and are required for the successful completion of cytokinesis. The delivery, targeting and fusion of REs to the furrow are controlled by RAB11 and RAB11FIP3 (also known as FIP3), which shares homology with Drosophila NUF.20 Thus, cells might use REs for the delivery of membrane to locations that are subject to dynamic reorganization. This process is likely mediated through interactions with the exocyst that is thought to recruit vesicles to areas of membrane fusion and growth.21,22

Questions still remain on how membrane trafficking events during cytokinesis are regulated. What signals target the RE vesicles to the cleavage site? What are the motors for their trafficking? In this review, we will highlight several new studies that provide a glimpse into how endocytic vesicles are likely regulated and transported during cytokinesis.

Recycling Endosomes Cluster at MTOC

Prior to cytokinesis, various membrane organelles including recycling endosomes are clustered at the pericentrosomal region near the microtubule organizing center (MTOC), where they can target vesicles to the site of cleavage. Clustering of the endosomes is dependent on the activity of microtubule motors.23 Several data show that the minus-end-directed motor dynein associates with REs24,25 and is responsible for the retrograde trafficking of these
vesicles, as overexpression of dynamitin, the p50 subunit of dynein activator dynactin, leads to scattered cytoplasmic distribution of recycling endosomes.\textsuperscript{26} Studies in \textit{C. elegans} embryos also show that depletion of DNC-2, the homolog of p50/dynamitin, disrupts the pericentrosomal localization of REs and results in cytoplasmic clumps of RAB-11-labeled recycling endosomes.\textsuperscript{27} These findings support the model that dynactin mediates the minus-end-directed transport of REs by dynein.\textsuperscript{28} (Fig. 1)
So how does the cell know to where the RE vesicles should be trafficked? Recent work identified a known scaffolding protein, RACK-1 (Receptor for Activated C Kinase 1), as a potential factor that may serve as a localization cue for the REs. RACK-1 localizes to the centrosomes and is required for proper delivery of REs during metaphase and anaphase. Depletion of RACK-1 in C. elegans embryos results in a scattered localization of the REs in the cytoplasm and subsequent cytokinesis failures. Moreover, RACK-1 can directly bind to DNC-2 in vivo, providing a biochemical basis for its role in anchoring and targeting RE and its motor to the MTs.

RACK-1 does not seem to function alone. Being an adaptor protein, mammalian RACK1 has been shown to interact with a number of signaling molecules including PKC-betaII. Interestingly, long-term activation of PKC-betaII results in sequestration of REs to the pericentrosomal region. RACK1 was identified as an anchor protein for PKC-II that keeps it in the activated form. This leads to a possible model that RACK1 may anchor PKC-betaII to the centrosome possibly mediating the recruitment of the REs by PKC signaling.

**RE Vesicles are Trafficked to the Cleavage Site**

Clusters of RE vesicles at the minus ends of microtubules (MTs) facilitate fast and robust transport of membrane to the cleavage sites. These vesicles are believed to be trafficked along microtubules, both astral MTs and midzone MTs. While dynemin serves as the minus-end-directed motor for endosomes, RE vesicles are likely trafficked to the cell surface via plus-end-directed kinesin motors. Kinesin-1 was proposed to mediate the delivery of RE vesicles into the midbody region. In this work, they show that after knockdown of Kinesin light chain, KLC1, transferrin-labeled endosomes are not present at the intercellular bridge and are retained at the minus-ends of microtubules. The authors also demonstrate that the Kinesin-1-dependent trafficking is controlled by the interaction between the small GTPase ARF6 and the JNK-interacting proteins JIP3/JIP4.

In order to add membrane at the right place and at the right time, the delivery of recycling endosomes to the furrow must be precisely controlled. Although the exact role of ARF6 at the cleavage furrow is not clear, it has been suggested that ARF6 may be required for the targeting of RE vesicles to the furrow. The exocyst complex has also been shown to target both Golgi-derived vesicles and REs to the cleavage site. Interestingly, SEC15 and SEC10, two components of the exocyst complex, bind to RAB11 and ARF6, respectively. More recently, the Centrospermin complex was shown to mediate the accumulation of RE vesicles at the midbody. CYK-4/MgcRacGAP was identified to interact with RAB11FIP3. The RhoGEP ECT2 competes with RAB11FIP3 for the interaction with CYK-4/MgcRacGAP. It is likely that multiple mechanisms function together to ensure the proper targeting and delivery of REs to the cleavage site.

While more and more studies are trying to answer how recycling endosomes are trafficked and regulated, this field remains open with a number of questions. The dynemin and dyactin complex may be responsible for the clearance of REs from the midbody region. Why and how are the RE vesicles recycled back from the cleavage site? Besides Kinesin-1, other kinesins have been shown to play key roles in cytokinesis. The Kinesin-II family member, KIF3, transports essential proteins, such as RhoA and RhoC regulators of membrane ruffles and Rab11.}

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