Endosome positioning during cytokinesis

Guillaume Montagnac*†1 and Philippe Chavrier*†
*Institut Curie, Centre de Recherche, Paris F-75248 France, and †CNRS, UMR144, Membrane and cytoskeleton dynamics, 26 rue d’Ulm, 75248 Paris Cedex 05, France

Abstract
In mammalian cells, completion of cytokinesis relies on targeted delivery of recycling membranes to the midbody. At this step of mitosis, recycling endosomes are organized as clusters located at the mitotic spindle poles as well as at both sides of the midbody. However, the mechanism that controls endosome positioning during cytokinesis is not known. Here, we discuss the possible mechanisms that drive the formation of endosomal clusters and the importance of this process for the targeted delivery of recycling membranes to the midbody.

Introduction
During mitosis of animal cells, the mitotic MT (microtubule) spindle drives the separation of chromosomes while the plasma membrane ingresses between the two nascent daughter cells. Additional to the mitotic spindle is the central spindle (or midzone MTs), a set of antiparallel MTs that become bundled between the separating chromosomes as the cleavage furrow continues to ingress [1]. This results in the formation of a narrow cytoplasmic bridge filled with MTs of the central spindle that are oriented with their fast growing plus-ends embedded in a central electron-dense matrix called the midbody. Completion of cytokinesis is the final step of mitosis, resulting in cleavage of the bridge in a process called abscission, which allows for proper separation of daughter cells.

Recent work focused on the role of membrane trafficking during cytokinesis and in particular it has been demonstrated that membrane insertion at the midbody is required for abscission of the bridge [2]. The probable scenario is that this intercellular channel is first plugged with delivered vesicles which then fuse together and with the plasma membrane, thus facilitating abscission.

The origin of membranes that are trafficked toward the midbody of mammalian cells is still not clear. While the necessity of post-Golgi trafficking for the completion of cytokinesis is a matter of debate [1,3], it is well documented that endocytic recycling is required for abscission [1,2].

During cytokinesis, recycling endosomes cluster around the two poles of the mitotic spindle as well as at both extremities of the central spindle [4–6]. Interestingly, the Golgi displays the very same distribution at this step of mitosis [7]. Recycling vesicles targeted to the midbody are likely to be transported from these endosomal clusters. In this regard, clusters proximal to the central spindle are ideally located because they face MT bundles that are penetrating into the bridge and because these bundles could provide tracks for the transport of vesicles toward the midbody. Thus it is essential to understand the mechanism of central spindle cluster formation as well as the importance of this process for the efficiency of membrane delivery to the midbody.

Clustering endosomes: finding MT minus ends
During interphase, clustering of various organelles, including Golgi cisternae, late endosomes/lysosomes as well as recycling endosomes in a juxtanuclear region close to the centrosome, relies on the MT network and on the activity of MT-dependent motors [8]. The minus end-directed motor dynein is particularly important in this regard as inhibition of its function results in both Golgi and perinuclear recycling endosome scattering throughout the cytoplasm [9].

The centrosome is the main MT-organizing centre as it has the ability to nucleate MTs and to anchor MT minus-ends [10]. MT nucleation depends on γ-tubulin, an essential component of centrosome, which provides a template for the assembly of α- and β-tubulin heterodimers [10]. Before mitosis, the centrosome duplicates to allow the formation of a bipolar mitotic spindle with the two centrosomes located at each spindle pole [11]. It is therefore not surprising to find endosome accumulation at the spindle poles as most of MT minus ends localize there.

The fact that endosomes also cluster at both ends of the central spindle is more puzzling. A subset of MTs of the central spindle emanates from the two mitotic spindle poles. The central spindle is also composed of shorter MTs decorated with γ-tubulin at their extremities [12], suggesting that minus ends also exist at the edges of the central spindle. Yet, how these minus ends are generated and stabilized at this location is not known. One hypothesis is that some mitotic spindle MTs are released from the poles to form a subset of midzone MTs, as was observed in Drosophila [13]. Another possibility is the de novo formation of MTs at the central spindle. Indeed, new MTs could be nucleated from the surface of preexisting mitotic spindle MTs in a process requiring γ-tubulin

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Abbreviations used: CLASP, cytoplasmic linker protein-associated protein; MT, microtubule.
†To whom correspondence should be addressed (email guillaume.montagnac@curie.fr).
[10]. In addition, it has recently been shown that Golgi membranes are able to nucleate MTs and this requires γ-tubulin and the plus end protein CLASP (cytoplasmic linker protein-associated protein) [14]. It was suggested that CLASP, beside its role at MT plus ends, could help anchoring MT minus ends to Golgi membranes [14]. In this context, it is interesting that the Drosophila orthologue of CLASP, Orbit, controls the dynamics of a subset of central spindle MTs during cytokinesis [13]. It is thus possible that Golgi stacks in the midzone area are organized by nucleating their own MTs or by capturing and stabilizing MTs released from the spindle poles.

These hypotheses are not mutually exclusive, since the highly dynamic reorganization of the MT network during mitosis probably involves complex mechanisms. However, regardless of the mechanism, the presence of MT minus ends at the boundaries of the central spindle is likely to provide a platform for Golgi and endosome clustering.

**Trafficking membranes from endosomes to the midbody**

Abscission of the cytoplasmic bridge relies on a robust and precise traffic of membranes towards the midbody. MT bundles of the central spindle could facilitate this process by providing tracks for endosomal transport. However, it is not clear whether endosome clustering is required for completion of cytokinesis or is rather a consequence of MT organization. The role of endosome clustering at the minus ends of central spindle MTs could be to ensure rapid and successful delivery of large amounts of membrane to the midbody. Yet a formal demonstration of the role of midzone endosome clustering during completion of cytokinesis is awaiting future work.

Additionally, whether vesicles transported inside the bridge derive from the central spindle clusters or from the more distant mitotic spindle clusters is not known. Schweitzer et al. [6] demonstrated that some membranes are trafficked from polar endosomal clusters to the central spindle clusters. It is thus possible that vesicles transported to the midbody originate from both types of endosomal clusters.

Finally, while dynein is probably as responsible for endosome clustering during cytokinesis as it is during interphase, the motor(s) involved in the trafficking of membranes from endosomal clusters toward the midbody are not known. This transport is likely to rely on plus-end directed kinesin motors. However, despite the fact that many kinesins are involved in the completion of cytokinesis [15], none of them were found to control membrane transport into the bridge.

In conclusion, while it is certain that endocytic recycling is required for the bridge abscission, little is known about the mechanism that regulates intracellular trafficking during cytokinesis. Thus further work should depict the machineries involved in endosomal clustering as well as in membrane trafficking toward the midbody and more generally to explain the importance of the endosomal compartment organization for the termination of mitosis.

**References**