Communicative & Integrative Biology 6:5, e25460; September/October 2013; © 2013 Landes Bioscience

Small GTPases as regulators of cell division

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Keywords: small GTPases, Rabs, cell division, mitosis, cytokinesis, chromosome segregation

The superfamily of small GTPases serves as a signal transducer to regulate a diverse array of cellular functions. The members of this superfamily are structurally and functionally classified into at least 5 groups (Ras, Rho/Rac, Rab, Arf, and Ran) and they are involved in the control of cell proliferation and differentiation, regulation of the actin cytoskeleton, membrane trafficking, and nuclear transport. It is widely reported that members of the Rab family participate in the control of intracellular membrane trafficking through the interaction with specific effector molecules. However, many Rabs and other small GTPases have also been shown to function in cell division. In this review, we discuss current knowledge about Rab proteins regulating different stages of the cell cycle, such as the congregation and segregation of chromosomes (during metaphase) and the final stage of cell division known as cytokinesis, in which a cell is cleaved originating 2 daughter cells.

Rab-GTPases in Cytokinesis

Rab proteins constitute a subfamily of small GTPases that play important roles in the regulation of intracellular vesicle transport.[1,2] Rab GTPases represent a large family of small guanosine triphosphate (GTP)-binding proteins that comprise more than 60 known members. In mammalian cells, it is well established that different Rab proteins localize on distinct membrane-bound compartments, where they regulate multiple steps in membrane traffic, including vesicle budding, movement, and fusion, through cycling between an active GTP-bound form and an inactive guanosine diphosphate (GDP)-bound form. GTP-bound GTPases are potent activators of intracellular signaling networks. GDP-GTP cycling is regulated by guanine nucleotide exchange factors (GEFs) and GAPs, which catalyze the activation and deactivation of GTPases, respectively.[2]

Cytokinesis is the terminal stage of eukaryotic cell division in which the cytoplasm of a dividing cell is partitioned between 2 daughter cells. Cytokinesis involves complex changes in cell shape, which require assembly and activation of a narrowing acto-myosin contractile-ring between the poles of the mitotic spindle. This contractile ring is a major driving-force for the physical partitioning of the cytoplasm.[3] While the acto-myosin contractile-ring is vital for ingression of the cleavage furrow, it is not the sole mechanism that drives this process as incorporation of new membrane into the furrow is also required. Thus, membrane trafficking is also crucial for abscission.[4,5] A comprehensive picture of how membrane trafficking participates during cytokinesis has been presented.[4] In fact, exocytic events have been documented with vesicles docking and fusing at the furrow area.[6] It is believed that membrane input is critical during abscission for nascent daughter cell separation.

In *C. elegans,* a role for Rab GTPases in the later stages of cell division has been documented.[7] siRNa-mediated depletion of Rab11, a Rab protein involved in endocytic vesicle recycling, leads to cytokinesis alterations, including furrow regression and abnormal scission.[8] Indeed, endosomes are suitable candidates to provide membrane during cytokinesis, and both Rab11 and its interacting protein Rab11-FIP3 localize to the cleavage furrow during cytokinesis.[9] In mammalian cells, it has also been demonstrated that Rab11- and FIP3-containing recycling endosomes accumulate near the cleavage furrow and that Rab11 is required for successful completion of cytokinesis.[10] In addition, 2 other GTPases, Arf1 and Arf6 have also been implicated in cytokinesis.[11-13] Interestingly, it has been shown that FIP3 is also required for Arf6 recruitment to the midbody during late telophase.[14] Of note, when both Rab11 and Arf6 are knocked-down, the furrow rapidly regresses and the cells are unable to form a stable midbody, suggesting that Rab11 and Arf6 function synergistically in allowing the furrow to progress to the terminal stage of abscission.[7]

Thus, as described above, several Rab proteins may contribute to cytokinesis. Rab35 is a newly discovered GTPase required for cytokinesis. Like Rab11, Rab35 localizes to the endocytic recycling pathway and regulates cytokinesis. However, during interphase, Rab35 does not colocalize exactly with the Rab11 compartment. Rab35 functions at an early endosome, prior to the relatively slow recycling endosome step regulated by Rab11.[15] Rab11 and Rab35 are localized to different subcellular compartments and control distinct endocytic recycling pathways.[16] Thus, it is likely that there are multiple endocytic routes that are individually essential for cytokinesis. In addition, Rab35 is required during cytokinesis to concentrate PIP2 at the cytokinetic bridge.[15] Furthermore, like Rab11, Rab35 is required during cytokinesis after furrow ingression to provide delivery of membrane vesicles derived from recycling endosomes to the cytokinetic bridge. This membrane would be required during abscission for nascent daughter cell separation.[10,15]

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Submitted: 06/14/13; Accepted: 06/19/13
Citation: Militello R, Colombo MI. Small GTPases as regulators of cell division. Commun Integr Biol 2013; 6: e25460; http://dx.doi.org/10.4161/cib.25460

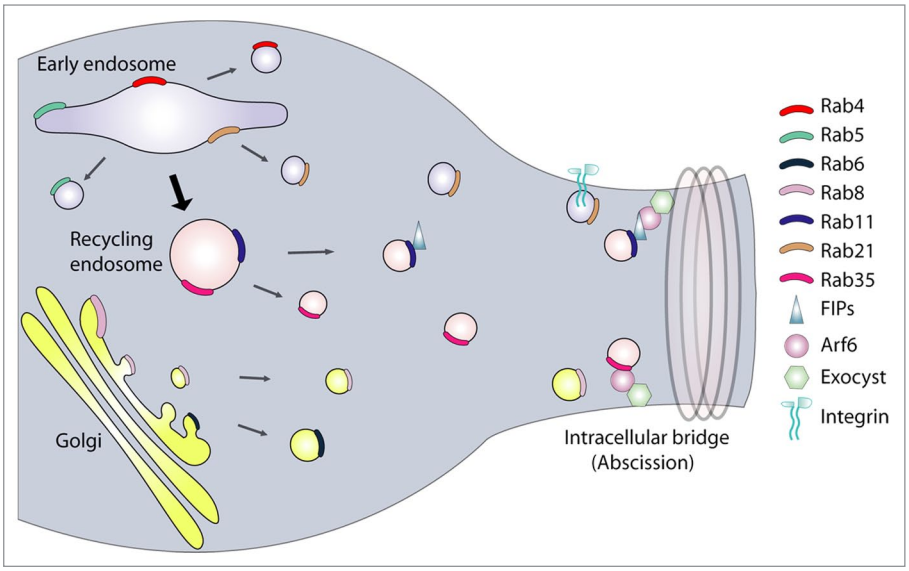

Figure 1. A schematic diagram illustrating the pathways and Rabs involved the exocytic/endocytic boundary in a cytokinesis cell: Modes of transport from early or recycling endosomes to the intracellular bridge (midbody), may involve different pathways regulated by Rab21, Rab11 or Rab35. Moreover, arrive to midbody transport vesicles from the exocutic pathway (labeled with Rab8). We also include in the scheme other Rabs protein (such as Rab4, Rab5 and Rab6) that regulate cell cycle as mentioned in the text.

In addition, it has been demonstrated that EPI64B (a GAP for Rab35), acts as an effector of ARF6 negatively regulating Rab35 activation.

This molecular mechanism controls the Rab35 pathway, including the localization of Rab35 at the intercellular bridge, and the completion of cytokinesis.[17]

In neurite outgrowth, another connection between Rab35 and Arf6 has been described, in response to nerve growth factor (NGF) stimulation. Rab35 accumulates at Arf6-positive endosomes and centaurin-β2 (also known as ACAP2) is recruited to the same compartment in a Rab35-dependent manner.[18]

The proper function of many Rabs requires continuous association and dissociation cycles from membranes. However, in the case of Rab4, it has been shown that this cycle (association and dissociation) is altered during mitosis and Rab4 accumulates in the cytoplasm through phosphorylation by a mitotic kinase. Mellman and collaborators[19] have demonstrated that reversible phosphorylation-dephosphorylation of Rab4 explains its localization during the cell cycle. Indeed, a mutation of Ser196 to glutamine or aspartic acid completely hampered Rab4 phosphorylation in mitotic cells preventing its translocation to the cytoplasm. Upon exit of cells from mitosis occurred dephosphorylation and reassociation of soluble Rab4 with membranes. Thus, phosphorylation of Ser196 is directly responsible for the reversible translocation of Rab4 into the cytosol in mitotic cells.[19] In addition, when using a dominant positive mutant of Rab4 that is permanently attached to the membranes, Van Der Sluijs and collaborators, demonstrated that the membrane-bound pool of Rab4 is targeted by a mitotic kinase.[20] Nevertheless, at present the function of Rab4 during mitosis remains unknown.

It has been shown that Rab21 activity and integrins targeting to the cleavage furrow, a process regulated by Rab21, are required for the completion of cell division. Loss of Rab21 gene expression in human cancer, leads to the accumulation of multinucleate cells. Importantly, reintroduction of Rab21 rescued this phenotype.[21] In addition, abnormal integrin traffic was linked with the generation of aneuploidy and cell transformation. In human prostate and ovarian cancer samples, downregulation of Rab21 correlates with increased malignancy. Loss-of-function experiments demonstrate that long-term depletion of Rab21 is sufficient to induce chromosome number aberrations in normal human epithelial cells.[22]

It is also important to mention that Rab6A' is required for normal transit through mitosis since a blockage in metaphase has been observed in Rab6A'-depleted cells.[23] Interestingly, depletion of GAPCenA, a Rab6 GAP that localizes to the centrosome,[24] leads to a similar phenotype to the one observed upon alteration of Rab6A' function. In our laboratory, we have evidence that Rab24 not only colocalizes with GAPCenA at the centrosome but also co-immunoprecipitates with this protein.[25] It has been proposed that GAPCenA may participate in a pathway involved in the metaphase/anaphase transition. Interestingly, depletion of Rab24 by a siRNA causes a marked accumulation of cells in metaphase.[25] It has been shown that Rab6-interacting protein1 (R6IP1), a Rab6-binding protein, also binds to Rab11, suggesting that this protein links Rab6 and Rab11 function.[26] Notably, R6IP1 function is also involved in controlling metaphase and cytokinesis, 2 events of the mitosis in which Rab6 and Rab11 have been involved. Preliminary results in our lab indicate that similar to Rab6 and Rab11, Rab24 also interacts with R6IP1 (unpublished result). Thus, this observation may explain, in part, the increased number of cells arrested in metaphase observed in Rab24 depleted cells.[25]

It was demonstrated by the yeast 2-hybrid system that Rab24 interacts with GABARAP (a human homolog of the LC3 protein) and also with cyclophilin A.[27] Interestingly, in a more recent publication, it was shown that cyclophilin A is a centrosome protein that undergoes cell cycle-dependent relocation to the midzone and midbody during cytokinesis, implicating a role of this protein during mitosis. In addition, depletion of cyclophilin A leads to cytokinesis defects through an inability to resolve intercellular brigdes, culminating in delayed or failed cytokinesis.[28] Our results indicate that in cells transiently overexpressing Rab24, the cells remain connected by a long cytoplasmic bridge and appear to be defective in abscission. Chromatin bridges between daughter cells were also observed in Rab24 silenced cells, but as described in the following section, we have

evidence that defects in cytokinesis are likely a consequence of errors previously observed during segregation and congression of chromosomes[25] (**Fig. 1**).

Rab-GTPases in Chromosome Segregation and Congression

Recent studies have shown the involvement of Rab5 in an early stage of cell division by modulating the congression and segregation of chromosomes.[29,30] Silencing Rab5 in U2OS cells causes defects in chromosome congression and marked prometaphase delay, due to a reduction in the localization of the protein CENP-F to kinetochores.[31] CENP-F, is a centromere-associated protein that contributes to the establishment of kinetochore-microtubule interactions. Another group demonstrated, almost simultaneously, that Rab5 is required for proper chromosome alignment before anaphase during Drosophila mitosis. It was shown that Rab5 associates in vivo with lamin altering lamin disassembly and causing relocation of Mud (mushroom body defect) to the poles during mitosis. Mud is the Drosophila counterpart of nuclear mitotic apparatus protein (NuMA), which is known to be important for spindle formation and maintenance in vertebrate cells.[32] Thus, surprisingly, through 2 different mechanisms, there is strong evidence that Rab5 is necessary for proper congression and alignment of the chromosomes in early stages of mitosis. In addition, it has been described that the protein DCDC5 (Doublecortin domain-containing protein 5) plays an important role in mediating dynein-dependent transport of Rab8-positive vesicles and in coordinating cell division.[33] In a recent study that reveals genes associated with cell division through a complete phenotypic profile, it has been reported that silencing of the Rab24 gene showed a binucleation phenotype and that the whole gene was essential for survival.[34]

We have recently demonstrated that the distribution of Rab24 changes during the cell cycle (Militello et al., 2013). In interphase, a fraction of Rab24 presents a reticular pattern following the microtubules distribution whereas this protein localizes to the mitotic spindle at metaphase, and to the midbody and cleavage furrow during cytokinesis. We have demonstrated that Rab24 actually associates with microtubules in vivo. In addition, we have demonstrated that Rab24 can associate to microtubules assembled in vitro with cytosolic or purified tubulin. Consistently, in a previous report, using the yeast two-hybrid system, it was shown that Rab24 interacts with human α 6 tubulin.[27] Interestingly, α 6 tubulin is present in mitotic spindle-fiber but absent in cytoskeletal microtubules in *Naegleria gruberi*.[35] We have also observed that depletion of Rab24 causes alterations in the symmetry and the aspect of the spindle during metaphase. Furthermore, similar to Rab5, depletion of Rab24 leads to defects in proper chromosome alignment during metaphase. In addition, Rab24 knock down significantly altered both mitotic and phase index with a concomitant increase in the number of binucleated cells, an indication of increased failure in cytokinesis. We have demonstrated that this protein is required for proper chromosome segregation and that the failures observed during cytokinesis, appear to be a result of errors in the segregation of the chromosomes. Thus, it is tempting to speculate that these defects might be related to the interaction between Rab24 and tubulin, at the mitotic microtubules.

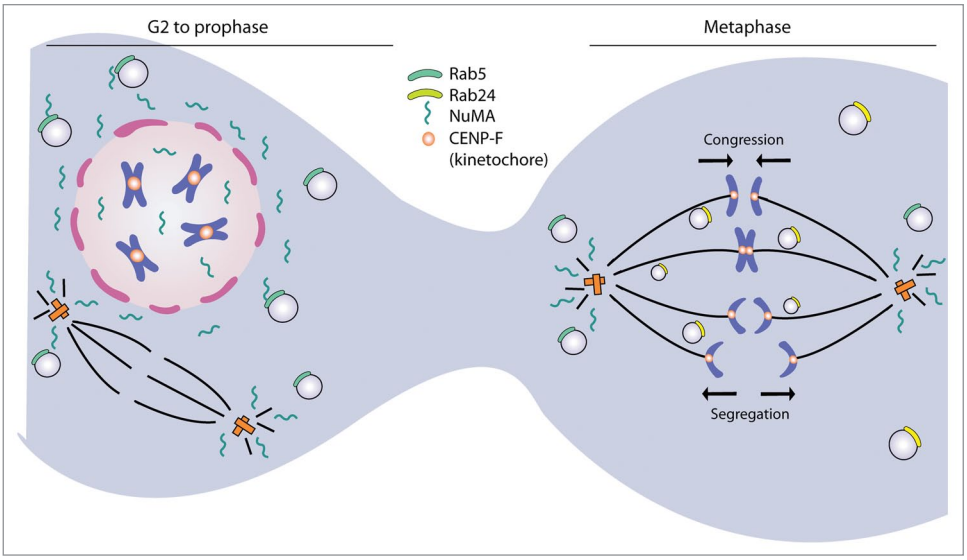

Figure 2. A schematic diagram depicting localization of different Rab proteins required for mitosis (G2/prophase to metaphase). Disassembly processes of the nuclear membrane and the congregation/segregation of chromosomes in the equatorial plane are regulated by Rab5 and Rab24 respectively.

It is interesting to mention that both Rab proteins (Rab5 and Rab24) have a high percentage (48%) of nucleotide sequence homology (database of PubMed gen). In addition, using a structural proteomic approach, Lambright and collaborators have demonstrated the interaction between Rabenosyn-5 (a Rab5 effector) and Rab24.[36] Thus, this connection between Rab5 and Rab24 or the structural analogies may account for some similar results described for both Rab proteins. A possible interaction between Rab24 and CENP proteins or a potential role of Rab24 in degradation of nuclear lamin, are important topics for future investigations (**Fig. 2**).

In this review we have presented enough evidence linking cellular transport proteins with cell division. As we mentioned above, through specific mechanisms, Rab proteins regulate different cell cycle stages, including cytocinesis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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