



HAL
open science

RhoA pathway and actin regulation of the golgi/centriole complex

Malgorzata Kloc, Ahmed Uosef, Jarek Wosik, Jacek Z. Kubiak, Rafik Mark
Ghobrial

► **To cite this version:**

Malgorzata Kloc, Ahmed Uosef, Jarek Wosik, Jacek Z. Kubiak, Rafik Mark Ghobrial. RhoA pathway and actin regulation of the golgi/centriole complex. *The Golgi Apparatus and Centriole. Results and Problems in Cell Differentiation*, 67, Springer Verlag, pp.81-93, 2019, 978-3-030-23173-6. 10.1007/978-3-030-23173-6_5. hal-02309655

HAL Id: hal-02309655

<https://hal-univ-rennes1.archives-ouvertes.fr/hal-02309655>

Submitted on 2 Dec 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

RhoA pathway and actin regulation of the Golgi/centriole complex

Malgorzata Kloc*^{1, 2, 3}, Ahmed Uosef ^{1, 2,9}, Jarek Wosik^{4,5}, Jacek Z Kubiak^{6,7,8}, Rafik Mark Ghobrial^{1, 2}

¹The Houston Methodist Research Institute, Houston, Texas 77030, USA; ²The Houston Methodist Hospital, Department of Surgery, Houston, Texas, USA; ³The University of Texas, M.D. Anderson Cancer Center, Department of Genetics, Houston Texas, USA; ⁴Electrical and Computer Engineering Department, University of Houston, Houston, Texas 77204, USA; ⁵Texas Center for Superconductivity, University of Houston, Houston, Texas 77204, USA; ⁶Laboratory of Epidemiology, Military Institute of Hygiene and Epidemiology (WIHE), Warsaw, Poland; ⁷Department of Regenerative Medicine and Cell Biology, Military Institute of Hygiene and Epidemiology (WIHE), Warsaw, Poland; ⁸Univ Rennes, UMR 6290, CNRS, Institute of Genetics and Development of Rennes, Cell Cycle Group, Faculty of Medicine, Rennes, France; ⁹ Ain Shams University School of Medicine, Cairo, Egypt.

*Corresponding Author

Malgorzata Kloc

email: mkloc@houstonmethodist.org

Abstract

In vertebrate cells, the Golgi apparatus is located in close proximity to the centriole. The architecture of the Golgi/centriole complex depends on a multitude of factors, including the actin filament cytoskeleton. In turn, both the Golgi and centriole act as the actin nucleation centers. Actin organization and polymerization also depends on the small GTPase RhoA pathway. In this chapter we summarize the most current knowledge on how the genetic, magnetic or pharmacologic interference with RhoA pathway and actin cytoskeleton directly or indirectly affects architecture, structure and function of Golgi/centriole complex.

1. Pericentriolar location of the Golgi apparatus

In vertebrate cells, the Golgi apparatus consists of stacks of flattened membranous cisternae interconnected by tubular cisternae into a continuous Golgi ribbon. The main function of the Golgi is the posttranslational modification of the proteins delivered from the endoplasmic reticulum (ER) and their subsequent sorting and delivery, within the Golgi-derived secretory vesicles, to the cell surface or to the endosomal–lysosomal system. During interphase, the Golgi apparatus is located near the nucleus, in close proximity to or surrounding the centrioles and their pericentriolar material (PCM; collectively called the centrosome). In preparation for cell division, during the G2 phase of the cell cycle, the Golgi ribbon separates into isolated stacks that migrate to the cell periphery and away from the centrioles that migrate to the opposite poles of the cell to nucleate the microtubules of the spindle (Colanzi et al., 2003, Cervigni et al., 2015; Sütterlin and Colanzi, 2010; Rios, 2014). In dividing cells, the centrioles and /or centrosomes function as the main microtubule organizing centers (MTOCs) for asters and the spindles. Additionally, in ciliated cells, the centrioles are converted into basal bodies and organize cilia (Bettencourt-Dias and Glover, 2007).

Although this subject is beyond the scope of this review, the Golgi itself is also able to nucleate microtubules (Miller et al., 2009; Maia et al., 2013, Rios, 2014). Interestingly, the Golgi and the centrosome share the molecules needed for microtubule assembly. One such molecule is the scaffolding protein kinase N-associated protein AKAP450, which recruits other proteins required for microtubule formation (El Din et al., 2005). Since Golgi-derived and centrosome-derived microtubule arrays differ in their geometry, it is believed that they may play complementary and/or different functions (Rios, 2014). For example, it was shown that Golgi-derived microtubules controlled the speed of cell migration during wound closure (Hutardo et al., 2011; Rios, 2014).

Physical contact between the Golgi and centriole is also required for cell polarization and movement when the Golgi and centriole move in unison to the leading edge of the cell. A striking example of the polarization of the Golgi/centriole complex is the formation of the immunological synapse between interacting immune cells during the immune

response (Kloc et al. 2014). Although we still do not fully understand the role of the association between the Golgi and centriole, it is believed that it facilitates polar/directional movement of the Golgi-derived secretory vesicles on the microtubules nucleated at the centrioles. Thus, it defines a polarity of secretion of various molecules (Rios, 2014) and the recycling and directional positioning of the receptors in the cell membrane.

In contrast to vertebrate cells, in yeast, invertebrates, and plants, the Golgi stacks are dispersed in the cytoplasm, do not form ribbon and are not located in the vicinity of the centrioles (daSilva et al., 2004; Kondylis and Rabouille, 2009; Preuss et al., 1992). This suggests that pericentriolar localization of the Golgi is not a prerequisite for its most basic functions.

The question that arises is how the peripherally located Golgi stacks are repositioned at the centriole and centrosome after cell division? It has been shown that the movement of the Golgi stacks from the cell periphery toward the cell center, and the maintenance of the pericentrosomal location of Golgi, depend on the microtubules and the motor protein dynein (Cole et al., 1996; Corthesy-Theulaz et al., 1992; Harada et al., 1998, Rios, 2014; Thyberg and Moskalewski, 1999), which is recruited to the Golgi by its resident membrane protein Golgin 160 (Hicks and Machamer, 2002; Yadav et al., 2009, 2012; Rios, 2014). The anchoring of Golgi stacks in the vicinity of centriole/centrosome depends on a cis-Golgi network-associated protein GMAP-210 (TRIP11) (Infante et al., 1999; Rios et al. 2004). It has been shown that depletion of GMAP-210 causes dispersion of Golgi stacks, while the experimental targeting of GAMP-210 to the mitochondria causes their clustering around the centrioles (Yadav et al., 2009, 2012; Rios, 2014).

Recently, it was shown that not only does the centriole affect the positioning and function of the Golgi but also that the Golgi regulates positioning of the centriole and centrosome (Bisel et al., 2008; Sütterlin and Colanzi, 2010). Bisel et al. (2008) showed that in the rat kidney (NRK) cell wound healing assay, the reorientation of the centrosome and accompanying Golgi toward the leading edge of the migratory cells depends on the phosphorylation of the Golgi structural protein GRASP65 by the extracellular signal-regulated kinase (ERK). This study showed that phosphorylation of

GRASP65 or treatment with brefeldin A, which both cause disassembly of the Golgi ribbon is necessary for centrosome orientation and subsequent polarization of migrating cells.

2. Golgi/centriole complex and actin

It is well established that the structure of the Golgi depends on centriole- and Golgi-derived microtubules (Kreis, 1990; Thyberg and Moskalewski, 1999; Miller et al., 2009; Maia et al., 2013, Rios, 2014). However, in the last decades, numerous studies have shown that actin filaments are also very important for the establishment and maintenance of the Golgi architecture (Colon-Franco et al., 2011; Egea et al. 2006; Guet et al., 2014; Lowe, 2011). Surprisingly, it has been shown that both the Golgi and the centriole are able to nucleate actin filaments (Chen et al., 2004; Dubois et al., 2005, Carreno et al., 2004; Guet et al, 2014; Obino et al., 2016).

2.1. Golgi and actin

The Golgi cisternae are stacked parallel to each other and have a very distinct morphology; they are flattened in the center and swollen at the rims, which bud off to form transporting vesicles. The actin filaments associated with the Golgi apparatus maintain the structural integrity of Golgi stacks, provide mechanical rigidity and flatness of the cisternae, form a physical barrier preventing the formation of transport vesicles at the center of the cisternae, and facilitate scission of the transport vesicles at the rims of the cisternae (Fig. 1). In addition, actin, together with the actin motor proteins, such as myosin II and VI, translocates the vesicles away from the Golgi to their intracellular or extracellular destinations (Fig. 1; Brownhill et al. 2009; Buss et al., 2002; diCampi et al., 1999; Egea et al., 2006; Fath, 2005; Guet et al., 2014; Miserey-Lenkei et al., 2010). Recently, Company et al. (2019) showed that the Golgi-associated myosin MYO1C, which co-localizes with the Golgi-associated actin, is necessary for actin stabilization, Golgi compaction, and anterograde and retrograde vesicular transport.

Depolymerization of actin filaments with cytochalasin D, latrunculin B or mycalolide B induces swelling of the cisternae, while stabilization of actin filaments with jasplakinolide causes fragmentation of the cisternae (Egea et al. 2006). Recently, Guet et al. (2014) used internalized microspheres trapped in optical tweezers to deform Golgi membranes

and showed that the alteration in actin dynamics and inhibition of actomyosin (myosin II) decreased the rigidity of Golgi membranes. They also showed that the mechanical force applied to the Golgi membranes disrupts the dynamics of Golgi-associated actin and decreases the formation of Golgi-derived vesicles (Guet et al., 2014). In addition, Makhoul et al. (2019) showed that actin regulation of the Golgi dynamics depends on the interaction between the golgin (trans-Golgi network (TGN), GCC88, protein) and its binding partner intersectin-1 (ITSN-1).

Another fascinating phenomenon dependent on actin/myosin is the stop-and-go motion of the Golgi described in plant cells (Nebenfuhr and Staehelin, 2001). Although the movement of Golgi through the cytoplasm is probably restricted to plant cells, where the Golgi stacks are dispersed in the cytoplasm, Breuer et al. (2017) showed that the Golgi apparatus oscillates (wiggles) in the hypocotyl cells of *Arabidopsis*. The authors believe that because “the wiggling resembles the searching behavior of foraging animals or microbes that has been suggested to optimize food search efficiency”, the Golgi may use this motion to more efficiently transport various compounds from the ER or/and distribute Golgi-derived material within the cell (Breuer et al., 2017). Further studies are needed to establish if the fascinating phenomenon of the Golgi oscillation is limited to the plant cells or is a universal phenomenon of all eukaryotic cells.

2.2. Centriole and actin

It has been known for years that the centriole and centrosome interact with actin filaments in interphase and dividing invertebrate and vertebrate cells, eggs and embryos. For example, it has been shown that actin is associated with the cnidarian sperm centrioles (Kleve and Cark, 1980) and that cortical actin plays a role in centrosome separation at the onset of mitosis in *Drosophila* embryos and 3T3-L1 adipocytes (Cao et. al., 2010; Wang et. al., 2008). In *Drosophila* syncytial blastoderm embryos, centrosomes direct the assembly and position of cortical actin caps and cytokinetic furrows (Stevenson et al., 2001). Ciliated cells in the neural tube have centriole-derived basal bodies that are anchored by actin filaments (Antoniades et al., 2014). However, although the results of proteomic analyses showed the presence of actin and actin-associated proteins at mammalian and human centrosomes (Andersen

et al., 2003; Firat-Karalar et al., 2014; Jacobsen et al., 2011), until recently, there was no direct proof that the centriole and centrosome could nucleate actin filaments. In 2016, Farina and coinvestigators, used isolated centrosomes from human Jurkat and HeLa cells to provide evidence that, indeed, the centrosome nucleates actin filaments through the action of the centrosomal Wiskott–Aldrich syndrome protein (WASP) family member, WASH (Zigmond, 2000), which stimulates the Arp2/3 complex (Farina et al., 2016). The Arp2/3 protein complex contains seven subunits. Two of these subunits, the actin-related proteins ARP2 and ARP3, serve as nucleation sites for actin filaments (Veltman and Insall, 2010). A question that arises concerns the roles of centrosome-derived actin filaments. Although further studies are needed to discover all potential functions, a recent study by Obino et al. (2016) showed that in the resting lymphocytes, the actin filaments nucleated by centrosomal Arp2/3 anchor the centrosome to the nucleus. Upon lymphocyte activation, which leads to its polarization and formation of the immunological synapse, the level of ARP2/3 at the centrosome decreases. This, in turn, reduces actin nucleation at the centrosome and allows centrosome detachment from the nucleus and its movement together with the accompanying Golgi complex to the immunological synapse (Obino et al., 2016). Ultimately, the positioning of the Golgi at the immunological synapse allows for the polar delivery of cytokines, enzymes, and receptors toward the lymphocyte target (Kloc et al., 2014). Recent studies by Inoue et al. (2019) on the actin present in the lymphocyte centrosomes showed that centrosomal actin physically blocks elongation of the centrosome-derived microtubules.

3. The role of RhoA pathway in Golgi and centriole architecture and functions

In all eukaryotic cells, the actin cytoskeleton is regulated by the small GTPase RhoA and its downstream effector ROCK1 p160 kinase. RhoA is activated by RhoA specific guanine exchange factors, GEFs that activate GTPases by stimulating the release of guanosine diphosphate (GDP) and binding of guanosine triphosphate (GTP; Cherfils and Zeghouf, 2013; Fig. 2A). RhoA is also reciprocally regulated by the Rho GTPase Rac1 and mTOR pathways (Fig.2A; Byrne et al., 2016; Laplante and Sabatini, 2009). Recent studies indicate that, surprisingly, ROCK1 kinase is not only the effector of RhoA but also an effector of Rac1 (Fig. 2A; Soriano-Castell et al., 2017). As previously

discussed, the actin cytoskeleton plays a major role in the structure and function of the Golgi/centriole complex. Thus, it should be expected that interference with the RhoA pathway would disrupt actin cytoskeleton and indirectly affect the Golgi/centriole complex. Indeed, studies from our laboratories showed that the inhibition of RhoA GEFs using Rhosin or Y16 inhibitors, the inhibition of ROCK1 using the Y2762 inhibitor, or the genetic deletion of RhoA reorganizes the actin cytoskeleton in mouse macrophages and disrupts the structure of the Golgi, which fragments, disperses and/or migrates from its perinuclear position to the macrophage tail (Fig. 2B, Figure 3; Chen et al., 2017, 2018; Liu et al. 2016a-c, 2017). Interestingly, actin disorganization does not cause repositioning of the centriole, which remains in the perinuclear position. Thus, the RhoA interference causes uncoupling of the Golgi from the centriole (Fig. 3). We also showed that the macrophage-specific deletion of RhoA caused defective recycling, which relies on the Golgi-derived vesicular pathway, of macrophage fractalkine receptor CX3CR1 (Liu et al., 2017). Interestingly, very similar effects on macrophage phenotype, the Golgi and receptors were achieved when the macrophages were exposed to the magnetic field gradient interference (Wosik et al. 2018). In these studies, we have designed a permanent rare-earth magnet setup with defined magnetic field-gradient patterns and investigated the effect of these fields on mouse peritoneal macrophages grown in in vitro culture. We observed that the magnetic force elongated macrophages and arranged them in distinctive rows/waves. The location and alignment of magnetic-field-elongated macrophages correlated very well with the simulated distribution and orientation of magnetic-force lines (Wosik et al. 2018). Also, the exposure of cultured RAW 264 cells (Abelson murine leukemia virus-transformed mouse macrophage) to the magnetic force had a similar effect (Uosef et al. unpublished). We also showed that similar to RhoA interference, the magnetic field exposure elongated macrophages, changed Golgi complex and cation channel receptor TRPM2 distribution, and modified the expression of macrophage molecular markers (Wosik et al. 2018).

All these studies suggested that the effect of RhoA pathway interference on the Golgi/receptor recycling was indirect, resulting from the changes to the actin cytoskeleton. However, there are studies indicating that RhoA and/or its binding partners are associated with the Golgi apparatus and thus are directly involved in the

regulation of some aspects of the Golgi structure and functions (Chi et al., 2013; Long and Simpson, 2017). Quassollo et al., (2015) showed that in the neuronal cells, RhoA, ROCK, and serine-threonine kinases, LIMK1 and PKD1, are necessary for the scission and fission of the dendritic Golgi outposts (GOPs) needed for the trafficking of synaptic receptors (Fig. 1). In another study, Zilberman et al. (2011) showed that the effects of the overexpression of RhoA, which causes dispersion of the Golgi ribbon and defects in the fission of the transporting vesicles, depend on a member of the formin protein, mDia1, localized at the Golgi membranes. Camera et al. (2003, 2008) showed that the Golgi apparatus of neural cells contains RhoA binding protein Citron-N, which controls actin filament polymerization through the local assembly of ROCK kinase and actin-binding protein, Profilin IIa. Another study showed a centriolar function of RhoA. Aoki et al. (2009) showed that the RhoA GEF ARHGEF10, is localized at the centrosome in G1/S and M phases of the cell cycle. The ARHGEF10-regulates RhoA and controls centrosome duplication through its binding partner- a motor protein KIF3B, which is colocalized with ARHGEF10 at the centrosome.

In summary, these data indicate that the RhoA pathway is both indirectly, through the actin cytoskeleton, and directly, through the Golgi/centriole associated factors, involved in the regulation of Golgi/centriole structure and functions.

Acknowledgments

The authors gratefully acknowledge support from the William and Ella Owens Medical Research Foundation to JW and MK, the William Stamps Farish Fund to MK and RMG, the State of Texas through the Texas Center for Superconductivity at the University of Houston to JW, Polish Ministry of National Defense project "Kościuszko" # 571/2016/DA to JZK and Polish Ministry of National Defense project "Kościuszko" # 5508/2017/DA to JZK. We also thank Kenneth Dunner Jr. for the electron microscopy work and acknowledge CCSG grant NIH P30CA016672 to MDACC High Resolution Electron Microscopy Facility.

References

1. Andersen JS, Wilkinson CJ, Mayor T, Mortensen P, Nigg EA, Mann M. (2003). Proteomic characterization of the human centrosome by protein correlation profiling. *Nature*. 426:570-574.
2. Antoniadou I, Stylianou P, Skourides PA. (2014). Making the connection: ciliary adhesion complexes anchor basal bodies to the actin cytoskeleton. *Dev Cell* 28:70-80
3. Aoki T, Ueda S, Kataoka T, Satoh T. (2009). Regulation of mitotic spindle formation by the RhoA guanine nucleotide exchange factor ARHGEF10. *BMC Cell Biol.* 10: 56.
4. Bettencourt-Dias, M., and Glover DM. (2007). Centrosome biogenesis and function: centrosomes brings new understanding. *Nat. Rev. Mol. Cell Biol.* 8:451–463.
5. Bisel B, Wang Y, Wei J-H, Xiang Y, Tang D, Miron-Mendoza M, Yoshimura S, Nakamura N, Seemann J. (2008). ERK regulates Golgi and centrosome orientation towards the leading edge through GRASP65. *J. Cell Biol.* 182: 837-843.
6. Breuer D, Nowak J, Ivakov A, Somssich M, Persson S, Nikoloski Z. (2017). System-wide organization of actin cytoskeleton determines organelle transport in hypocotyl plant cells. *Proc Natl Acad Sci U S A.* 114(28):E5741-E5749.
7. Brownhill, K., Wood, L., and Allan, V. (2009). Molecular motors and the Golgi complex: staying put and moving through. *Semin. Cell Dev. Biol.* 20, 784–792.
8. Buss F, Luzio JP, Kendrick-Jones J: Myosin VI, an actin motor for membrane traffic and cell migration. *Traffic* 2002, 3:851-858.
9. Byrne KM, Monsefi N, Dawson JC, Degasperi A, Bukowski-Wills J-C, Volinsky N, Dobrzyński M, et al. (2016) Bistability in the Rac1, PAK, and RhoA Signaling Network Drives Actin Cytoskeleton Dynamics and Cell Motility Switches. *Cell Syst.* 2: 38–48.
10. Camera P, da Silva JS, Griffiths G, Giuffrida MG, Ferrara L, Schubert V, Imarisio S, Silengo L, Dotti CG, Di Cunto F (2003). Citron-N is a neuronal Rho-associated protein involved in Golgi organization through actin cytoskeleton regulation. *Nat Cell Biol.* 5:1071-1078.
11. Camera P, Schubert V, Pellegrino M, Berto G, Vercelli A, Muzzi P, Hirsch E, Altruda F, Dotti CG, Di Cunto F. (2008). The RhoA-associated protein Citron-N controls dendritic spine maintenance by interacting with spine-associated Golgi compartments. *EMBO Rep.*9:384-392.

12. Capmany A, Yoshimura A, Kerdous R, Caorsi V, Lescure A, Nery ED, Coudrier E, Goud B, Schauer K. (2019). MYO1C stabilizes actin and facilitates arrival of transport carriers at the Golgi apparatus. *J Cell Sci.* 2019 Mar 14. pii: jcs.225029. doi: 10.1242/jcs.225029. [Epub ahead of print]
13. Cao J, Crest J, Fasulo B, Sullivan W. (2010). Cortical actin dynamics facilitate early-stage centrosome separation. *Curr Biol.* 20:770-776
14. Carreno, S., Engqvist-Goldstein, A.E., Zhang, C.X., McDonald, K.L., and Drubin, D.G. (2004). Actin dynamics coupled to clathrin-coated vesicle formation at the trans-Golgi network. *J. Cell Biol.* 165: 781–788.
15. Cervigni RI, Bonavita R, Barretta ML, Spano D, Ayala I, Nakamura N, Corda D, Colanzi A. (2015). JNK2 controls fragmentation of the Golgi complex and the G2/M transition through phosphorylation of GRASP65. *J Cell Sci* 128: 2249-2260
16. Chen, J.-L., Lacomis, L., Erdjument-Bromage, H., Tempst, P., and Stamnes, M. (2004). Cytosol-derived proteins are sufficient for Arp2/3 recruitment and ARF/coatomer-dependent actin polymerization on Golgi membranes. *FEBS Lett.* 566: 281–286
17. Chen W, Ghobrial RM, Li XC, Kloc M. Inhibition of RhoA and mTORC2/Rictor by Fingolimod (FTY720) induces p21-activated kinase 1, PAK-1 and amplifies podosomes in mouse peritoneal macrophages. (2018). *Immunobiology.* Jul 7. pii: S0171-2985(18)30046-9. doi: 10.1016/j.imbio.2018.07.009. [Epub ahead of print]
18. Chen W, Li XC, Kubiak JZ, Ghobrial RM, Kloc M. (2017). Rho-specific Guanine nucleotide exchange factors (Rho-GEFs) inhibition affects macrophage phenotype and disrupts Golgi complex. *Int. J. Biochem. Cell Biol.* 93:12-24
19. Cherfils J, Zeghouf M (2013). Regulation of small GTPases by GEFs, GAPs, and GDIs. *Physiological Reviews.* 93 (1): 269–309.
20. Chi X, Wang S, Huang Y, Stamnes M, Chen JL.(2013). Roles of rho GTPases in intracellular transport and cellular transformation. *Int J Mol Sci.* 14:7089-7108
21. Colanzi, A., C. Sütterlin, and V. Malhotra. (2003). Cell-cycle-specific Golgi fragmentation: how and why? *Curr. Opin. Cell Biol.* 15:462–467
22. Cole NB, Sciaky N, Marotta A, Song J, Lippincott-Schwartz J. (1996) Golgi dispersal during microtubule disruption: regeneration of Golgi stacks at peripheral endoplasmic reticulum exit sites. *Mol. Biol. Cell* 7: 631–650.

23. Colón-Franco JM, Gomez TS, Billadeau DD. (2011). Dynamic remodeling of the actin cytoskeleton by FMNL1 γ is required for structural maintenance of the Golgi complex. *J Cell Sci.* 2011 Sep 15; 124(18): 3118–3126.
24. Corthesy-Theulaz I, Pauloin A, Pfeffer SR. (1992). Cytoplasmic dynein participates in the centrosomal localization of the Golgi complex. *J. Cell Biol.* 118: 1333–1345.
25. daSilva LL, Snapp EL, Denecke J, Lippincott-Schwartz J, Hawes C, Brandizzi F. (2004). Endoplasmic reticulum export sites and Golgi bodies behave as single mobile secretory units in plant cells. *Plant Cell* 16, 1753–1771.
26. di Campli, A., Valderrama, F., Babia, T., De Matteis, M.A., Luini, A., and Egea, G. (1999). Morphological changes in the Golgi complex correlate with actin cytoskeleton rearrangements. *Cell Motil. Cytoskeleton* 43, 334–348.
27. Dubois, T., Paleotti, O., Mironov, A.A., Fraisier, V., Stradal, T.E.B., De Matteis, M.A., Franco, M., and Chavrier, P. (2005). Golgi-localized GAP for Cdc42 functions downstream of ARF1 to control Arp2/3 complex and F-actin dynamics. *Nat. Cell Biol.* 7: 353–364.
28. Egea, G., Lazaro-Dieguez, F., and Vilella, M. (2006). Actin dynamics at the Golgi complex in mammalian cells. *Curr. Opin. Cell Biol.* 18: 168–178.
29. El Din El Homasany BS, Volkov Y, Takahashi M, Ono Y, Keryer G, Delouée A, Looby E, Long A, Kelleher D. (2005). The Scaffolding Protein CG-NAP/AKAP450 Is a Critical Integrating Component of the LFA-1-Induced Signaling Complex in Migratory T Cells. *J Immunol* 175 : 7811-7818
30. Farina F, Gaillard J, Guérin C, Couté Y, Sillibourne J, Blanchoin L, Théry M. (2016). The centrosome is an actin-organizing centre. *Nat Cell Biol.* 18:65-75.
31. Fath KR: Characterization of myosin-II binding to Golgi stacks in vitro. *Cell Motil Cytoskeleton* 2005, 60:222-235.
32. Firat-Karalar EN, Sante J, Elliott S, Stearns T. (2014). Proteomic analysis of mammalian sperm cells identifies new components of the centrosome. *J Cell Sci.* 127:4128-133.
33. Guet D, Mandal K, Pinot M, Hoffmann J, Abidine Y, Sigaut W, Bardin S, Schauer K, Goud B, Manneville J-B. (2014). Mechanical Role of Actin Dynamics in the Rheology of the Golgi Complex and in Golgi-Associated Trafficking Events. 24: 1700-1711

34. Harada A, Takei Y, Kanai Y, Tanaka Y, Nonaka S, Hirokawa N. 1998 Golgi vesiculation and lysosome dispersion in cells lacking cytoplasmic dynein. *J. Cell Biol.* 141, 51–59.
35. Hicks SW and Machamer CE. (2002). The NH₂-terminal Domain of Golgin-160 Contains Both Golgi and Nuclear Targeting Information. *J. Biol. Chem.* 277: 35833-35839.
36. Hurtado L, Caballero C, Gavilan MP, Cardenas J, Bornens M, Rios RM. (2011). Disconnecting the Golgi ribbon from the centrosome prevents directional cell migration and ciliogenesis. *J. Cell Biol.* 193: 917–933.
37. Infante C, Ramos-Morales F, Fedriani C, Bornens M, Rios RM. (1999). GMAP-210, A cis-Golgi network associated protein, is a minus end microtubule binding protein. *J. Cell Biol.* 145: 83–98
38. Inoue D, Obino D, Pineau J, Farina F, Gaillard J, Guerin C, Blanchoin L, Lennon-Duménil AM, Théry M. (2019). Actin filaments regulate microtubule growth at the centrosome. *EMBO J.* Mar 22. pii: e99630. doi: 10.15252/embj.201899630. [Epub ahead of print]
39. Jakobsen L, Vanselow K, Skogs M, Toyoda Y, Lundberg E, Poser I, Falkenby LG, Bennetzen M, Westendorf J, Nigg EA, Uhlen M, Hyman AA, Andersen JS. (2011). Novel asymmetrically localizing components of human centrosomes identified by complementary proteomics methods. *EMBO J.* 30:1520-1535.
40. Kleve MG, Clark WH Jr. (1980). Association of actin with sperm centrioles: isolation of centriolar complexes and immunofluorescent localization of actin. *J Cell Biol.* 86:87-95.
41. Kloc M, Kubiak JZ, Li XC, Ghobrial RM. (2014). The newly found functions of MTOC in immunological response. *J Leukoc Biol.* 95:417-430.
42. Kondylis V, Rabouille C. (2009). The Golgi apparatus: lessons from *Drosophila*. *FEBS Lett.* 583: 3827– 3838.
43. Kreis, TE. (1990). Role of microtubules in the organization of the Golgi apparatus. *Cell Mot. Cytoskeleton.* 15:67-70
44. Laplante M, Sabatini DM. (2009). mTOR signaling at glance. *J Cell Sci.* 122: 3589-3594
45. Liu Y, Chen W, Wu C, Minze LJ, Kubiak JZ, Li XC, Kloc M, Ghobrial RM (2017). Macrophage/monocyte-specific deletion of RhoA down-regulates fractalkine receptor and inhibits chronic rejection of mouse cardiac allografts. *J Heart Lung Transpl.* 36:340-354.

46. Liu Y, Chen W, Minze LJ, Kubiak JZ, Li XC, Ghobrial RM, Kloc M. (2016a). Dissonant response of M0/M2 and M1 bone marrow derived macrophages to RhoA pathway interference. *Cell Tissue Res.* 366: 707-720
47. Liu Y, Minze LJ, Mumma L, Li XC, Ghobrial RM, Kloc M. (2016b). Mouse macrophage polarity and ROCK1 activity depend on RhoA and non-apoptotic Caspase 3. *Exp Cell Res.* 341:225-36
48. Liu Y, Tejpal N, You J, Li XC, Ghobrial RM, Kloc M. (2016c). ROCK inhibition impedes macrophage polarity and functions. *Cell Immunol.* 300:54-62.
49. Long M, Simpson JC. (2017). Rho GTPases operating at the Golgi complex: Implications for membrane traffic and cancer biology. *Tissue Cell.* 49:163-169
50. Lowe, M. (2011). Structural organization of the Golgi apparatus. *Curr. Opin. Cell Biol.* 23: 85–93.
51. Maia AR, Zhu X, Miller P, Gu G, Maiato H, Kaverina I. (2013). Modulation of Golgi-associated microtubule nucleation throughout the cell cycle. *Cytoskeleton (Hoboken)* 70: 32–43.
52. Makhoul C, Gosavi P, Duffield R, Delbridge B, Williamson NA, Gleeson PA. (2019). Intersectin-1 interacts with the golgin GCC88 to couple the actin network and Golgi architecture. *Mol Biol Cell.* 2019 Feb 1;30(3):370-386. doi: 10.1091/mbc.E18-05-0313. Epub 2018 Dec 12.
53. Miller PM, Folkmann AW, Maia AR, Efimova N, Efimov A, Kaverina I. (2009). Golgi-derived CLASP dependent microtubules control Golgi organization and polarized trafficking in motile cells. *Nat. Cell Biol.* 11: 1069–1080.
54. Miserey-Lenkei, S., Chalancon, G., Bardin, S., Formstecher, E., Goud, B., and Echard, A. (2010). Rab and actomyosin-dependent fission of transport vesicles at the Golgi complex. *Nat. Cell Biol.* 12, 645–654.
55. Nebenfuhr A, Staehelin LA (2001) Mobile factories: Golgi dynamics in plant cells. *Trends Plant Sci* 6:160–167
56. Obino D, Farina F, Malbec O, Sáez PJ, Maurin M, Gaillard J, Dingli F, Loew D, Gautreau A, Yuseff M-I, Blanchoin L, Théry M, Lennon-Duménil A-M. (2016). Actin nucleation at the centrosome controls lymphocyte polarity. 2016; *Nature Com.* 7:10969-10983
57. Preuss, D., J. Mulholland, A. Franzusoff, N. Segev, and D. Botstein. (1992). Characterization of the *Saccharomyces* Golgi complex through the cell cycle by immunoelectron microscopy. *Mol. Biol. Cell.* 3:789–803.

58. Quassollo, G., Wojnacki, J., Salas, D.A., Gastaldi, L., Marzolo, M.P., Conde, C., Bisbal, M., Couve, A., Cáceres, A. (2015). A RhoA signaling pathway regulates dendritic Golgi outpost formation. *Curr. Biol.* 25, 971–982.
59. Rios RM. (2014). The centrosome–Golgi apparatus nexus. *Phil. Trans. R. Soc. B* 369: 20130462.
60. Rios RM, Sanchis A, Tassin AM, Fedriani C, Bornens M. (2004). GMAP-210 recruits gamma-tubulin complexes to cis-Golgi membranes and is required for Golgi ribbon formation. *Cell* 118: 323–335.
61. Soriano-Castell D, Chavero A, Rentero C, Bosch M, Vidal-Quadras M, Pol A, Enrich C, Tebar F. (2017). ROCK1 is a novel Rac1 effector to regulate tubular endocytic membrane formation during clathrin-independent endocytosis. *Sci Rep.* 7:6866.
62. Stevenson VA, Kramer J, Kuhn J, Theurkauf WE. (2001). Centrosomes and the Scrambled protein coordinate microtubule-independent actin reorganization. *Nat Cell Biol.* 3:68-75.
63. Sütterlin C and Colanzi A. (2010). The Golgi and the centrosome: building a functional partnership. *The Golgi and the centrosome: building a function. J Cell Biol.* 188:621-628.
64. Thyberg J, Moskalewski S. (1999). Role of microtubules in the organization of the Golgi complex. *Exp. Cell Res.* 246: 263–279.
65. Yadav S, Puthenveedu MA, Linstedt AD. (2012). Golgin160 recruits the dynein motor to position the Golgi apparatus. *Dev. Cell* 23: 153–165.
66. Yadav S, Puri S, Linstedt AD. (2009). A primary role for Golgi positioning in directed secretion, cell polarity, wound healing. *Mol. Biol. Cell* 20: 1728–1736.
67. Veltman DM, Insall RH (2010). "WASP family proteins: their evolution and its physiological implications". *Mol. Biol. Cell.* 21: 2880–93.
68. Wang W, Chen L, Ding Y, Jin J, Liao K. (2008). Centrosome separation driven by actin-microfilaments during mitosis is mediated by centrosome-associated tyrosine-phosphorylated cortactin. *J Cell Sci.* 121:1334-1343.
69. Wosik J, Chen W, Qin K, Ghobrial RM, Kubiak JZ, Kloc M. (2018) Magnetic field changes macrophage phenotype. *Biophysical J.* 114:2001-2013.
70. Zigmond SH. (2000). How Wasp Regulates Actin Polymerization *J Cell Biol.* 150: 117–120.

71. Zilberman, Alieva, N.O., Miserey-Lenkei, S., Lichtenstein, A., Kam, Z., Sabanay, H., Bershadsky, A. (2011). Involvement of the Rho/mDia1 pathway in the regulation of Golgi complex architecture and dynamics. *Mol. Biol. Cell* 22, 2900–2911.

Figure Legends

Fig. 1. Diagram of actin filament and RhoA role in the scission from the Golgi cisternae and in the transport of the cargo-containing vesicles.

The actin filaments associated with the Golgi provide mechanical rigidity and flatness of the cisternae, form a physical barrier preventing formation of transport vesicles at the center of the cisternae, and facilitate scission of the transport vesicles at the rims of the cisternae. Actin polymerization is regulated by RhoA pathway. The vesicles transport their cargo, such as various receptors, to the cell membrane using actin filament and actin motors, or alternatively, the microtubules and microtubule motors.

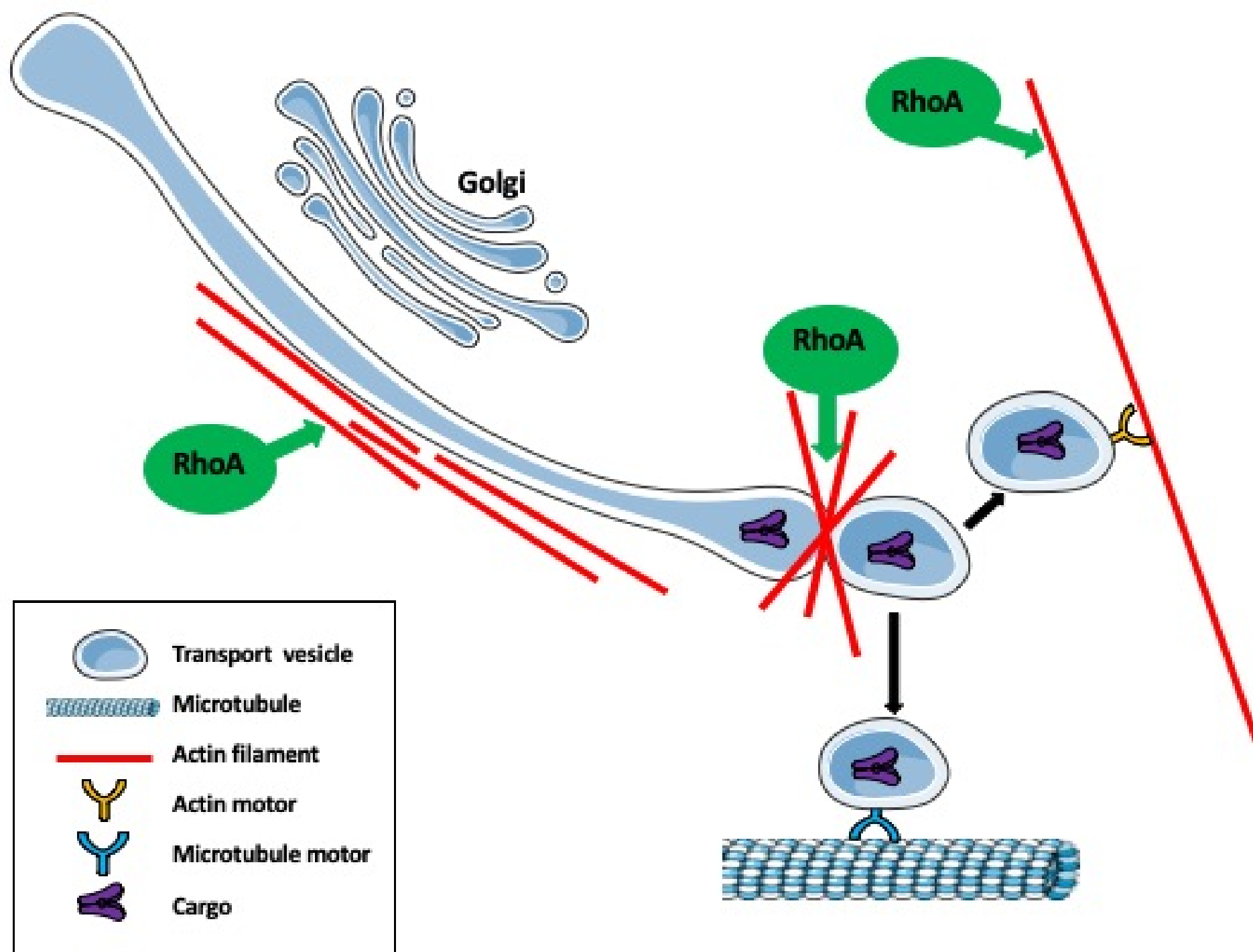
Fig. 2. Diagram of the RhoA pathway, actin, centriole and Golgi.

- A) In normal, untreated cells, RhoA is activated by RhoA specific GEFs and is reciprocally regulated by mTOR and the Rac 1 pathway. RhoA regulates actin filament polymerization and organization through its downstream effector ROCK kinase. Recently, it has been shown that ROCK is also a direct downstream effector of Rac1 (Soriano-Castell et al., 2017). A properly organized actin cytoskeleton and fully functioning RhoA pathway support perinuclear organization of Golgi and centrioles, the Golgi-derived vesicular transport of various molecules and receptors, and the recycling of the receptors from the membrane back to the Golgi. B) Pharmacologic, genetic or magnetic interference with the components of the RhoA pathway such as: GEFs inhibition with Rhosin or Y16, Rock inhibition with Y27632, RhoA deletion or the exposure of macrophages to the magnetic field gradient, elongate macrophage, disrupt actin

cytoskeleton, disperse Golgi and/or cause translocation of its fragments into the macrophage tail. These interferences do not affect perinuclear localization of the centrioles.

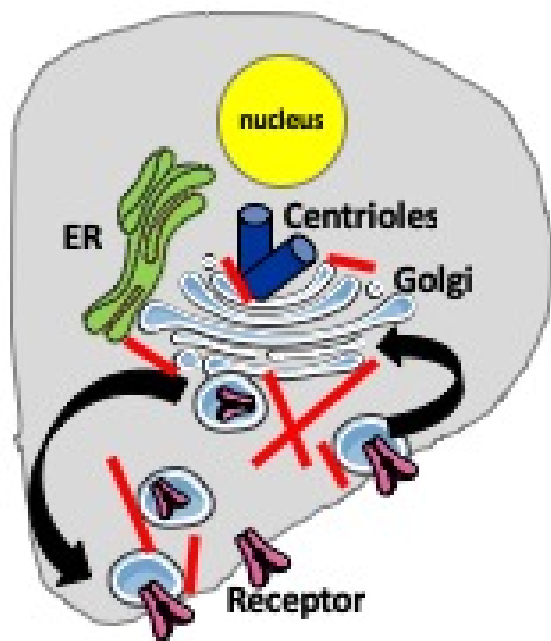
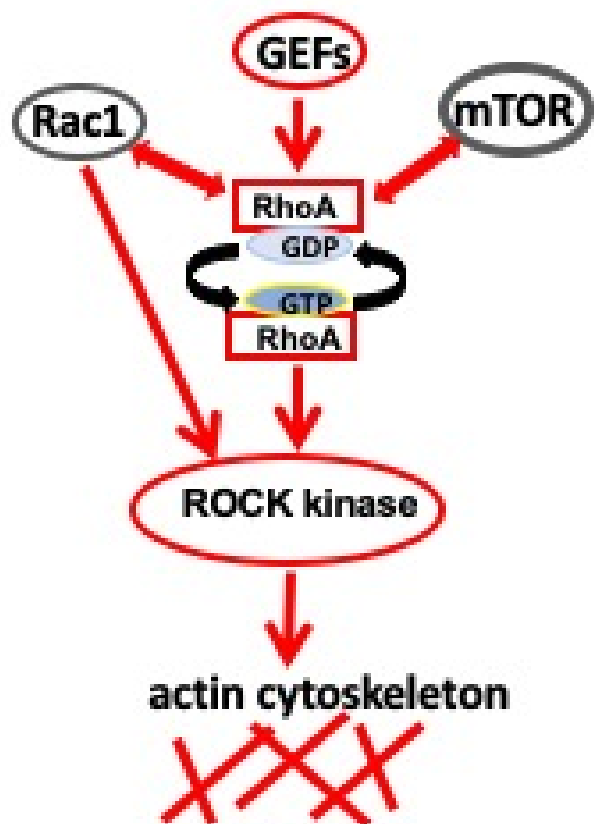
Fig. 3. Golgi and centrioles in mouse macrophages

- A)** Transmission electron microscopy. In untreated macrophages, centrioles are surrounded by the stacks of Golgi cisternae and positioned in the vicinity of the nucleus. Bar is equal to 500nm. **B).** Light microscopy. Untreated mouse macrophage immunostained with Golgi marker anti-GM130 antibody and FITC-conjugated secondary antibody (green) and counterstained with Rhodamine phalloidin to visualize actin (red) and DAPI to visualize nucleus (blue). Golgi is visible in the vicinity of the nucleus. Bar is equal to 50 μ m. **C)** Transmission electron microscopy RhoA deleted macrophages treated with GEFs' inhibitor, Rhosin. The centrioles and remnants of dispersed Golgi were visible in the vicinity of the nucleus, while the majority of the dispersed Golgi moved away from the nucleus toward the macrophage tail (not shown here). Bar is equal to 500nm. **D)** Light microscopy. Mouse macrophage treated with GEF inhibitor Rhosin and immunostained with Golgi marker anti-GM130 antibody and FITC-conjugated secondary antibody (green), and counterstained with DAPI to visualize nucleus (blue). Golgi fragments are displaced from the perinuclear position to the macrophage tail. Bar is equal to 18 μ m. Panel D is from the Fig. 6 in Chen et al. (2017) with the permission of Elsevier.



A

Untreated cell

**B**

RhoA interference

