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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; diCampli et al.,1999; Egea et al., 2006; Fath, 2005; Guet et al., 2014; Miserey-Lenkei et al., 2010). Recently, Campany 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.

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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.

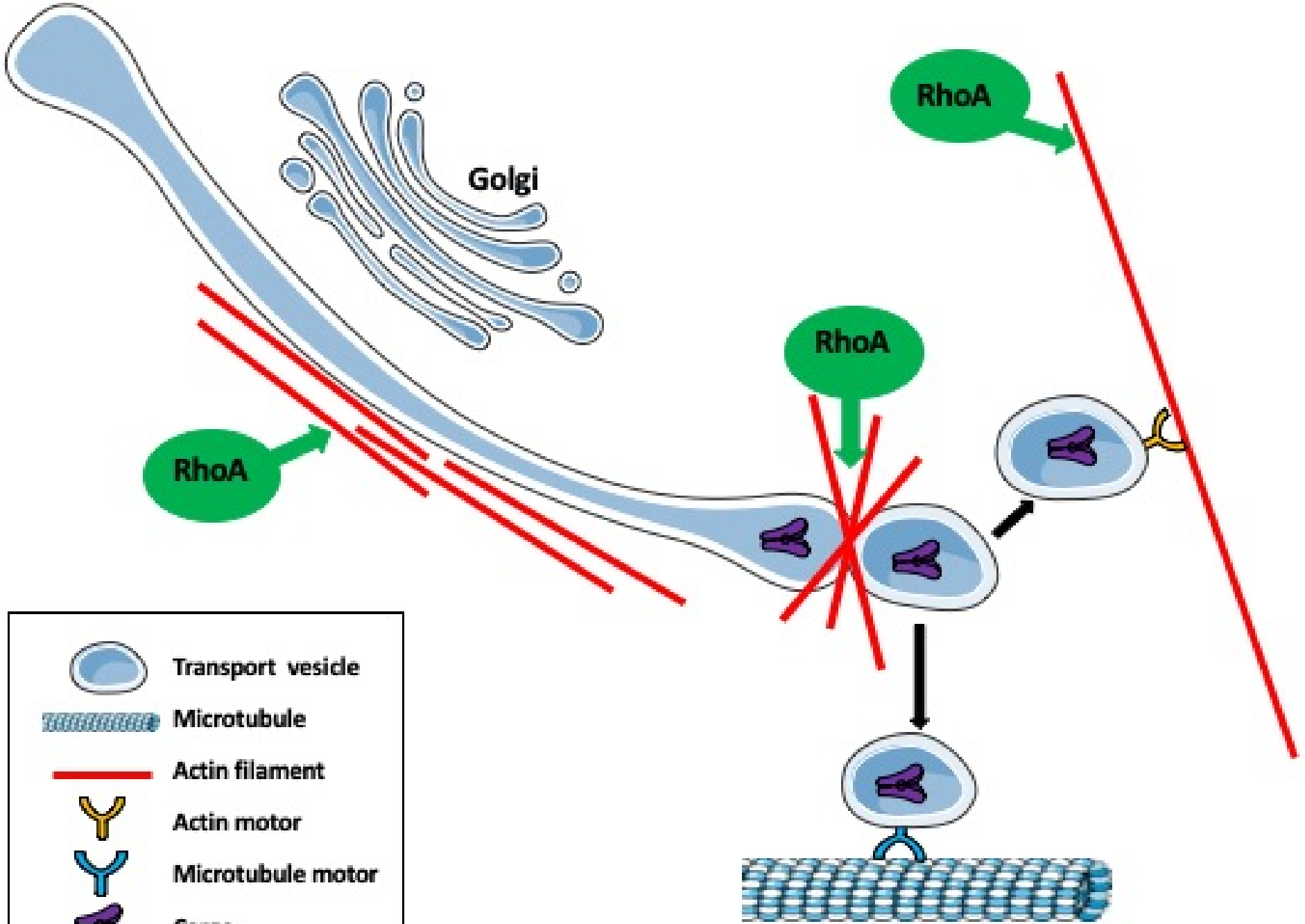
Fig. 2. Diagram of the RhoA pathway, actin, centriole and Golgi.

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 50μm. 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 18μm. Panel D is from the Fig. 6 in Chen et al. (2017) with the permission of Elsevier.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RhoA
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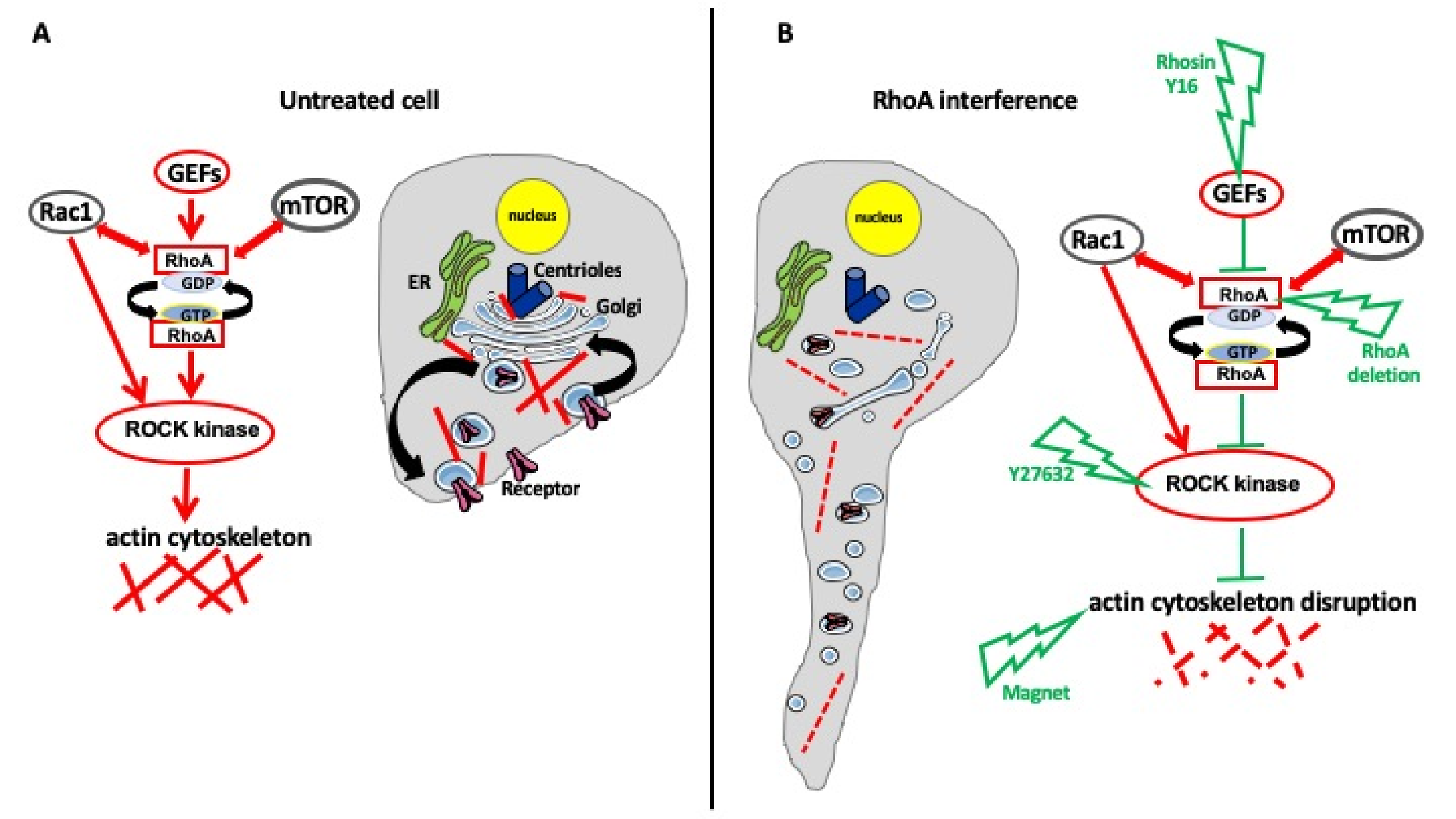
Microtubule
Actin filament
Actin motor
Microtubule motor
Cargo


A
Untreated cell
GEFs
Rac1
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RhoA
ROCK kinase
actin cytoskeleton
nucleus
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Centrioles
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Receptor
B
RhoA interference
Rhosin
Y16
GEFs
Rac1
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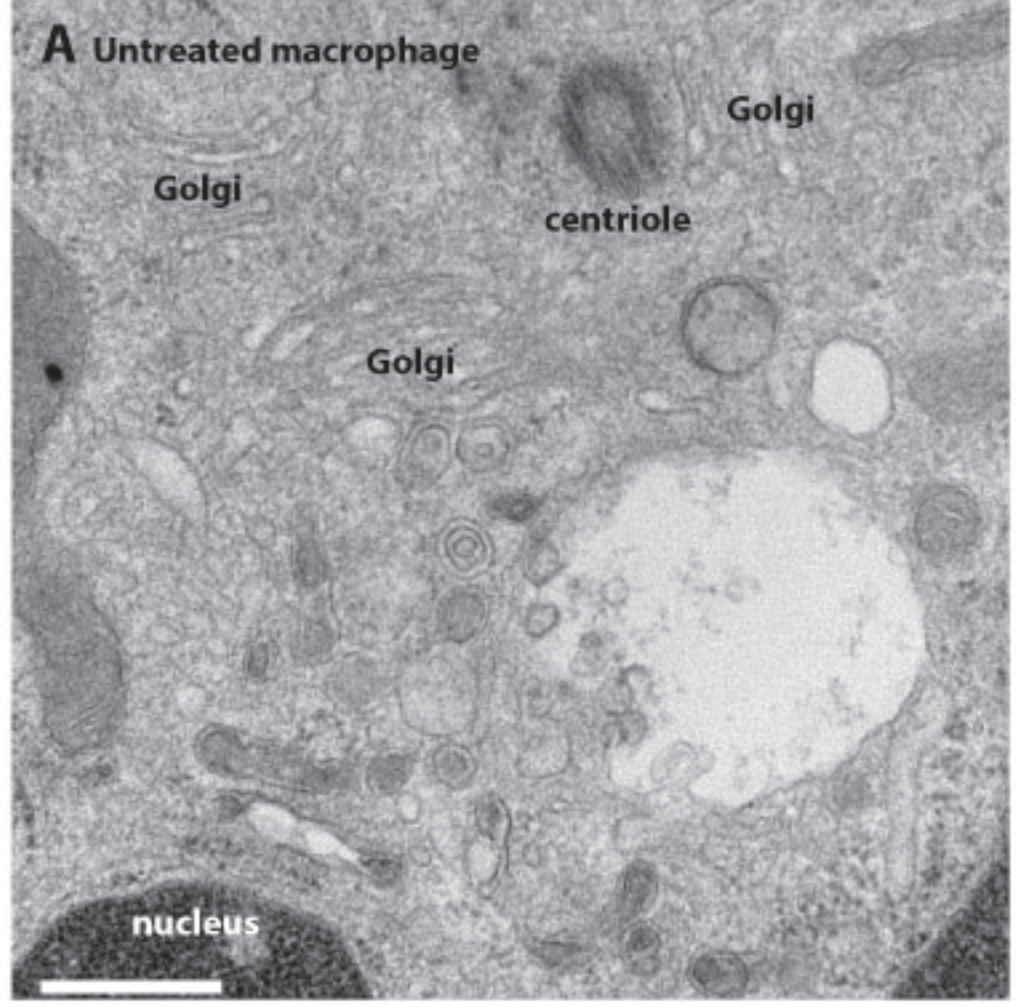
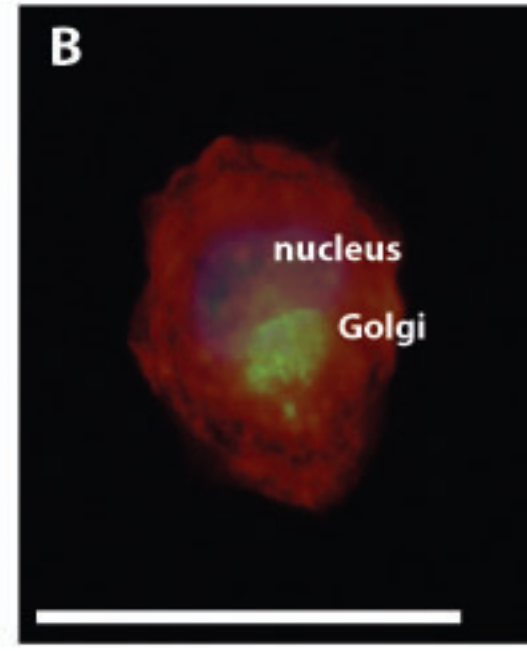

A Untreated macrophage
Golgi
Golgi
centriole
Golgi
nucleus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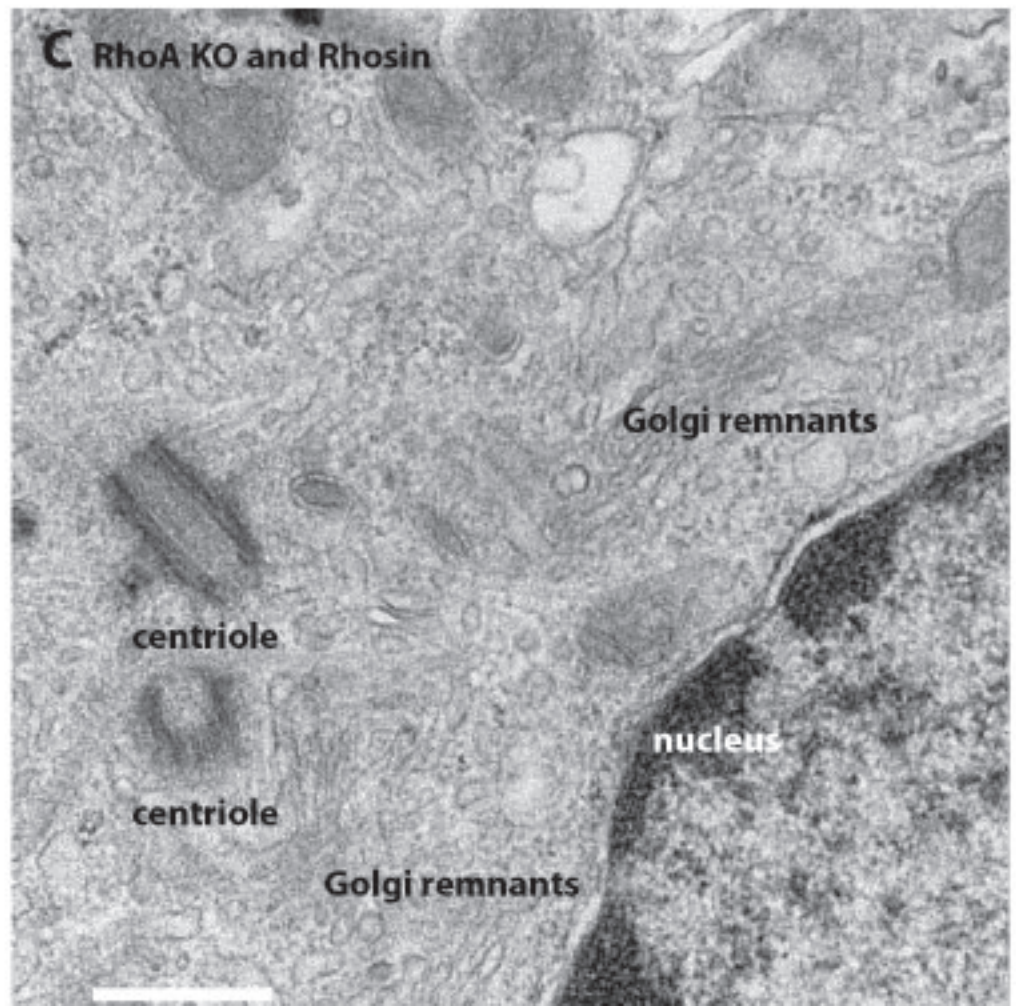
C RhoA KO and Rhosin
Golgi remnants
centriole
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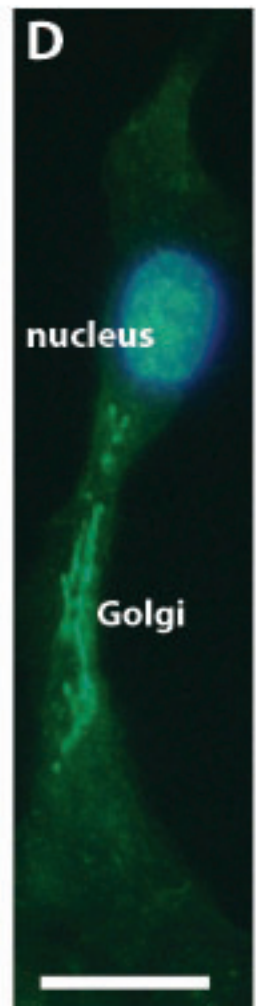
nucleus
Golgi remnants


D
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