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# Cdc42 and cellular polarity: Emerging roles at the Golgi

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## Abstract

Cdc42 belongs to the Rho family of small GTPases and plays key roles in cellular events of polarity. This role of Cdc42 has typically been attributed to its function at the plasma membrane. However, Cdc42 also exists at the Golgi complex. In this review, we summarize major insights that have been gathered in studying the Golgi-pool of Cdc42 and propose that Golgi-localized Cdc42 enables the cell to diversify the function of Cdc42, which in some cases represent new roles, and in other cases act to complement the established roles of Cdc42 at the plasma membrane. Studies on how Cdc42 acts at the Golgi also suggest key questions to address in the future.

### Keywords

Cdc42; Golgi; COPI; polarity; cell migration

## Cdc42 acts not only at the plasma membrane but also at the Golgi

Small GTPases play integral roles in intracellular signal transduction pathways. Thus, they are involved in the regulation of virtually all cellular processes [1–4]. Small GTPases typically act as molecular switches, cycling between the active (GTP-bound) and the inactive (GDP-bound) state. In their active state, these GTPases are typically localized to cellular membranes. As such, a current concept has been that compartmentalization of signaling molecules through membrane localization provides a major mechanism by which the outcome of intracellular signal transduction can be modulated [4–6].

In the case of Cdc42, a member of the Rho family of small GTPases [7,8], distinct pools have been found in different subcellular membrane compartments. These include the plasma membrane, the Golgi complex, and the endoplasmic reticulum (ER) [9,10]. The pool of Cdc42 at the plasma membrane has been well documented to play key roles in polarity and

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in the regulation of the actin cytoskeleton (Box 1) [6]. Interest developed in understanding the role of the Golgi pool, when cell-based studies uncovered that this pool is activated under certain circumstances [11]. Studies have suggested three general ways that Cdc42 can act at the Golgi: i) acting as a reservoir to replenish the pool at the plasma membrane, ii) having a function independent of the pool at the plasma membrane, and iii) coordinating with the pool at the plasma membrane to achieve polarity events (summarized in Figure 1). Key studies that have contributed to this understanding are discussed below.

## The Golgi pool acts as a reservoir for the plasma membrane pool

The possibility that the Golgi pool of Cdc42 could serve as a reservoir for the pool at the plasma membrane was initially suggested by a yeast study that sought to examine how Cdc42 acts in polarity in the absence of external cues [12]. In this study, the targeted delivery of Cdc42 to a localized region of the plasma membrane was found to require the actin cytoskeleton and also a myosin motor [12]. Delivery of Cdc42 was also found to be dependent on the exocyst [12], which is a multimeric complex that acts to dock Golgi-derived vesicles with the plasma membrane [13]. Thus, these findings suggested that Cdc42 at the Golgi is transported to the plasma membrane to exert cellular polarity.

Subsequently, mammalian studies also suggested that Cdc42 at the Golgi can be transported to the plasma membrane to exert function. In one study, a reporter construct was generated by fusing a domain from an effector of Cdc42, Wiskott-Aldrich Syndrome Protein (WASP), to a fluorescent dye. This reporter changed its fluorescence intensity in proportion to the level of active Cdc42. Using this approach, the study found that activation of Cdc42 at the Golgi coincided with activation of Cdc42 at the plasma membrane [11]. Moreover, the disruption of microtubules affected total cellular activity of Cdc42 [11]. Thus, because microtubules are involved in transport from the Golgi to the plasma membrane, the collective considerations suggested that mammalian Cdc42 at the Golgi could be replenishing the pool at the plasma membrane. Further supporting this possibility, a recent study has found that blocking transport from the Golgi to the plasma membrane decreases the activity of Cdc42 at the leading edge of migrating cells [14].

In an interesting twist, the small GTPase ADP-Ribosylation Factor 6 (ARF6) has also been found to be required for the replenishment of cdc42 at the cell surface [15]. ARF6 acts in the endocytic pathway by promoting endocytosis at the plasma membrane, and also recycling, which involves transport from a sub-compartment of the early endosome (known as the recycling endosome) to the plasma membrane [16]. How can a requirement for ARF6 be reconciled with studies that have suggested the importance of delivery from the Golgi? A potential explanation comes from the consideration that transport from the Golgi to the plasma membrane can occur indirectly, involving transit through the recycling endosome compartment [17]. Indeed, it was shown that delivery of Cdc42 together with beta-PIX (a guanine nucleotide exchange factor that activates Cdc42) is ARF6-dependent [15]. Thus, the collective observations suggest that Golgi-localized Cdc42 can be delivered to the plasma membrane in two general ways, either directly or indirectly, through the recycling endosome.

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Although studies on mammalian Cdc42 have been conducted using different cell types, the results are predicted to be generalizable, as transport from the Golgi to the plasma membrane and cell migration are fundamental cellular processes. A replenishing role for the Golgi pool of Cdc42 could serve another major purpose. A well-established mechanism of regulating the recruitment of Cdc42 to the plasma membrane involves the guanine nucleotide dissociation inhibitor (GDI), which acts to bind cytosolic Cdc42 in modulating its recruitment to target membrane. However, a replenishing role for Cdc42 suggests yet another regulatory mechanism, in this case involving transport from the Golgi to the plasma membrane. Related to this possibility, one can also envision that regulators and effectors of Cdc42 can be delivered from the Golgi to the plasma membrane in modulating the function of Cdc42 at the plasma membrane.

## The Golgi pool acts independently of the plasma membrane pool

The Golgi pool of Cdc42 can also act independently of its pool at the plasma membrane. In mammalian cells, the Golgi is formed by a series of flattened cisternae, which can be subdivided into the cis, medial, and trans regions [18]. Early studies had suggested that transport within the Golgi stacks occurs through vesicles formed by the Coat Protein I (COPI) complex [19]. Subsequently, intra-Golgi transport was found to be more complex, with the movement of the Golgi stacks mediating anterograde transport (known as cisternal maturation), and vesicles formed by the COPI complex mediating retrograde transport [20].

An initial hint that Cdc42 acts on intra-Golgi transport came from the observation that Cdc42 interacts with coatomer [21], which is a multimeric complex that constitutes the core components of the COPI complex [22]. Specifically, Cdc42 was found to compete with cargo proteins for binding to coatomer [21]. Thus, it was speculated that Cdc42 acts to inhibit retrograde Golgi transport by inhibiting the sorting of retrograde cargoes into COPI vesicles [21]. Recently, a more refined understanding of how Cdc42 acts in Golgi transport has been achieved. Besides generating vesicles for retrograde Golgi transport, COPI has been found to generate tubules, which connect the Golgi stacks in promoting anterograde Golgi transport [23].

Following up on this discovery, a recent study has found that Cdc42 not only inhibits the sorting of retrograde cargoes into COPI vesicles, but also promotes the formation of COPI tubules to enhance anterograde Golgi transport [24]. Although vesicle and tubule formation are both initiated by the formation of COPI buds from Golgi membrane, vesicle formation involves the subsequent constriction of the bud neck that eventually results in vesicles being released from organellar membrane, a process known as vesicle fission. Cdc42 was found to promote COPI tubule formation through two complementary mechanisms, promoting the initial budding process and also inhibiting the subsequent fission process, which then diverts COPI vesicle formation toward tubule formation [24]. Overall, by modulating the two major functions of the COPI complex (Figure 2), cargo sorting and carrier formation, Cdc42 polarizes transport at the Golgi to favor the anterograde direction [24].

Other studies have suggested additional ways that Cdc42 at the Golgi could impact on COPI transport. Cdc42 has been found to modulate the recruitment of dynein onto COPI vesicles,

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which suggests a microtubule-based mechanism that can dictate the directionality of COPI transport at the Golgi [25]. The same study also found that actin dynamics play a role in the recruitment of dynein to COPI carriers [25]. Further defining how Cdc42 acts, another study has uncovered an actin-based mechanism, which involves WASP promoting COPI transport [26]. Notably, because COPI had been thought for many years to act only in generating vesicles for retrograde Golgi transport, both the actin- and the microtubule-based mechanisms were only studied in the context of retrograde COPI vesicular transport. However, in light of the more recent appreciation that COPI also promotes anterograde Golgi transport through the generation of tubules [23], how the different cytoskeleton-based mechanisms affect COPI transport will need to be re-evaluated.

## The Golgi pool acts in conjunction with the plasma membrane pool

Golgi-localized Cdc42 can also act by coordinating its function with the pool at the plasma membrane for complex events of cellular polarity. One such role involves Cdc42 controlling the positioning of the Golgi. In directed cell migration, it is well known that the Golgi polarizes towards the direction of cell movement, and that altering this polarization impairs cell migration [27,28]. This role of Cdc42 has been found to be dependent on microtubules, and also under the control of ARHGAP21, a protein that catalyzes the deactivation of Cdc42 [29]. More recently, AKAP350, a Golgi-localized protein involved in the nucleation of microtubules, has been found to interact with CIP4, which acts as an effector of Cdc42 in promoting the polarity of migrating cells [30].

To ascertain that Cdc42 at the Golgi acts in conjunction with the pool at the plasma membrane for polarity events, studies have also sought to track the different sub-cellular pools of Cdc42 more directly. Spatial activation of Cdc42 has been directly visualized through reporters that are based on fluorescence resonance energy transfer (FRET) [31]. These reporters, known as Raichu, are single polypeptides generated by linking Cdc42 to an effector domain and coupling this fusion construct to a fluorescent dye. Using this approach, a recent study has revealed that the leading edge of migrating cells have higher levels of active Cdc42 as compared to its trailing edges [14]. Notably, this asymmetry of Cdc42 activity was found to be dependent on the presence of active Cdc42 at the Golgi and also on membrane transport from the Golgi to the plasma membrane. Thus, the ability of Cdc42 to position the Golgi allows the polarized delivery of secretory traffic to specific regions of the plasma membrane in achieving directional cell migration.

Another consideration is that the post-Golgi traffic should result in the expansion of membrane at the cell surface. Thus, if more membrane is delivered than Cdc42, the net effect could be a dilution of Cdc42 concentration on the plasma membrane. In this regard, it has been shown in yeast that a local autocatalytic positive feedback together with an endocytosis-based corralling mechanism ensures that exocytosis is a driving factor for polarization [32,33]. Whether a similar mechanism exists in mammalian cells remains to be elucidated.

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## Concluding Remarks

We have highlighted above three general ways that the Golgi pool of Cdc42 can act (summarized in Figure 1). As such, it appears that the Golgi-localized Cdc42 enables the cell to diversify the function of this small GTPase, which in some cases represent new roles, and in other cases act to complement its established roles at the plasma membrane. The elucidation of how Cdc42 acts at the Golgi also suggests key issues for further clarification in the future (see Outstanding Questions).

First, whether Golgi-localized Cdc42 acts in a replenishing role or in a coordinating role with the pool at the plasma membrane need not be mutually exclusive. Indeed, the collective results on how Cdc42 acts in directional cell migration suggest that both mechanisms likely act in concert in achieving this complex example of polarity. A better understanding of their relative contribution will likely come from the ability to track distinct pools of Cdc42 in the cell. Indeed, FRET-based microscopy has been used recently to uncover that GM130, a Golgi-localized protein, regulates Cdc42 at the Golgi without affecting its pool at the plasma membrane [14] (Figure 3). Similarly, a variation of the FRET technique, known as fluorescence lifetime imaging microscopy (FLIM), has been used recently to provide more conclusive evidence that Cdc42 at the Golgi affects the interaction between cargo and the COPI complex [24]. Thus, we anticipate that the further application of these advanced approaches of microscopy will provide better clarity in the future regarding the relative contributions of Cdc42 at the Golgi versus that at the plasma membrane in complex examples of polarity, such as directional cell migration.

Second, it will be interesting to determine whether the ability of Cdc42 to modulate bidirectional COPI transport at the Golgi is important for directional cell migration. Although Cdc42 has been found to promote anterograde Golgi transport by a recent study, the study has further defined that Cdc42 acts to promote a faster rate of anterograde transport than the basal rate achieved by cisternal maturation [20]. Thus, further work will be needed to determine whether a faster rate of anterograde Golgi transport induced by Cdc42 is critical for directional cell migration, or might the basal rate of transport mediated by cisternal maturation be sufficient.

Third, the involvement of the actin and the microtubule cytoskeleton has been a recurrent theme for how Golgi-localized Cdc42 acts. However, most studies have examined the roles of these two cytoskeletal processes independent of each other. Thus, a more sophisticated understanding will likely be attained in the future by examining how both cytoskeleton-based mechanisms acts in concert to achieve specific circumstance of cellular polarity that require the Golgi pool of Cdc42. Future studies will also be needed to explore other Cdc42 effectors that have been found to have a Golgi pool, such as IQGAP [34]. In this regard, a method with strong potential involves optogenic tools that can spatiotemporally modulate the actions of Cdc42. The feasibility of such an approach is illustrated by recent advances where optogenetic approaches were used to control organelle positioning [35], as well as the activity of another Rho family GTPase, Rac1 [36].

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Fourth, a general mechanistic paradigm has been that distinct functions of small GTPases can be achieved through coupling to their key classes of regulators, guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). Besides playing critical roles in catalyzing the activation and deactivation of small GTPases, these key regulators also act to specify the location and the interacting partners for specific pools of small GTPases [3]. In this regard, although GEFs and GAPs are predicted to exist for the Golgi pool of Cdc42, their identification has not been straightforward. FDG1, a GEF for Cdc42 has been localized to the Golgi [37]. However, whether it acts on the Golgi pool of Cdc42 has been questioned [14]. Another GEF that was previously reported to localize to the Golgi is TUBA [38]. However, this finding has also been controversial, as multiple other groups have not been able to replicate the finding [14, 39, 40]. Similarly, with the exception of ARHGAP21 (also known as ARHGAP10), which has been localized to the Golgi to some extent [41], how other GAPs can potentially regulate the Golgi pool of Cdc42 needs to be investigated. Besides GEFs and GAPs, other mechanisms of regulating Cdc42 are emerging. For example, Golgi-localized Cdc42 has been found recently to be regulated by a Ras GEF, known as RasGRF, which involves its binding to Cdc42 to prevent activation by relevant GEF(s) [14]. Another possibility comes from studies on the Ras small GTPase. It has been suggested that active Ras at the Golgi is an “echo” of Ras that has been activated at other cellular locations [42]. Thus, we anticipate that additional novel mechanisms in regulating Cdc42 at the Golgi will be uncovered in the future.

Fifth, Cdc42 has been found recently to possess an intrinsic ability to bend membrane in promoting the formation of COPI tubules at the Golgi [24]. This discovery is remarkable, as it is uncovering an effector function of Cdc42, in contrast to its currently known functions, which involve roles as upstream regulator of cellular events. Thus, an intriguing prospect is that other examples of effector function for Cdc42 will be uncovered in the future.

Finally, we note that a better understanding of how Cdc42 acts at the Golgi has shed key insights into pathophysiology, such as cancer biology. The finding that Cdc42 plays a critical role in cellular transformation has led to the discovery that its Golgi pool plays a key role in this process through interaction with coatomer [21], which modulates bidirectional COPI transport to achieve the polarization of Golgi transport [24]. Moreover, another study has shown recently that the aberrant regulation of Cdc42 at the Golgi by GM130 potentially contributes to colonic and breast cancer [14, 43]. As such, the different lines of future investigation that we have outlined above have the prospect of advancing not only a basic understanding of how Cdc42 acts at the Golgi, but also how this pool plays key roles in human diseases.

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### Box 1: Cdc42 and its roles at the plasma membrane

As a small GTPase, Cdc42 is GDP-bound in its inactive state and is kept soluble in the cytosol by binding to GDIs. Cdc42 is activated upon interaction with GEFs. In its GTP-bound active state, Cdc42 engages effectors to promote its biologic functions. The vast majority of research has been centered on studying the roles of Cdc42 at the plasma membranes. Several polarity-inducing stimuli activate Cdc42, such as receptor tyrosine kinases, adhesion molecules (cadherins), and G-protein coupled receptors [8]. Active Cdc42 binds to the plasma membrane, preferably to domains enriched in phosphatidylinositol (4,5) bis-phosphate, which are maintained by PTEN [44]. Cdc42 binds to WASP and thereby activating the Arp2/3 complex resulting in the formation of membrane protrusions, such as filopodia [8]. Cdc42 also activates the Par complex, which contains atypical protein kinase C (aPKC). Active aPKC suppresses Glycogen synthase kinase 3β leading to the association of the adenomatous polyposis coli protein (APC) with microtubule plus-ends and their stabilization at the leading edge of migrating cells [8]. Plus-ends of microtubules are also stabilized by Cdc42-dependent recruitment of IQGAP and the plus-end protein CLIP-170 [45]. The stabilization of microtubules at the leading edge of migrating cells promotes the reorientation of the MTOC towards the direction of migration.

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### Outstanding questions

- How is Golgi-localized Cdc42 regulated?
- Does Golgi-localized Cdc42 modulate cytoskeletal dynamics and is this relevant for cell polarity and migration?
- Is the ability of Cdc42 to modulate bidirectional COPI transport at the Golgi is important for directional cell migration?
- What is the functional impact of dysregulation of Cdc42 at the Golgi in tumors and can this be targeted therapeutically?
- Does Cdc42 have any other effector functions besides its ability to impart membrane curvature on Golgi membranes?

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## Trends Box

Cdc42 exists and functions at the plasma membrane and at the Golgi.

Cdc42 can replenish the pool at the plasma membrane via transport from the Golgi to the plasma membrane, which can occur either directly or indirectly by additional transit through the recycling endosome.

Cdc42 can act distinctly from the pool at the plasma membrane. A major way involves Cdc42 regulating bidirectional Golgi transport, acting to prevent retrograde cargoes from being sorted into COPI vesicles and also promoting the formation of COPI tubules that act in anterograde Golgi transport.

Cdc42 can act in conjunction with the pool at the plasma membrane. One example is Cdc42 regulating the positioning of the Golgi complex. By coordinating this role with Cdc42 acting at the plasma membrane, surface protrusions are generated at the leading edge of migrating cells.

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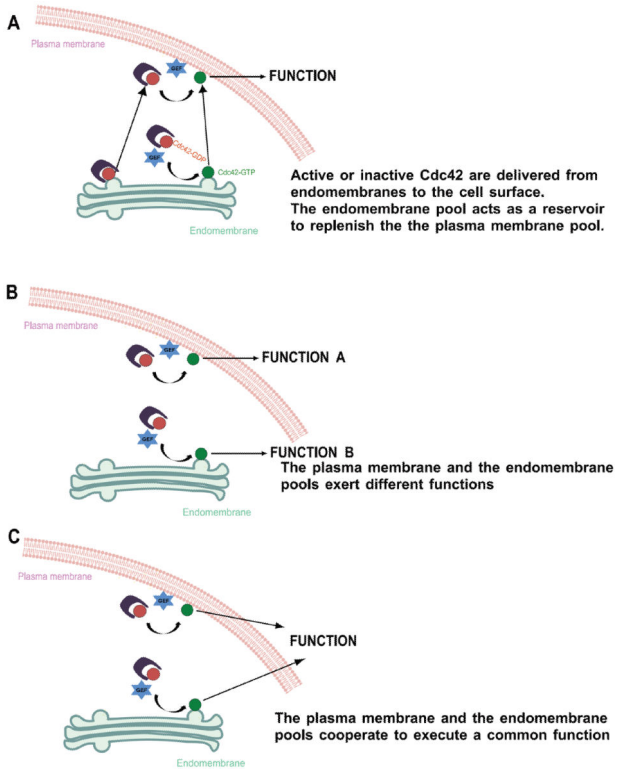


**Figure 1.**

Schematic illustration of the three basic modes of action of Cdc42 at endomembranes. *A*, the endomembrane pools acts as a reservoir to replenish the plasma membrane. Inactive Cdc42 (red circle) is GDI bound in the cytosol and becomes membrane associated upon activation. Active Cdc42 (green circle) might be delivered from the Golgi to the plasma membrane. Likewise, inactive Cdc42 might be delivered to the plasma membrane to be activated there. The biologic effects of Cdc42 are exerted at the plasma membrane. *B*, the endomembrane Cdc42 pool exerts different functions than the plasma membrane pool. Cdc42 is activated locally on both endomembranes as well as on the plasma membrane separately. Each pool exerts a different biologic function. *C*, Cdc42 is activated locally on both endomembranes as well as on the plasma membrane separately and both pools cooperate to exert a common biologic output.

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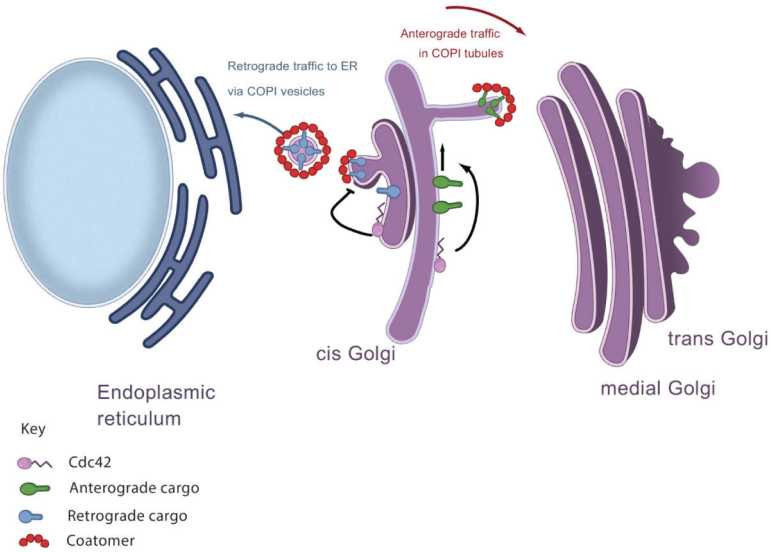


**Figure 2.**

Schematic illustration of the opposing role Cdc42 on regulation of anterograde and retrograde COPI trafficking. The COPI coat mediates retrograde transport to the ER in small vesicles and this is suppressed by active Cdc42 at the Golgi. At the same time, anterograde intra-Golgi trafficking is mediated via COPI coated tubules and this trafficking step is supported by active Cdc42 at the Golgi.

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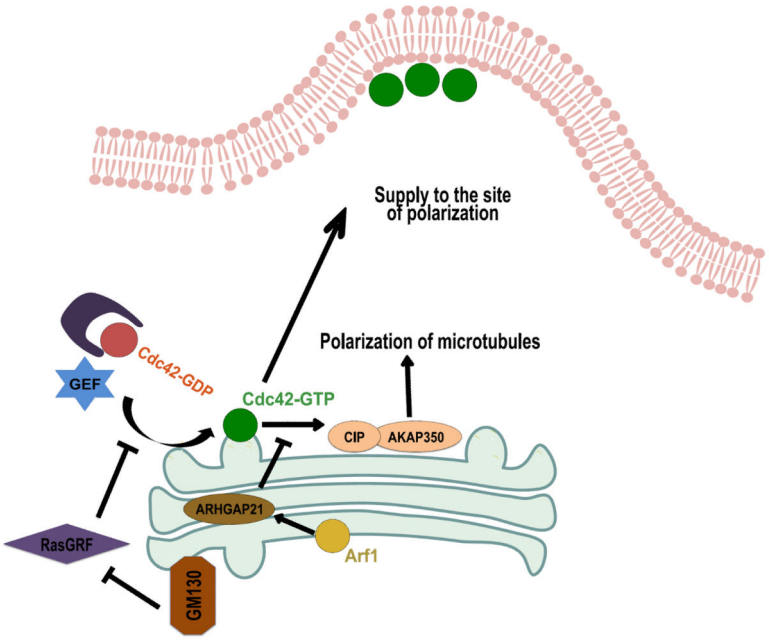


**Figure 3.**

Illustration of the regulation of Cdc42 at the Golgi and its role in cell polarization. The Golgi matrix protein GM130 sequesters RasGRF, an inhibitor of Cdc42 activation. Cdc42 at the Golgi may lead to recruitment and activation of the CIP-AKAP350, which promotes the polarization of microtubules towards the site of polarization. Active Cdc42 maybe itself delivered to the site of polarization, thereby sustaining polarity. At the Golgi, Cdc42 is subject to deactivation by ARHGAP21, which is recruited to the Golgi in a manner dependent on the small GTPase Arf1.