

Control of the cytokinetic apparatus by flux of the Rho GTPases

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Abstract

Cytokinesis in animal cells is powered by the cytokinetic apparatus, a ring of filamentous actin and myosin-2 that underlies the plasma membrane and closes between the separating chromosomes. Formation of the cytokinetic apparatus is at least partially dependent on the small GTPase, Rho. Similar to other small GTPases, Rho cycles between the active (GTP-bound) and inactive (GDP-bound) states. Because of this switch-like behaviour, Rho and other members of the Rho GTPase family, such as Rac and Cdc42, have long been thought to work in a manner such that their activation and inactivation are not tightly coupled. That is, a given Rho-dependent event, such as cytokinesis, has been thought to be initiated by activation of Rho, and then, many minutes later, terminated by inactivation of Rho. Here we discuss evidence suggesting that in fact Rho undergoes rapid movement through the GTPase cycle throughout the entire process of cytokinesis, and that this cycling is necessary for proper cytokinetic apparatus function.

Introduction

Cytokinesis is the essential cellular process in which two daughter cells are physically separated from each other at the end of the cell division cycle. In animal cells, cytokinesis is powered by the closure of the cytokinetic apparatus, an array of F-actin (filamentous actin) and myosin-2 that assembles at and is anchored to the equatorial plasma membrane. The cytokinetic apparatus is a fascinating structure: it is called into existence within the space of 1–2 min, it rapidly closes inward, bringing the plasma membrane with it, and then it disassembles. Thus the mechanisms that control cytokinetic apparatus assembly and function have been the subject of intensive investigation for many years.

Formation of the cytokinetic apparatus is at least partly controlled by the small GTPase, Rho. Not only is Rho activated in a precisely defined ‘zone’ at the cell equator prior to cytokinesis in a variety of organisms and cell types [1], but also prevention of Rho activation and/or disruption of Rho regulators likewise impairs cytokinesis in a variety of organisms and cell types (e.g. [1–4]). Furthermore, several Rho targets, such as Rho-activated kinase, which promotes myosin-2 activation, and formins, which promote assembly of unbranched actin networks, are needed for proper assembly of the cytokinetic apparatus (e.g. [5,6]).

Rho, similarly to other members of the RhoGTPase family, including Rac and Cdc42, cycles between active (GTP-bound) and inactive (GDP-bound) states. *In vitro*, both GTP hydrolysis and exchange of GDP for GTP are extremely slow.

However, *in vivo*, the two steps are accelerated by GAPs (GTPase-activating proteins) and GEFs (guanine-nucleotide-exchange factors) respectively. A third class of Rho GTPase regulators, the GDIs (guanine-nucleotide-dissociation inhibitors) can further modulate the cycle, by binding to the GDP-bound form of the Rho GTPases and slowing exchange for GTP. The cycle is coupled to differential targeting of Rho GTPases to the plasma membrane, such that in the active form they associate with the plasma membrane, but in the inactive form they are cytosolic and associated with GDIs.

Results and discussion

Given the switch-like behaviour of Rho GTPases *in vitro*, and classic studies showing assembly of F-actin-rich stress fibres is triggered by mutant forms of Rho that cannot hydrolyze GTP (and are thus constitutively active) [7], it has long been thought that Rho controls contractile processes in a stepwise manner. That is, in response to a particular signal, a Rho GEF is activated and then activates Rho. Active Rho then binds effector proteins (such as Rho-activated kinase and formins) to start the process in question. Rho remains active until the process is completed, at which time a Rho GAP is activated. This GAP inactivates Rho, thereby causing termination of the process. An important feature of this view is that the activation and inactivation of Rho are temporally uncoupled. To use the specific example that is most relevant to this article, the currently favoured model for cytokinesis proposes that cytokinesis is initiated by a GEF called Ect2. Ect2 is thought to activate Rho at the equator. Active Rho then promotes myosin-2 activation and actin assembly, thereby driving formation of the cytokinetic apparatus. Active Rho remains associated with the equator as the contractile apparatus closes. Once closure is complete, Rho

Key words: cell division, cytokinesis, filamentous actin (F-actin), myosin-2, Rho, signalling.

Abbreviations used: F-actin, filamentous actin; GAP, GTPase-activating protein; GEF, guanine-nucleotide-exchange factor; GDI, guanine-nucleotide-dissociation inhibitor.

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is thought to be inactivated by a GAP called MgcRacGAP [8,9]. Again, in this model for cytokinesis, Rho activation and inactivation are thought to be temporally separated.

While this model is in keeping with traditional views of how Rho GTPases work, it also has important shortcomings. The first comes from the observation that the width of the zone of active Rho is constant, even as its intensity increases [1]. Given the measured rate of GTP hydrolysis by Rho *in vitro* (1 GTP per Rho per 50 min [10]), it would be expected that the Rho zone would broaden over time, as more active Rho is generated and it diffuses from the site of activation. In principle, zone broadening could be counteracted by anchoring active Rho at the plasma membrane. However, a mechanism based entirely on anchoring and activation has its own problem: the amount of active Rho within a zone is constantly decreasing as the contractile apparatus closes. To put this in concrete terms, a Rho zone in a dividing sea urchin blastomere would initially occupy $\sim 10\,000\ \mu\text{m}^2$, but within ~ 5 min, occupies $\sim 5000\ \mu\text{m}^2$, representing a 50% reduction in active Rho. However, based on measured rates of GTP hydrolysis by Rho *in vitro* (see above), the maximum expected loss of Rho activity during this time frame is 5%.

The second major problem with a model in which Rho activation and inactivation are uncoupled is that the cytokinetic apparatus as well as the Rho zone show considerable plasticity. That is, micromanipulation studies have shown that if a spindle is physically displaced after the assembly of the cytokinetic apparatus, the original cytokinetic apparatus rapidly disappears and a new one reforms in a position corresponding to the midplane of the repositioned spindle [11]. The Rho zone shows similar behaviour in spindle displacement experiments, as well as instances where the spindle spontaneously repositions itself: the zone is rapidly lost from its original position and then reforms over the midplane of the repositioned spindle [1]. It is difficult to imagine how Rho zones could quickly respond to changes in spindle position if they are generated by a mechanism that is dependent only on Rho activation and anchoring.

Based on these and other considerations, we have proposed the GTPase flux model [12]. In this model, Rho zones do not simply represent sites of local Rho activation, but instead sites where Rho is being rapidly driven through the entire GTPase cycle. Although counterintuitive, mathematical modelling shows that reducing the half-life of active Rho along with limiting its diffusion is much better at mimicking the observed spatial and temporal dynamics of Rho zones than simply limiting diffusion [12]. That is, limited diffusion alone results in zones that take many minutes to reach steady state and which are accompanied by progressive broadening. In contrast, a combination of limited diffusion and rapid turnover yields zones that reach steady state within a minute or two, and which maintain a narrow focus.

The flux model also explains how zones can respond quickly to spindle displacement. Specifically, because Rho turns over quickly within a zone, constant Rho activation is required to maintain the zone. When the spindle is moved, the stimulus is displaced to a new region where a new zone

forms, while at the same time turnover erases the original zone. Such a mechanism would allow zones to adjust quickly to even small changes in the spindle position, ensuring that the cytokinetic apparatus itself maintains the tight focus originally described more than three decades ago [13].

Assuming that the GTPase flux model is correct, it becomes essential to identify and investigate mechanisms that might ensure rapid Rho turnover within the zone. One simple means of achieving rapid Rho turnover would be to combine both GEF and GAP activity. This could be accomplished if the GAP activity of MgcRacGAP is active from the beginning of cytokinesis, rather than just at the end. Consistent with this notion, it has been reported that Aurora B kinase phosphorylates MgcRacGAP and increases its activity as a Rho GAP [9]. The authors of that study envisaged that the Rho GAP activity of MgcRacGAP would be used to disassemble inactive Rho at the end of cytokinesis and thereby promote disassembly of the cytokinetic apparatus; however, as Aurora B is both active and localized to the same region as MgcRacGAP early in cytokinesis, it is also possible that phosphorylation of MgcRacGAP by Aurora B could promote the Rho GTPase flux.

It remains to be determined whether or not the GAP activity of MgcRacGAP is important for Rho GTPase flux. Previous studies have indicated that the GAP activity of MgcRacGAP may [14], or may not be [15] required for cytokinesis, but in no case has the role of the GAP domain in Rho zone dynamics been assessed. However, preliminary results from the *Xenopus* embryo system suggest that the GAP activity of MgcRacGAP is indeed important for regulating the Rho zone in cytokinesis (A.L. Miller, unpublished work). Nevertheless, it is important to keep in mind that while MgcRacGAP is an obvious candidate to mediate Rho GTPase flux, it is not the only one. For example, p190 Rho GAP is cell cycle regulated and disruption of its function has been shown to inhibit cytokinesis [16], although, as with MgcRacGAP, it is not yet known if perturbation of the GAP activity of p190 Rho GAP perturbs Rho zone dynamics.

Negative cross-talk between the Rho GTPases could also potentially help sculpt the cytokinetic Rho zone. For example, it has been reported that Rac and Cdc42 activity are elevated in regions outside the equator during cytokinesis [17]. Further, during polar body emission in amphibians, the zone of active Rho circumscribes a circular patch of active Cdc42 over the forming polar body [18]. This sort of mutually exclusive localization of the different active Rho GTPases could reflect mutually inhibitory cross-talk. That such negative cross-talk occurs is not in doubt, but its molecular underpinnings are poorly understood. One potential mechanism is provided by phosphorylation of RhoGDI by Pak, an effector of Rac [19]. Pak-dependent RhoGDI phosphorylation results in suppression of RhoGDI binding to Rac, while still permitting Rho binding [19]. A potential *in vivo* outcome of such an event could be elevation of Rho turnover in regions where Rac activity is high, since (presumably) more RhoGDI would be available to interact with Rho. Although classically RhoGDIs are thought to bind to Rho-GDP and would thus not be

expected to modulate Rho turnover until after its inactivation by a GAP, there is some evidence that GDIs can also interact with GTP-bound versions of the Rho GTPases [20].

Finally, while the above discussion is focused on cytokinesis, similar reasoning applies to a variety of other biological processes controlled by the Rho GTPases. For example, during both cellular wound repair [21] and phagocytosis [22], Rho GTPases are transiently activated in zones that, similar to the cytokinetic Rho zone, undergo rapid, continuous reduction in overall size. We therefore suggest that Rho GTPase flux is probably generally used by cells to control actomyosin dynamics [12].

In summary, while much attention has been focused on the means by which Rho is activated during cytokinesis, this almost surely represents only part of the story. Proper control of the Rho zone probably requires mechanisms that promote inactivation as well, so as to ensure that Rho moves rapidly through the GTPase cycle.

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