nature reviews molecular cell biology

https://doi.org/10.1038/s41580-023-00682-z

Review article

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Patterning of the cell cortex by Rho GTPases

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Abstract

The Rho GTPases — RHOA, RAC1 and CDC42 — are small GTP binding proteins that regulate basic biological processes such as cell locomotion, cell division and morphogenesis by promoting cytoskeleton-based changes in the cell cortex. This regulation results from active (GTP-bound) Rho GTPases stimulating target proteins that, in turn, promote actin assembly and myosin 2-based contraction to organize the cortex. This basic regulatory scheme, well supported by in vitro studies, led to the natural assumption that Rho GTPases function in vivo in an essentially linear matter, with a given process being initiated by GTPase activation and terminated by GTPase inactivation. However, a growing body of evidence based on live cell imaging, modelling and experimental manipulation indicates that Rho GTPase activation and inactivation are often tightly coupled in space and time via signalling circuits and networks based on positive and negative feedback. In this Review, we present and discuss this evidence, and we address one of the fundamental consequences of coupled activation and inactivation: the ability of the Rho GTPases to self-organize, that is, direct their own transition from states of low order to states of high order. We discuss how Rho GTPase self-organization results in the formation of diverse spatiotemporal cortical patterns such as static clusters, oscillatory pulses, travelling wave trains and ring-like waves. Finally, we discuss the advantages of Rho GTPase self-organization and pattern formation for cell function.

Sections

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Introduction

The Rho GTPases — RHOA, RAC1, CDC42 and their close relatives — are small GTP binding proteins of the Ras superfamily that are best known for their regulation of actin filaments (F-actin) and the motor protein myosin 2 (ref. 1). Similar to other members of the superfamily, Rho GTPases undergo cycles of GTP binding and hydrolysis (Fig. 1a), and these cycles are linked to their ability to signal to their targets: Rho GTPases are active when bound to GTP and can bind to and stimulate so-called effector proteins which, in turn, signal to F-actin and myosin 2. Following hydrolysis of GTP to GDP, the Rho GTPases can no longer bind to their effectors and become inactive, until they exchange GDP for GTP, completing the cycle. In vitro, GTP hydrolysis and GDP–GTP exchange are extremely slow; in vivo these steps are accelerated by GAPs (GTPase activating proteins) and GEFs (guanine nucleotide exchange factors), respectively. The GTPase cycle has a distinct spatial context within the cell, with active (GTP-bound) Rho GTPases associating with the plasma membrane via their carboxy-terminal prenyl groups. Following inactivation, the GTPase can be extracted from the plasma membrane by RhoGDI (Rho guanine nucleotide dissociation inhibitor), which encloses the prenyl group and maintains the inactive GTPase in a soluble, cytosolic form[2–4] (Fig. 1a).

Targeting of the Rho GTPases to the plasma membrane is of particular significance because it gives them access to the cell cortex. The cell cortex is the outermost layer of the cell and includes both the plasma membrane and the layer of cytoplasm just beneath it[5]. The cortex is typically rich in F-actin ('cortical F-actin') and myosin 2 ('cortical myosin 2') and is of great importance to the cell because it drives cell shape changes needed for an enormous variety of processes including cell migration, phagocytosis, polarity establishment, cytokinesis and morphogenesis. The Rho GTPases enable the cell shape changes required for these processes, by virtue of their ability to rapidly remodel the cortical F-actin and myosin 2 via activation of their effectors at the plasma membrane[6–10]. Consequently, the subcellular patterning of Rho GTPases in the cortex and how this is controlled have long been the subject of intense interest.

The dominant model for Rho GTPase patterning has been one in which pattern control is exercised in an essentially linear manner, with a given upstream signal such as a growth factor stimulating a GEF in a particular region of the plasma membrane, which then stimulates the Rho GTPase in the same region. The high Rho GTPase activity then triggers a particular response such as cytokinesis (Fig. 1b). In this activation-centric view, GEFs are the primary drivers of the response, whereas GAPs are considered to simply restrain or terminate the response. However, recent studies in various model systems indicate that upstream signals result in the engagement of both Rho GTPase GEFs and GAPs at the same time, and show that this results in self-organization of the Rho GTPases into cortical patterns such as stable clusters, travelling waves and oscillatory pulses[11–21]. Thus, it is not simply activation of the Rho GTPases that matters but the GTPase cycle itself and the resultant pattern (Fig. 1c).

The objective of this Review is to present and discuss the evidence that self-organization plays a major role in the regulation of the Rho GTPases. Towards this end, we first discuss Rho GTPase patterns and provide a brief overview of self-organizing patterns. We consider the role of positive and negative feedback in such patterns, both in generic terms and then in terms of the Rho GTPase GEFs and GAPs known to engage in feedback in cells. We then present a series of examples of self-organizing Rho GTPase patterns, drawing on recent studies of diverse processes and model systems. Finally, we discuss the advantages that arise from the use of self-organization for signalling at the cell cortex.

Rho GTPase zones and Rho GTPase flux

Early imaging studies of Rho GTPase dynamics using different approaches (Box 1) revealed that during processes including yeast budding[22], cytokinesis[23], plasma membrane repair[24], exocytosis[25] and cell locomotion[26,27], RHO and CDC42 are activated in cortical 'zones' — regions of the cell cortex where Rho GTPase activity is highly elevated.

These zones are local patterns such as stripes, patches and rings that are highly enriched in GTPase activity relative to the immediately surrounding areas. They can emerge and disappear within seconds to minutes, even when occupying thousands of square micrometres. Superficially, it might seem reasonable that such patterns could be generated by Rho GTPase activation alone simply by localization of a GEF at the site of the zone in the absence of GAP activity or other mechanisms for removal of active GTPases from the plasma membrane. Indeed, Rho GEFs are often targeted to distinct subcellular locations[28]. However, any accumulation of active Rho GTPase at the plasma membrane will be counteracted by diffusion of the GTPase away from the site of activation, thereby degrading the pattern. The very low intrinsic rate of GTP hydrolysis by Rho GTPases will exacerbate this problem as the active GTPases can potentially diffuse very far away from the site of activation, essentially raising the background level of GTPase activity and thereby further degrading the pattern.

These observations led to the 'GTPase Flux Hypothesis', which posits that Rho GTPase activation and inactivation are tightly coupled within zones to counteract the effects of GTPase diffusion[29]. An independent modelling study demonstrated that the high activity and fast turnover of small GTPases characteristic of activity zones requires simultaneous GEF and GAP action[30]. Confirmation of these concepts was provided by demonstrations that suppression of GAP expression does not simply increase GTPase activity but, rather, disrupts GTPase patterns or patterns of their targets[31,32], and by studies showing that Rho GTPases have very short (several seconds or less) half-lives at the plasma membrane[33–35]. Put simply, steady-state maintenance of the GTPase activity zones requires continuous delivery of inactive GTPase, its activation and compensatory inactivation and removal. Consequently, the concept of GTPase flux was further extended from local GTPase cycling to GTPase transport and nucleotide cycling on a cellular scale[36].

Thus, activation and inactivation must be temporally coupled to account for the existence of Rho GTPase zones (Fig. 1c). In one common implementation of this requirement, Rho GTPase GEFs and GAPs can be targeted to complementary compartments (for example, an apical GEF and a basolateral GAP in epithelial cells)[37–40]. However, recent studies based on improved live imaging approaches, combined with theoretical modelling and experimental manipulations, have revealed another, less intuitive mechanism cells use to generate dynamic Rho GTPase patterns: spatial coupling of the GEFs and GAPs. Specifically, studies in many systems including budding and fission yeast[11,12], worms[13], flies[14], echinoderms[15,16], frogs[15,17], cultured mammalian cells[18–20] and cell-free extracts[21] reveal that Rho GTPases exhibit periodic (cyclic) activity patterns including single travelling waves, travelling wave trains and oscillatory pulses (Fig. 2). Behaviours such as these are hallmarks of signalling networks that couple positive and negative feedback to drive self-organized pattern formation[41,42].

Self-organizing patterns of Rho activity

Self-organization is the spontaneous acquisition of order by a previously disordered system arising from local interactions of system constituents[43]. Self-organization requires continuous energy

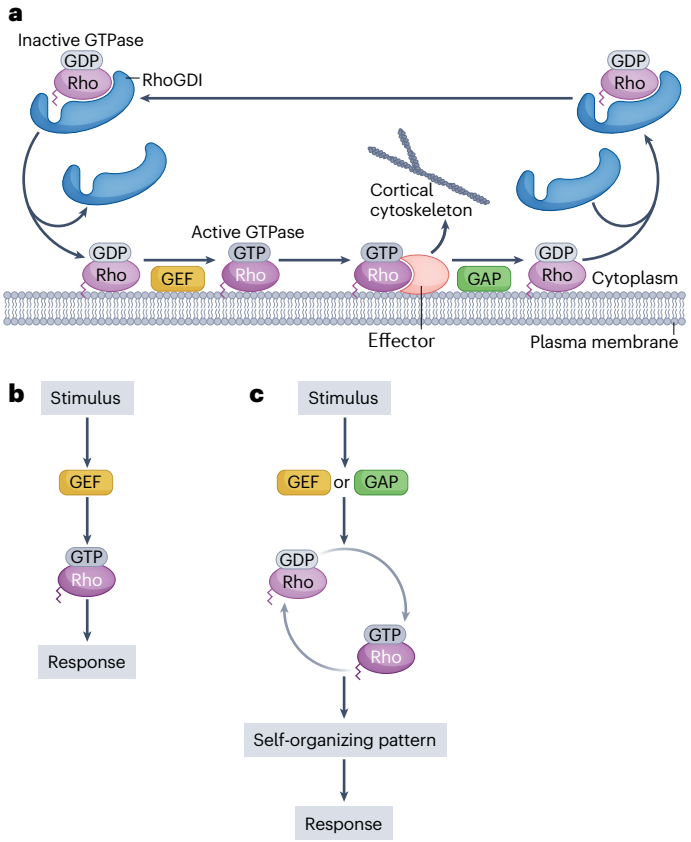


Fig. 1 | Basic principles of Rho GTPase regulation. a, The Rho GTPase cycle. Activation of Rho GTPase (that is, exchange of GDP for GTP) results from interaction with a GEF (guanine nucleotide exchange factor). The active GTPase can then bind to an effector resulting in changes in the cortical cytoskeleton. GTPase inactivation results from interaction with a GAP (GTPase activating protein) and is followed by extraction of the GTPase from the plasma membrane by RhoGDI, rendering the GTPase soluble in the cytoplasm. RhoGDI then somehow returns the inactive Rho to the plasma membrane, completing the cycle. **b**, Activation-centric view of Rho GTPase signalling. In this view, the path from the stimulus to the response is essentially linear, with the stimulus activating a GEF, the GEF activating the GTPase and the active GTPase directing the response, whereas the contributions of GAPs to the response are considered to merely limit or terminate the response. **c**, Self-organizing view of Rho GTPase signalling. In this view, the path from the signal to the response is highly non-linear, with the stimulus activating both the GEF and the GAP resulting in continuous GTPase cycling and self-organization of the GTPases into patterns which then dictate the response. In the figure, Rho indicates RHOA, RAC1 or CDC42.

investment[44], and thus represents a state far from equilibrium, even when an apparently stationary organization is achieved. This contrasts with self-assembly, wherein the process usually proceeds to equilibrium[44]. At the subcellular level, self-organization is most famously associated with formation of the mitotic spindle[45,46], but it is becoming increasingly apparent that self-organization is ubiquitous[47] with the potential to contribute to many aspects of cell behaviour, including patterning of the plasma membrane[48].

To understand how Rho GTPases form self-organizing patterns requires familiarity with patterning by so-called 'activator-inhibitor' systems[49,50]. Activator-inhibitor systems are generic, idealized models of pattern formation, which can be used to explain how Rho GTPases self-organize, with the active GTPase serving as the activator and GTP hydrolysis providing the requisite energy investment (Box 2).

It is important to realize that mechanisms such as the activator-inhibitor system are idealizations, and patterning in living cells, by Rho GTPases or other self-organizing systems, is typically subject to many influences. Such influences include positional cues that can direct pattern formation to certain areas of the cell, often acting by increasing the local concentration of the pattern-forming elements. For example, as described in more detail below, formation of the CDC42-GTP cluster during yeast budding is normally confined to the site of previous budding by so-called landmark proteins. In another example, the mitotic spindle directs the concentration of high-amplitude, complementary RHOA-GTP and F-actin waves at the equator during cytokinesis. Molecular noise is another influence in self-organizing patterns. Molecular noise is the natural (spontaneous) fluctuation of local protein concentration and has the potential to impact all aspects of cell biology[51]. In the context of self-organizing GTPase pattern formation, the role of noise is expected to be particularly prominent when and where the parameters of the pattern-forming network are close to the onset of pattern formation and in which normal positional cues for pattern formation are lacking. Thus, in the budding yeast in which landmark proteins have been experimentally removed, molecular noise determines where the bud forms[52]. Similarly, in the absence of external guidance cues, noise determines which ends are chosen to be the front and the back of migrating cultured cells plated on narrow adhesive stripes[53,54].

The patterns presented in Box 2 are only a small sample of the patterns that the mathematical models of non-equilibrium systems can produce in silico[55,56], prompting the question of how self-organizing patterns are identified in vivo. Although there are no absolute rules, two or more of the following criteria are typically applied. The first is identification of pattern dynamics that are characteristic of activator-inhibitor systems such as waves or oscillatory pulses simply because these are often most easily explained by self-organization. Conversely, underlying self-organization can be revealed by the onset of periodic behaviours following some manipulation. For example, the appearance of oscillatory behaviour following the removal of a pattern regulator is often taken as a sign of missing feedback[57]. Given the importance of feedback, a second common criterion is evidence that system component interactions can generate feedback. A third criterion is evidence that the pattern can form spontaneously, even when upstream cues are compromised. For example, during the budding process, budding yeast normally form a cap of CDC42-GTP close to the site of previous cell division due to the influence of landmark proteins; when these proteins are genetically deleted, the cap still forms, but it is mislocalized[58] (see also below). A fourth criterion is the ability of a theoretical model based on the principles of self-organization to capture the features of the experimental pattern or, better still, to make testable predictions about the pattern that are confirmed by experiment. A fifth (and as yet extremely challenging) criterion is the successful reconstitution of the pattern in vitro from an initially homogeneous mixture of the components[59–61].

Rho GTPase feedback loops

Activator-inhibitor mechanisms for patterning typically presuppose the existence of both positive and negative feedback in Rho GTPase regulation, a supposition that is fulfilled by the observation that Rho GTPases can both positively and negatively regulate their own GEFs and GAPs[62–65].

Indeed, a wealth of such feedbacks have been described (Table 1 and Fig. 3). The feedback mechanisms can be grouped based on the number of steps between the GTPase and the GEF or GAP (Fig. 3). First is direct feedback, where the active GTPase itself binds to a GEF and modifies its activity[62–65]. For example, at least seven Rho GEFs[66–68] and at least one CDC42 GEF[69] interact allosterically with RHO-GTP or CDC42-GTP, respectively, an interaction which directs the GEF to the plasma membrane and increases its activity. Such interactions drive positive feedback (the GTPase activates its GEF, generating more active GTPase); to date there are no reported examples of active Rho GTPases binding to and stimulating their own GAPs (see also below). The second step is effector-based feedback, where the GTPase effector modifies the activity or localization of a GEF or GAP. For example, active GTPases often bind scaffold proteins that also bind and stimulate their upstream GEF to drive positive feedback[70–73]. Last is effector target-based feedback, where a downstream target of a given effector modifies the activity or localization of a GEF or GAP. For example, F-actin, a downstream target of actin regulatory proteins such as formins (RHO and CDC42 effectors) and N-WASP (a CDC42 effector), can modulate the activity or localization of both GEFs and GAPs[72,74–77].

These examples are by no means exhaustive, and the network depicted in Fig. 3 and the examples presented in Table 1 omit other potential feedback mechanisms such as those that work through RhoGDI[78]. Nonetheless, a striking observation emerges: based on the number of participants in a feedback loop and the number of levels at which feedback acts, feedback itself is a fundamental outcome of the Rho GTPase signalling. That is, not only do the GEFs act as effectors in direct feedback but all of the major classes of effectors also participate in feedback, including formins[79], N-WASP[72], Rho-associated protein kinase (ROCK)[80], p21-activated kinases (PAKs)[81,82] and protein kinase N (PKN)[83]. With respect to downstream targets, in addition to F-actin, monomeric actin (G-actin) participates in feedback[84], as does

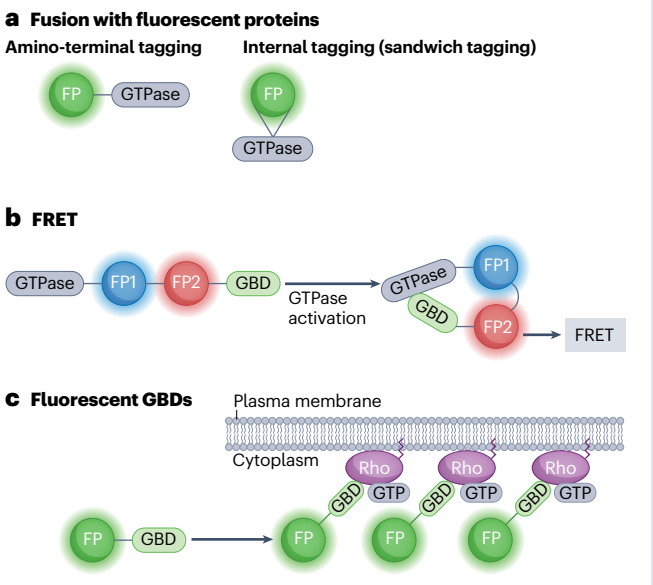
Box 1

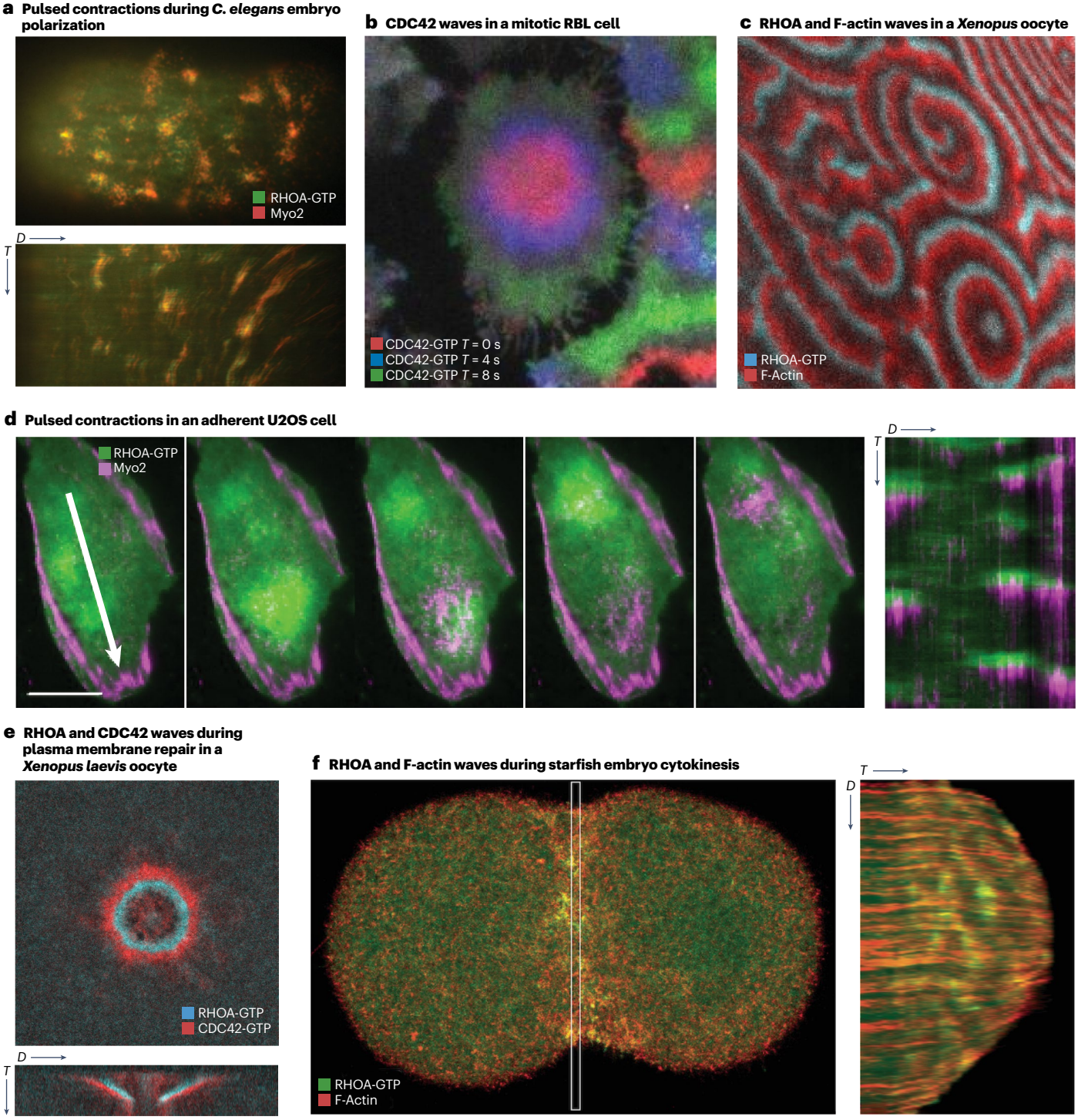
Live cell imaging approaches for Rho GTPases

Three general approaches have been used for live cell imaging of Rho GTPases. One is expression of the GTPases amino-terminally tagged with fluorescent proteins[14,33,35,274] (carboxy-terminal tagging prevents prenylation) (see the figure, part **a**). This technique is simple and permits fluorescence recovery after photobleaching or photoactivation to monitor GTPase turnover[33]. However, it does not distinguish between active and inactive GTPases, and in some cases the fusion proteins do not properly reflect normal Rho GTPase localization or function[34,275,276]. Additionally, expression of exogenous Rho GTPases can upset the stochiometric balance of the Rho GTPases with RhoGDI (Rho guanine nucleotide dissociation inhibitor)[277], resulting in aggregation and degradation of the GTPases[277]. Insertion of the fluorescent protein (FP) into an exposed surface loop of the GTPase (sandwich or internal tagging) improves localization and function[34,191,276], and the balance with RhoGDI can be maintained by either gene replacement[34,276] or coexpression with RhoGDI[191].

Förster resonance energy transfer (FRET)-based probes[27,278,279] are more complex, in that they typically contain the GTPase of interest, a GTPase binding domain (GBD) that binds specifically to the activated GTPase and two FPs, one a donor and one an acceptor (see the figure, part **b**). When the GTPase within the probe is activated by a GEF, the FPs are brought together, permitting the donor to excite the receptor and thus generating FRET fluorescence. Comparing the local ratio of donor fluorescence with acceptor fluorescence reveals areas where the probe is preferentially activated. These probes can be GTPase subtype-specific (distinguishing, for example, RHOA from RHOB) and do not interfere with the function of the endogenous Rho GTPase. However, they often have limited dynamic range, making it difficult to visualize the FRET signal against background, and they do not report on the endogenous GTPase activity per se but, rather, the local GEF availability[27]. Their dynamic range can be improved by modifications in probe design[280].

Fluorescent GBDs work by recruitment of these probes from the cytoplasm to areas of high GTPase activity at membranes[22,24–26,31] (see the figure, part **c**). They are simple to use and report on endogenous, active GTPases. However, at high levels they interfere with endogenous GTPase function, and they do not distinguish between GTPase subtypes (for example, RHOA versus RHOB). Moreover, their utility in different cell types varies widely[255]. Their performance can be improved by total internal reflection microscopy or confocal microscopy[18,19,24], by tight control of expression[18], by including a volume marker[31], and by increasing the number of GBDs or fluorophores per probe[200,255,256]. In two recent, very useful studies[256,257], mammalian cell-based assays were used to vet various GBDs. The interested reader is strongly encouraged to read these studies before embarking on Rho GTPase imaging in living cells.





myosin 2 (ref. 85) and phosphatidylinositol (3,4,5)-trisphosphate (PIP3)[86–88] – a downstream target of RAC and CDC42 (refs. 89,90). Further, depending on the context, a given effector or target can participate in diverse feedbacks. For example, myosin 2 can engage in both positive and negative feedback with RHOA[18,80] whereas F-actin engages in negative feedback with RHOA via binding to at least two different Rho GAPs[13,18]. It is also apparent that the potential for non-linear positive feedback is high, which is important because theoretical studies show that positive feedback is required to be non-linear for the formation of GTPase activity zones[91]. Both theory[36] and experiment[67] indicate that mechanisms which result in recruitment of a GEF to the plasma membrane or the cortical cytoskeleton, either via allosteric interaction with a GTPase or by other means, result in non-linear positive feedback.

Fig. 2 | Self-organizing Rho GTPase patterns. a, Pulsed contractions in a *Caenorhabditis elegans* embryo. Top: single frame from TIRF movie showing RHOA-GTP (green) and myosin 2 (red); anterior end of the embryo on the left, posterior on the right. Bottom: kymograph derived from the embryo in the top panel; total elapsed time 200 s. Pulses are evident in the kymograph as streaks which, on average, move towards the anterior end of the embryo over time. RHOA activity rises before myosin 2 in the contractions. **b**, Mitotic CDC42-GTP wave in an RBL cell from a TIRF movie. Image shows a composite of three successive timepoints with each time point coloured differently to reveal movement (red, $T = 0$ s; blue, $T = 4$ s; green, $T = 8$ s). The image captures a target pattern wave (that is, one that forms from a spot and spreads outward from the spot) of CDC42 activity. **c**, Experimentally induced RHOA-GTP and F-actin waves in frog oocytes. Single frame from a timelapse light sheet movie showing travelling waves of RHOA-GTP (cyan) chased by F-actin waves (red) in frog oocyte expressing the RHOA GEF Ect2 and the RHOA GAP RGA-3/4. Both target and spiral wave patterns are evident. **d**, Pulsed contractions in a U2OS cell. Left: series of images from timelapse TIRF movie of a nocodazole-treated U2OS cell showing RHOA-GTP (green) and myosin 2 (magenta); images taken 30 s apart. Right: kymograph corresponding to the white arrow on leftmost image; elapsed time 770 s. RHOA activity rises ahead of myosin 2 recruitment in the pulsed contractions. **e**, Travelling waves of Rho GTPase activity in a wounded frog oocyte. Top: single frame from a confocal movie of wounded frog oocyte showing a CDC42-GTP wave (red) and a RHOA-GTP wave (cyan); the CDC42-GTP wave encircles the RHOA-GTP wave. Bottom: kymograph from the cell depicted in the top panel; elapsed time 240 s. Single waves of RHOA-GTP and CDC42-GTP converge on the wound. **f**, Cytokinetic RHOA and F-actin waves in starfish blastomere. Left: single frame from a confocal movie of dividing starfish blastomere showing RHOA-GTP (green) and F-actin (orange). The cell is undergoing cytokinesis and the RHOA-GTP and F-actin waves are confined to the equatorial cortex. Right: kymograph taken from the area indicated by a box in the central region of the dividing cell on the left; elapsed time 960 s. Furrow waves of RHOA-GTP and chasing F-actin waves are evident as angled lines. *D*, distance; *T*, time. Source of images: panel **a** courtesy of John Michaux and Ed Munro, University of Chicago; panel **b** courtesy of Cheesan Tong and Min Wu, Yale University; panel **c** courtesy of Ani Michaud, Promega Corp.; panel **d** courtesy of Melanie Graessl, Perihan Nalbant, and Leif Dehmelt, University of Duisburg and Technical University of Dortmund; panel **e** courtesy of Lila Hoachlander-Hobby, University of Wisconsin–Madison; panel **f** provided by the authors.

A further point implicit in the different categories of feedback are differences in feedback onset: feedback with more and slower steps will take longer than feedback with fewer, faster steps. Thus, the potential for generating different lengths of delay (and thus variation in the period length of oscillatory patterns) is also high. Notably, the only examples of direct feedback are positive, with the active GTPase stimulating GEFs, meaning that when paired with any negative feedback, a delay between the feedbacks is possible. But this is not the only way to achieve a delay: in principle, as long as the negative feedback has more steps, or slower steps than the positive feedback, a delay is expected. Further, differences in the extent of the delay are expected to produce differences in the pattern. Thus, pairing a direct positive feedback mechanism with an effector-based negative feedback would be more likely to produce higher frequency patterns (that is, waves or pulses with a shorter period) than pairing the same direct feedback mechanism with a downstream target-based negative feedback. This is simply because the target-based mechanism has more steps than the effector-based mechanism. This consideration leads to a final, related point: perhaps one of the virtues of Rho GTPase crosstalk — the communication between different GTPases — is that it makes longer cycles possible as feedback going through two or more GTPases would be inevitably slower than feedback routed through a single GTPase.

Rho GTPases have what seems to be an excessively large repertoire of regulators: at least 80 different GEFs and 70 different GAPs[92] regulating the 3 most abundant GTPases — RHOA, RAC1 and CDC42. By way of comparison, the three Ras GTPases, HRAS, KRAS and NRAS, are regulated by three GEFs and six GAPs[93]. We suggest that the explanation for the abundance and diversity of Rho GTPase GEFs and GAPs is that they enable a diversity of feedbacks which, in turn, enable a potentially limitless repertoire of self-organizing Rho GTPase patterns.

Self-organized Rho GTPase patterns in cells

In this section, we present recent examples of self-organizing Rho GTPase patterns in more detail with the goal of revealing how such patterning works in different contexts. The examples were chosen from studies that used high temporal resolution (<10 s sampling intervals) imaging of Rho GTPase activity, which is needed to reveal many of the patterns considered here, and based on their satisfaction of two or more of the criteria for self-organizing patterns alluded to above.

Polarized growth in yeast

Polarized growth in fungi provides a paradigmatic example of a morphogenetic process pre-patterned by a circular cluster ('cap') of Rho GTPase activity[94–97]. Budding yeast has two mutually exclusive morphogenetic programmes that strictly require polarized growth: budding and shmooing, that is, formation of the mating protrusion. Fission yeast can also exhibit bipolar growth at the two opposite cell tips that can grow simultaneously. Early studies demonstrated that CDC42 and its GEF CDC24 are both strictly required for and localize to the zones of polarized growth[98–101]. The field was revolutionized by the introduction of CDC42 activity reporters[22,26,102–104] that demonstrated that CDC42 is highly active at a disc-shaped cluster with a diameter of 1–3 µm that marks the nascent protrusion sites. CDC42-GTP via its numerous effectors directly drives all morphogenetic processes, including formation of polarized actin cables, vesicle secretion and, in the case of budding, establishment of the septin ring[105,106]. From the initiation of bud protrusion, the CDC42-GTP cluster translocates into the growing daughter cell and disassembles at mitosis when the bud growth ceases[96,105]. Observations of the CDC42 and RAC1 clusters at the tips of cellular protrusions in other fungi suggest that they ubiquitously drive morphogenesis of polarized growth zones across the entire fungal kingdom[94,104,107–109]. Similarly, in plants, the ROP ('Rho of plants') GTPases are active and enriched at the tips of growing pollen tubes and root hairs[110–113].

The mechanism of CDC42-GTP cluster formation attracted much attention over several decades[96,97,114,115]. Early work employing cytoskeletal poisons showed that neither microtubules nor actin are necessary for the cluster emergence[96,116]. Instead, the CDC42-GTP cluster location on the membrane is influenced by a system of landmark proteins converging on the Ras-like small GTPase RSR1 (also known as BUD1), which directly recruits CDC24 as its effector[58]. In the context of shmoo formation, the CDC42 cluster position is biased by the G protein signalling activated by the mating pheromone receptor[117]. However, deletion of RSR1 results in random bud positioning, but not failure of bud formation, whereas mutation of pheromone sensing abrogates chemotropic growth to a partner but not shmoo formation[117]. These

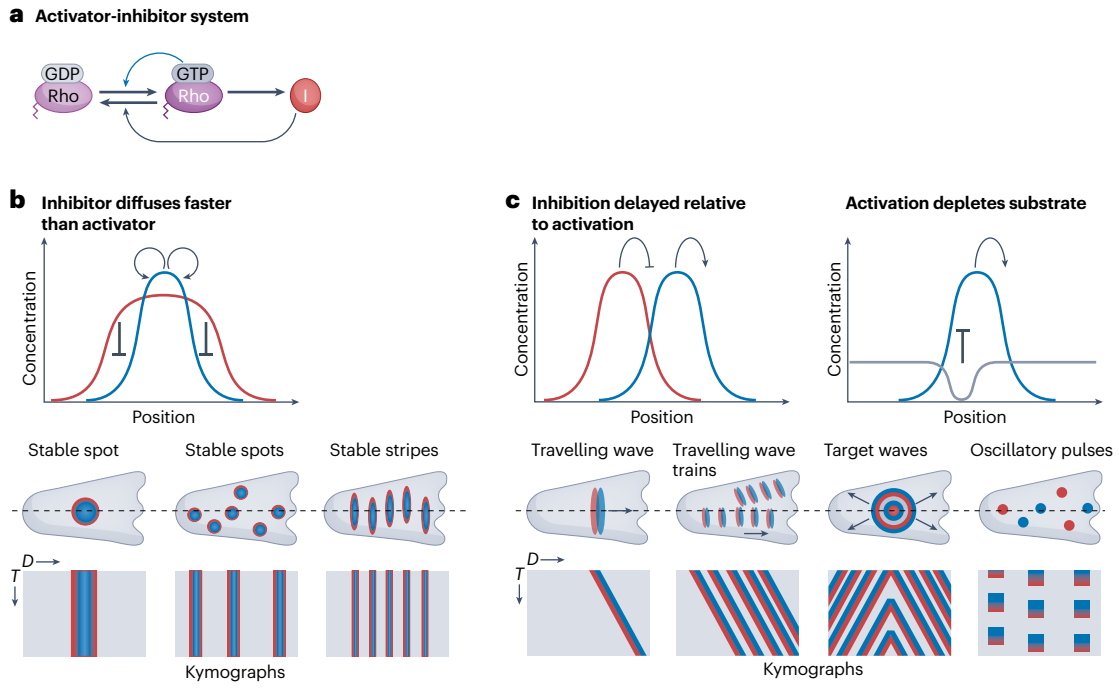
Box 2

Self-organizing Rho patterns

Self-organization is a process in which a disordered system spontaneously acquires some form of order, such as a pattern, due to interactions between system parts. Self-organized patterns commonly arise as a result of combined positive and negative feedback. One well-studied example of how positive and negative feedback gives rise to self-organized patterns is the 'activator-inhibitor' system (see the figure, part **a**). In activator-inhibitor systems, an activator (in this case, the Rho GTPase in its active, GTP-bound state) stimulates its own activation via positive feedback (for example, by stimulation of a GEF) while also stimulating the production of an inhibitor (I) which antagonizes the activity of the GTPase via negative feedback (for example, by stimulation of a GAP). A variation of this theme is the 'activator-depleted substrate' system in which negative feedback arises not from an inhibitor but from consumption of a limiting substrate needed for activation of the activator. Because the active GTPase is produced from the inactive GTPase, active and inactive forms of any GTPase always make an activator-depleted substrate pair.

To avoid having the positive and negative feedbacks simply cancel each other out, they operate on different length or time scales. For example, if the inhibitor diffuses faster than the activator, stationary patterns such as stripes or spots can be produced as the activator (and thus positive feedback) becomes confined to islands surrounded by seas of fast-moving inhibitor (see the figure, part **b**). A common way to analyse such patterns over time is by use of kymographs (also known as 'space–time plots'). Kymographs are generated by making a very narrow slice (represented by a dotted line) on a movie file and then collecting one slice for each time point of the movie. The slices are then positioned next to each other in order, similar to a montage but without the border. One arrow indicates which axis is time (*T*); the other indicates which axis is distance (*D*) (or space). Stable patterns will appear as vertical stripes in the kymograph; moving patterns will be angled in the kymograph.

If the production of the inhibitor (and negative feedback) is delayed relative to the positive feedback, various dynamic patterns can be produced such as waves or oscillatory pulses (see the figure, part **c**). Here, the wave of activator moves away from a wave of inhibitor into regions free of inhibitor (that is, the inhibitor 'chases' the activator). For activator–substrate depletion systems, waves can form as the activator moves away from areas of substrate depletion into areas of high substrate concentration.



results argued that the upstream signals serve only as spatial cues but are otherwise not required for the CDC42 cluster formation. In the following years the CDC42-GTP cluster emerged as a manifestation of self-organized cellular polarization and, thus, symmetry breaking[91,118]. A pivotal point was the discovery of the positive feedback loop mediated by the scaffold effector Bem1 that simultaneously binds

CDC42-GTP and its activator, the GEF CDC24 (refs. 71,119) (Fig. 4a). The key role of Bem1 in CDC42 polarization had been extensively confirmed by genetic perturbations[11,120,121] and, more recently, by direct optogenetic recruitment[122]. This feedback is also conserved in fission yeast, where the CDC42 effector SCD2 recruits the GEF SCD1 (ref. 123). Several other non-mutually exclusive feedback loops have been suggested in the literature[34,124] (for detailed review see ref. 91).

Modelling has provided essential insight into which mechanisms could, in principle, account for CDC42-GTP cluster formation. First, a complete model of CDC42 cluster formation needs to describe spontaneous symmetry breaking[91]. Second, as polarizing yeast cells need only a single bud or a shmoo, such a model needs also to explain this uniqueness. Early models provided several physically plausible mechanisms of symmetry breaking but they required actin cable-mediated delivery of CDC42 and did not address either nucleotide cycling of CDC42 or the uniqueness of the CDC42 cluster[22,125,126]. The first fully mechanistic model of spontaneous CDC42-GTP cluster formation[36] was derived from the reaction network consisting of nucleotide

Table 1 | Feedback to Rho GTPase GEFs and GAPs

GEF/GAP	GTPase	Feedback from	Feedback type	Refs.
GEF				
p190-RhoGEF; p115-RhoGEF; PRG; LARG; GEF-H1; LBC	RHOA	RHOA-GTP	Direct, positive	66,67,263,264
ECT2	RHOA	RHOA-GTP	Direct, positive	68
ABR	RHOA	RHOA-GTP	Direct, positive	192,199
DOCK180	CDC42 or RAC1	CDC42-GTP	Direct, positive	69
p190-RhoGEF	RHOA	RAC1-GTP	Direct, positive, crosstalk	263
PIX	CDC42 or RAC1	CDC42-GTP	Direct, positive, crosstalk	265
ARHGEF7	CDC42 or RAC1	Coronin 1a	Effector, positive	75
Intersectin	CDC42 or RAC1	N-WASP	Effector, positive	72,74
LARG	RHOA	DIA1	Effector, positive	79
TRIO	RHOA	Filamin	Effector, positive	266
PREX2	CDC42 or RAC1	PAK1	Effector, negative	81
DOCK180	CDC42 or RAC1	PIP3	Target, positive	86
DOCK2	CDC42 or RAC1	PIP3	Target, positive	87
DOCK4	CDC42 or RAC1	PIP3	Target, positive	88
FGD1	CDC42 or RAC1	Cortactin	Target, positive	267
PIX	CDC42 or RAC1	Paxillin	Target, positive	268
TIAM1	CDC42 or RAC1	ARP2/3	Target, positive	269
TRIO8	CDC42 or RAC1	SESTD1	Effector, negative	270
PREX1	CDC42 or RAC1	PAK1	Effector, negative	271
GEF-H1	RHOA	F-actin, myosin 9	Target, negative	18
GEF-H1	RHOA	myosin 2	Target, negative	18
BETA-PIX, VAV, TIAM1, DBS	CDC42 or RAC1	myosin 2	Target, negative crosstalk	85
GAP				
ARHGAP18	RHOA	PKN	Effector, positive	83
p190-RhoGAP	RHOA	RND3	Effector, positive	80
ARHGAP15	CDC42 or RAC1	PAK1, PAK2	Effector, negative	82
ARHGAP9, ARHGAP12, ARHGAP15, ARHGAP27, ARHGAP32, ARHGAP33	CDC42 or RAC1	G-actin	Target, negative	84
RGA-3/4	RHOA	F-actin	Target, negative	13,214
OPHN1	RHOA or RAC1	F-actin	Target, negative	272
ARHGAP12, ARHGAP25	CDC42 or RAC1	PIP3	Target, negative	273
ARHGAP15	CDC42 or RAC1	PIP3	Target, negative	82
ABR	CDC42 and or RAC1	RHO-GTP	Direct, negative crosstalk	192,199

PAK, p21-activated kinase; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; PKN, protein kinase N.

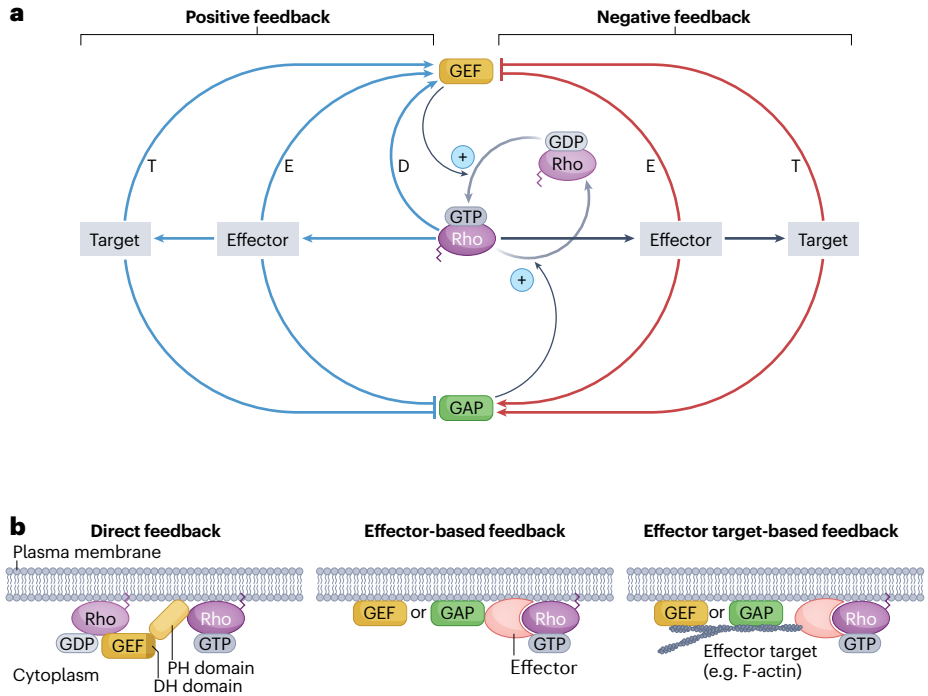


Fig. 3 | Feedback to Rho GTPase GEFs and GAPs. a, Overview of known mechanisms of positive and negative feedback of Rho GTPases acting through their GEFs and GAPs. Feedback is considered positive if the end result is an increase in the activity of the Rho GTPase or negative if the end result is a decrease in the activity of the GTPase. Thus, stimulation of a GEF by its target GTPase is considered positive feedback whereas stimulation of a GAP by its target GTPase is considered negative feedback. Positive interactions indicated by arrows with pointed ends; negative interactions indicated by arrows with flat ends. See Table 1 for specific examples. **b**, Example mechanisms of the different classes of feedback. Direct feedback: an active Rho GTPase binds allosterically to its GEF via the PH domain in the GEF, thereby targeting it to the plasma membrane and exposing the active site (DH domain) which can then activate an inactive GTPase. Effector-based feedback: an active GTPase binds an effector, which binds a GEF or GAP, targeting it to the plasma membrane. Effector target-based feedback: an active GTPase stimulates an effector which promotes formation of (in this case) F-actin which, in turn, targets the GEF or GAP to the plasma membrane. Direct feedback has only been described for positive feedback; effector-based feedback and effector target-based feedback can be either positive or negative. In the figure, Rho indicates the Rho GTPase RHOA, RAC1 or CDC42. D, direct feedback (active GTPase binds GEF); E, effector-based feedback (effector binds or modifies GEF or GAP); T, effector target-based feedback (downstream target of effector binds to or modifies GEF or GAP).

cycling and membrane-cytoplasmic shuttling of CDC42. In agreement with experiment, this model did not require F-actin as part of positive feedback. Instead, it introduced the notion of spatiotemporal GTP hydrolysis-driven CDC42 flux that continuously renews the membrane-bound CDC42-GTP cluster. The flux concept also explained the uniqueness of the bud as two or more GTPase clusters cannot grow simultaneously in the same cell if they compete for the limited cellular pool of the GTPase and its GEF. The competition between several CDC42 clusters was confirmed experimentally[11,121,127,128] and actively studied theoretically[129–133]. Multiple variations and extensions of the original models based on CDC42 nucleotide cycling[36,134] were proposed in the following years[91,135–144].

Addition of negative feedback turns stationary GTPase clusters into moving or oscillating clusters[22,105,145–148]. CDC42 GAPs bound to septin polymers recruited by CDC42-GTP via its effectors GIC1/2 were shown to form a negative feedback loop in the context of budding[105]. (Fig. 4a). Vesicle insertion into the plasma membrane has also been extensively studied as a potential negative feedback diluting CDC42-GTP on the membrane[127,149–151] and as a mechanism driving patterning of the septin ring where it plays a positive feedback role by diluting septin polymers[105]. Similarly, in fission yeast, vesicle insertion into the plasma membrane plays a positive role in CDC42 cluster formation by pushing the GAP Rga4 away from the centre of the CDC42 cluster[152]. Furthermore, in the presence of negative feedback, the competition of two CDC42 clusters can change from antagonistic (winner takes all) to oscillatory (out-of-phase coexistence) — the mechanism which was proposed to explain the discovery of the tip-to-tip CDC42-GTP oscillations in fission yeast[12,153,154].

Pulsed contractions

Pulsed contractions driven by focal activation and accumulation of myosin 2 and F-actin are a common feature of developing animal embryos, where they drive cell and tissue shape changes[155–159] and contribute to polarization via advection[160]. The contractions are generally ascribed to transient, localized bursts of RHOA activity which result in F-actin polymerization via formins[161] and myosin 2 filament assembly via ROCK[162].

Pulsed contractions are particularly striking in early *Caenorhabditis elegans* embryos, where they engage in a complex interplay with Par proteins to help specify the developmental fate of the blastomeres[160,163]. In a recent study[13], the mechanism of pulsed contractions was analysed in the *C. elegans* embryos via a combination

of TIRF microscopy and single molecule tracking. These approaches made it possible to distinguish between the contributions of actin and myosin filament assembly, disassembly and contraction to the formation of individual pulses (Fig. 2a). Pulses have a periodicity of ~30 s and are initiated by focal RHOA activation. RHOA activation was dependent on positive feedback (Fig. 4b), based on both pulse kinetics and RHOA depletion which showed that pulsing behaviour requires that the RHOA concentration exceeds a defined threshold. RHOA-dependent myosin 2 and F-actin assemblies accumulate in pulses, ~5–6 s after RHOA activation. RHOA activity begins to fall coincident with the recruitment of F-actin and myosin 2 and before the onset of contraction, suggesting that the loss of RHOA activity is contraction-independent. This point was confirmed by myosin 2 depletion, which failed to arrest RHOA pulsing although it prevented contraction. Rather, loss of RHOA activity was driven by a delayed negative feedback loop based on F-actin and RGA-3/4, two functionally redundant RHOA GAPs[164]: RGA-3 was recruited to pulses coincident with loss of RHOA activity and depletion of RGA-3/4 resulted in an arrest of pulsing and uniformly high cortical levels of RHOA activity[13]. Further, RGA-3 co-localized with F-actin within pulses, and pharmacological disruption of F-actin resulted in loss of cortical RGA-3 as well as cessation of pulsing. An activator-inhibitor model based on RHOA positive feedback and delayed negative feedback through F-actin and RGA-3/4 captured all of the features of pulse dynamics, leading the authors to conclude that pulsed contractions are governed by a self-organizing cortical network (Fig. 4b).

Pulsed contractions based on focal myosin 2 activation are not restricted to embryos but have also been observed in various cultured cell types[165], where they have been linked to processes such as focal adhesion, stress fibre formation[166–168] and endocytosis[168]. In a recent study of U2OS cells, myosin 2 pulses which developed spontaneously were associated with and dependent on a self-organizing network that controls RHOA activity[18] (Fig. 2d): myosin 2 and Rho pulses were dependent on the Rho GEF GEF-H1, and direct positive feedback from active Rho to GEF-H1 was demonstrated via expression of GEF-H1 with a non-functional RHOA-GTP-binding site (Fig. 4b). Delayed negative feedback occurred via two motor proteins: the unconventional myosin 9, which has a C-terminal Rho GAP domain, and by myosin 2 itself. The period of the RHOA-GTP waves was ~80 s, and dual label imaging and cross-correlational analysis revealed that the formin FHOD1 was recruited ~6 s after RHOA-GTP, F-actin (the presumptive recruiter of myosin 9) recruitment occurred ~11 s after RHOA-GTP and myosin 2 recruitment occurred ~40 s after RHOA-GTP[18]. Strikingly, the amplitude of the oscillatory pulses was dependent on the stiffness of the extracellular matrix, demonstrating that the signalling network was capable of responding to external signals. In a follow-up study[169], chemo-optogenetic targeting[170] and modelling were employed to further probe the relationship between GEF-H1 and RHOA-GTP. Experimental recruitment of RHOA-GTP to the plasma membrane was sufficient to recruit GEF-H1, directly confirming that this GEF and RHOA-GTP engage in positive feedback. Graded release of GEF-H1 from mitochondrial sequestration via optogenetics demonstrated that whereas a minimum level of GEF-H1 was necessary for periodic pulses of RHOA activity, an excess of GEF-H1 reduced the wave amplitude. Modelling revealed that this effect is dependent on myosin 2-based noise.

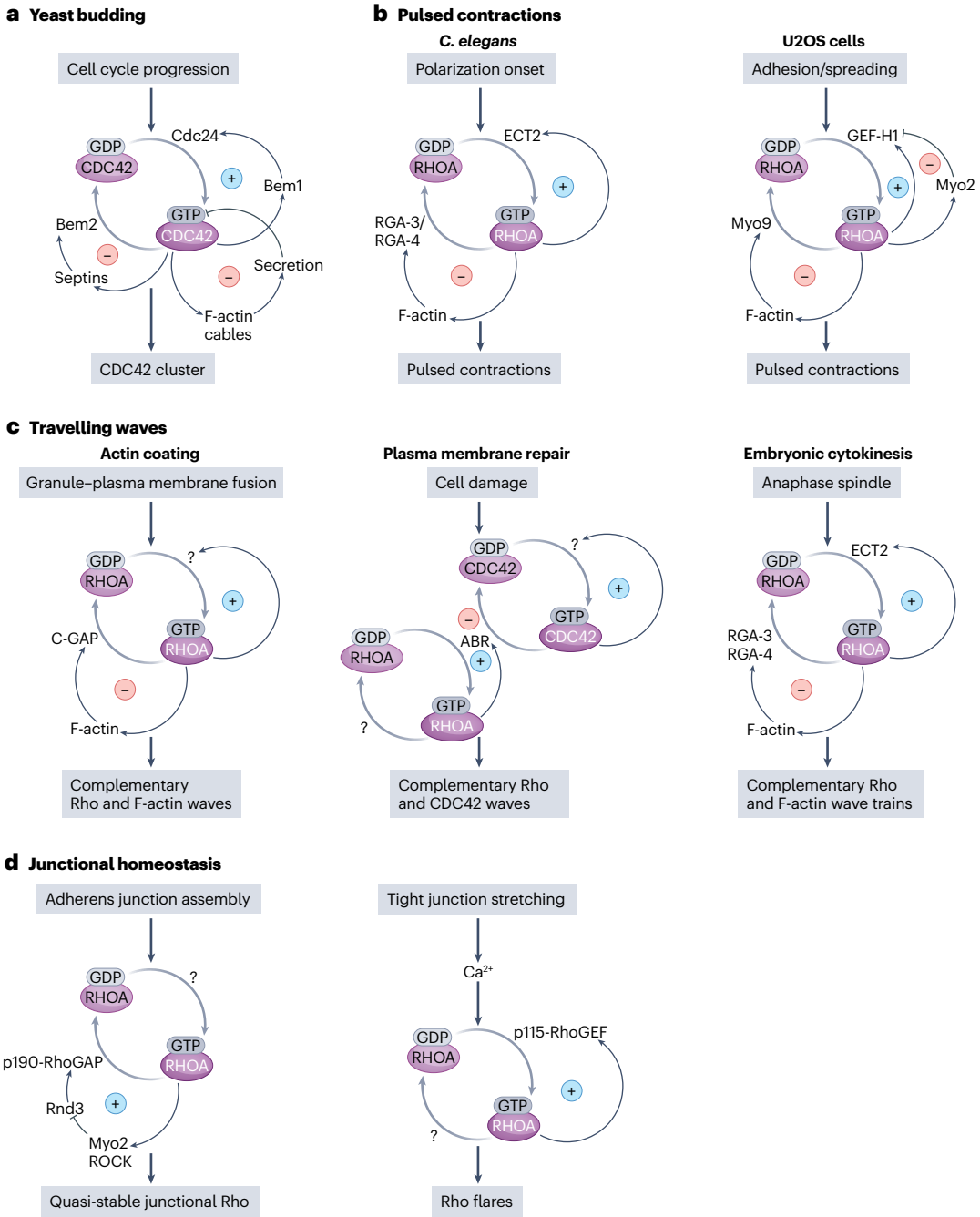
Travelling waves

Travelling waves of cortical F-actin are associated with various dynamic cellular phenomena[41,171]. Such waves propagate by new actin assembly at their leading edge (that is, the front of the wave) and disassembly of actin at their trailing edge (that is, the back of the wave). Although in many cases their upstream control mechanisms are unclear, travelling actin waves are often generated by complementary waves of Rho GTPase activation and inactivation[15,17].

Travelling actin waves can assume various forms, one of which is the actin 'coat'. Actin coating refers to the process wherein secretory granules become enveloped by F-actin after fusing with the plasma membrane[172]. Coating is associated with exocytosis of secretory granules that contain bulky, insoluble content[172] and has been observed in exocytosis of cortical granules in frog eggs[25], pancreatic[173] and salivary[174] acinar cells, pancreatic β-cells[175], alveoli[176] and endothelial cells[177]. Coating is triggered by rapid, fusion-dependent[178] activation of Rho GTPases on the membrane of the exocytic granules[172,174,179]. Following fusion, the active GTPases recruit various effectors[172,174,180–182] that direct accumulation of F-actin, myosin 1 (ref. 183) and myosin 2 (refs. 177,179,180). Once formed, the coat contracts and compresses the granule, which powers expulsion of the granule contents and retrieval of the granule membrane to maintain plasma membrane homeostasis[174,175,184,185].

How is coat contraction coupled to RHOA-GTP dynamics? This question was addressed using the *Drosophila* salivary gland, where exocytosis of so-called glue proteins is accompanied by and dependent on actin coating[174]. As expected, RHOA activation is essential for coat formation and contraction. Accordingly, Rho suppression impairs recruitment of F-actin, ROCK and myosin 2 (ref. 174). More interestingly, however, inhibition of myosin 2 or ROCK[76,174] does not simply stall coat contraction. Instead, it results in oscillatory cycles of RHOA activation and inactivation and consequent F-actin accumulation and loss from the fused vesicles, with the cycles of the same length as normally required to attain full coat contraction[76]. This observation suggested delayed negative feedback, prompting a screen for coat-localized Rho GAPs. The screen identified C-GAP, which was recruited to exocytic granules ~5 s after F-actin. Further, C-GAP recruitment was F-actin-dependent, as was Rho inactivation (Fig. 4c). Strikingly, suppression of C-GAP expression resulted in the arrest of coat contraction, although active Rho and the actin coat persisted, demonstrating that RHOA inactivation as well as RHOA activation are required for coat contraction. Based on these results, a model was proposed in which granule compression results from what is essentially a travelling wave of RHOA-GTP activity that is chased by a wave of negative feedback in the form of F-actin-dependent recruitment of C-GAP[76].

Single travelling GTPase waves are also evident during plasma membrane repair. In this process, damage to the plasma membrane triggers local activation of RHOA, CDC42, RAC1 or all three in model systems including yeast[186], worms[187], flies[14], frogs[24] and cultured human muscle cells[188]. Rho GTPase signalling during plasma membrane repair has been most intensely studied in frog oocytes[189] and syncytial fly embryos[190]. In these large cells, live cell imaging using GTPase binding domain (GBD) activity reporters of Rho GTPase[24] (Box 1) or directly labelled Rho GTPases[14,191] (Box 1) has shown that the GTPases are activated within ~20–60 s of plasma membrane damage and then organize into concentric zones of activity. The zone of RHOA activity borders the wound edge, and CDC42 and RAC1 activity forms a broader zone that circumscribes the RHOA zone (Fig. 2e). Not surprisingly, activation of the GTPases is dependent on wound-recruited GEFs[192,193], although RHOA may also be activated via wound-induced production of reactive oxygen species[188]. The complementary GTPase zones direct the



formation of a contractile actomyosin-based ring that closes over the wound site in concert with the GTPase zones. This results in repair of the cortical cytoskeleton and expulsion of material damaged by the wound[24,194–196].

Studies in frog oocytes show that movement (closure) of the Rho GTPase zones around wounds arises from the fact that the CDC42 and RHOA zones are actually circular travelling waves that move at ~80 nm s^{-1} with preferential GTPase activation at their leading edges[17]. The leading-edge activation of the GTPase drives the zones forward, even under conditions where actomyosin-based contraction is completely suppressed. The trailing edge of the RHOA zone is defined by an approximately threefold higher rate of RHOA inactivation where it abuts the CDC42 zone[17]. At the same time, the CDC42 zone is apparently limited by direct extraction of active CDC42 by RhoGDI[191]. Modelling and imaging results[197,198] show that the zones self-organize near the wound edge due to spatially restricted bistability, such that within the area around the wound, RHOA or CDC42 activity is stable in one of only two states — high activity within the zones or low activity outside the zones. The bistability results from positive feedback: positive feedback within the RHOA zone arises from RHOA-GTP-dependent recruitment of the dual GEF–GAP ABR[192] (Fig. 4c). ABR has an amino-terminal GEF domain that, in vitro, targets RHOA, CDC42 and RAC1, and

Fig. 4 | Proposed feedbacks for examples of self-organization. For each example, the upstream signal is indicated at the top and the pattern produced is indicated at the bottom. **a**, Formation of the polarizing CDC42 cluster in budding yeast relies on at least one positive feedback loop (via CDC42-GTP to Bem1 to the GEF (guanine nucleotide exchange factor) Cdc24) and two negative feedback loops (from CDC42-GTP to septins and the GAP (GTPase activating protein) Bem2 and from CDC42-GTP to F-actin cables and secretory vesicles). **b**, Pulsed contractions in *Caenorhabditis elegans* may arise from direct positive feedback from RHOA-GTP to the GEF Ect2 and from negative feedback from RHOA-GTP to F-actin to the GAP RGA-3/4. Pulsed contractions in U2OS cells arise from direct positive feedback from RHOA-GTP to the GEF GEF-H1 and two negative feedback loops: from RHOA-GTP to myosin 2 which inhibits GEF-H1, and from RHOA-GTP to F-actin to the GAP myosin 9. **c**, Travelling waves during actin coating of secretory vesicles arise from negative feedback from RHOA-GTP to F-actin to the GAP C-GAP; the basis of positive feedback has yet to be identified. Travelling waves during plasma membrane repair arise from positive feedback from RHOA-GTP to the dual GEF–GAP ABR; ABR is also responsible for negative crosstalk from RHOA-GTP to CDC42-GTP and participates in positive feedback for RHOA. CDC42-GTP is responsible for negative crosstalk to RHOA-GTP by an as yet unidentified RHOA GAP (not shown). Travelling waves during embryonic cytokinesis arise from direct positive feedback from RHOA-GTP to the GEF Ect2 and from negative feedback from RHOA-GTP to F-actin that engages in negative feedback with the GAP RGA-3/4. **d**, Homeostasis in adherens junctions relies on positive feedback from RHOA-GTP to Rho-associated protein kinase (ROCK) and myosin 2, which negatively regulate non-canonical Rho GTPase RND3 which positively regulates the GAP p190-RhoGAP. The basis of the negative feedback has not been identified. Homeostasis in tight junctions is restored following junction stretching via positive direct positive feedback from RHOA-GTP to p115-RhoGEF. The basis of negative feedback is unknown but may be dependent on ROCK. Pointed arrows indicate positive regulatory interactions; flat-headed arrows indicate negative regulatory interactions. ? indicates players assumed but not yet identified. Circled plus signs indicate positive feedback loops; circled minus signs indicate negative feedback loops.

a C-terminal GAP domain that targets RAC1 and CDC42 (ref. 199). The basis for the positive feedback within the CDC42 zone is unclear. The spatial restriction of the feedback to the wound-proximal region is thought to be due to the formation of a wound-induced 'playing field' – a region within which bistability is enabled; this is likely based on elevated intracellular free calcium and protein kinase Cβ (PKCβ)[24,197,200,201]. Segregation of RHOA and CDC42 zones results from reciprocal negative crosstalk between RHOA and CDC42: GAP activity of ABR suppresses CDC42 activity in the RHOA zone. Accordingly, expression of GAP-dead ABR results in CDC42 invading the RHOA zone whereas ABR overexpression expands the RHOA zone at the expense of the CDC42 zone[192]. Conversely, the CDC42 zone suppresses RHOA activity, apparently by recruiting a GAP. This was inferred by the higher inactivation rate of RHOA in the CDC42 zone[17], and by the demonstration that CDC42 suppression spreads and intensifies the RHOA activity zone[202]. Thus, the RHOA zone (wave) is chased by a wave of CDC42-dependent negative feedback.

Travelling Rho GTPase waves are also evident during cell division, but as wave trains (multiple travelling waves in the same cell) rather than single waves. One example is provided by cytokinesis which, in animal cells, is initiated by the activation of RHOA in a narrow zone at the equatorial cortex[23,203,204] and suppression of RAC activity in the same region[205]. RHOA activation results from spindle-mediated concentration of the Rho GEF ECT2 at the equatorial plasma membrane[5,28,206]. ECT2 localization is controlled, at least in part, by its interaction with the centralspindlin complex, which comprises the microtubule motor MKLP1 and MgcRacGAP (also known as CYK4)[203,207]. The role played by MgcRacGAP is complex and controversial[208] but, at a minimum, it contributes to cytokinesis by localizing ECT2 (refs. 203,207) and suppressing activation of Rac in the equatorial cortex[205,209,210] while also somehow promoting ECT2 activation[211,212], and maintaining RHOA activity within a focused, narrow zone[57].

High spatiotemporal resolution imaging in starfish embryos revealed that the RHOA zone is constituted from travelling RHOA activity waves that first appear in anaphase and are rapidly amplified and concentrated at the equatorial cortex by the mitotic spindle[15] (Fig. 2f). The RHOA-GTP waves have a period of ~60–80 s and give rise to and are chased by waves of F-actin that likewise concentrate at the equatorial cortex. The waves persist as the formation of the cytokinetic furrow is initiated, and then eventually transition into a state in which RHOA activity is more uniformly high as the furrow deepens. Modelling and experiments indicated that the waves reflect a self-organizing, activation-inhibition system wherein ECT2 and RHOA-GTP are responsible for positive feedback[15]. This conclusion is supported by the observation that ECT2 is allosterically activated by binding to RHOA-GTP via its PH domain[68]. In what was at the time a major surprise, the negative feedback was found to be F-actin-dependent[15]. Similar results were obtained in *Xenopus* embryos, with the added feature of high-amplitude waves of F-actin that persist throughout the cell cycle but which are excluded from the equatorial cortex as the cytokinetic RHOA-GTP waves develop[15,213]. In both starfish and frog, the waves are regulated not only by the mitotic spindle but also by CDK1 activity independently of the spindle[15,213].

F-actin-dependent negative feedback in cytokinetic RHOA waves was recently shown to rely on RGA-3/4 (ref. 214) (Fig. 4c). RGA-3/4 was previously implicated in negative RHOA regulation during cytokinesis in human cells and *C. elegans*[215,216]. In both starfish and frog, RGA-3/4 was shown to form waves that chase RHOA-GTP waves in an F-actin-dependent manner; similar to the RHOA-GTP waves, the RGA-3/4 waves are focused and amplified at the equatorial cortex during anaphase. Moreover, ectopic expression of ECT2 and RGA-3/4 in immature frog oocytes, which do not naturally display excitable cortical waves[15,214], was sufficient to induce high-amplitude, travelling waves of RHOA-GTP that are chased by waves of F-actin and RGA-3/4. A model based on activation-inhibition captured not only the basic features of the cytokinetic waves but also the more complex wave dynamics seen in immature oocytes. Reconstitution of cortical excitability in vitro using frog egg extract on supported lipid bilayers produced stationary RHOA activity pulses and solitary propagating waves, further supporting the self-organized nature of cortical excitability[21].

Wave trains are not restricted to embryos but have also been observed in adherent mammalian cells. Studies of Rho GTPase dynamics in a cultured mammalian mast cell model (RBL cells) revealed that this cell type generates self-organized oscillatory pulses (standing waves) and travelling waves of CDC42 activity in response to antigen exposure in interphase[19]. In metaphase, travelling CDC42 waves are also observed (Fig. 2b). These are accompanied by low-amplitude travelling RHOA activity waves or pulses[20]. The mitotic CDC42 waves are regulated by cell adhesion and have been linked to spindle position control in these cells[20] whereas the mitotic Rho waves

may be related to those involved in cytokinesis in other cell types (see above).

Recently, mitotic Rho dynamics were analysed in detail in n RBL cells treated with nocodazole, a manipulation that both increases Rho wave amplitude due to activation of GEF-H1 (ref. 217) and arrests the cells in M phase, permitting a detailed analysis of Rho wave control mechanisms[218]. Mitotic Rho waves varied dramatically in terms of period and amplitude in different cells in the population, with long-period (~3 min), high-amplitude waves in some cells and short-period (~30 s), low-amplitude waves in others. In a limited number of cells, mixed-mode waves were observed, in which the fast and slow wave cycles were superimposed. By manipulating the levels of specific phosphoinositides, it was found that different wave types could be interconverted, allowing the contribution of different feedback relationships to wave dynamics to be deduced. It was concluded that the fast waves are regulated by PI3K and PIP3 via an activator-inhibitor mechanism (Box 2), whereas the slow waves are regulated by PI(4)P via an activator-depleted substrate mechanism (Box 2) in which PI(4,5)P2 is the rate-limiting consumed substrate. Exactly how these phospholipids regulate Rho remains to be determined, but GEF-H1 and ECT2, the two Rho GEFs likely to be involved, have been previously linked to PI3K (ref. 219) and PI(4,5)P2 (ref. 220), respectively. More importantly, the results show that different classes of feedback (activator-inhibitor and activator-depleted substrate) can produce quantitatively different GTPase patterns in the same cell and, based on the presence of mixed-mode oscillations can coexist, resulting in different oscillation patterns occurring at the same time.

Cell–cell junction self-organization and epithelial homeostasis

Vertebrate epithelial cells are linked by tight junctions, which provide barrier function, and adherens junctions, which mechanically integrate the cells. These junctions are linked to a contractile apical actomyosin network that supports epithelial tissue integrity and drives cell shape changes. In order to maintain epithelial homeostasis, cell–cell junctions must dynamically respond to changes in tissue tension, cell density and other insults such as tissue damage or disease that threaten normal barrier function.

Live cell imaging using Rho GBD activity reporters[80,221] (Box 1) or a Förster resonance energy transfer (FRET) sensor[222] (Box 1) revealed that a zone of RHOA activity encircles the apical surface of each epithelial cell, regulating the contractility of the apical actomyosin bundle. Both adherens junctions and tight junctions are sites of complex and dynamic Rho GTPase signalling[223–225]. The normal balance of junctional Rho GTPase activity is maintained by a long list of GEFs, GAPs and scaffolding proteins that act in different epithelial contexts[225,226]. In the adherens junctions of both mammalian and frog epithelial cells, the key regulators include several proteins originally identified as conserved cytokinesis regulators: the RHOA GEF ECT2 (ref. 227), the centralspindlin component MgcRacGAP[228] and the scaffolding protein anillin[221,229]. Despite the outwardly static appearance of junctional complexes, recent work[33] indicates that the turnover of RHOA within the cell–cell junction zones is quite rapid ($t_{1/2}$ of ~1 s) and revealed that one factor that contributes to junctional RHOA dynamics is anillin, the knockdown of which reduced the half-life of junctional RHOA even further[33]. Anillin locally concentrates PI(4,5)P2 in the plasma membrane, which increases the membrane retention of active RHOA, allowing for extended downstream signalling from active RHOA to its effectors and their targets[33]. Another mechanism that regulates junctional RHOA dynamics is a complex but fascinating feedback mechanism in which junctional myosin 2 maintains a balance of Rho activation and inactivation via ROCK-dependent phosphorylation of the non-canonical Rho GTPase RND3, which, in turn, modulates the recruitment of p190B-RhoGAP to the junctions[80] (Fig. 4d). A model based on diffusion counteracted by myosin 2-powered advection explains how the tight localization of RHOA-GTP to the junction is maintained in the face of rapid turnover[230].

In addition to the zone of Rho activity that supports cell–cell junction homeostasis, the tight junctions of the frog embryonic epithelium also exhibit local, transient bursts (or 'flares') of RHOA activation[221,231]. These RHOA flares occur in response to the mechanical strain imposed by developmentally controlled cell shape changes, which cause local tight junction leaks. The RHOA flares direct the repair of the junctions by promoting local accumulation of actomyosin, and are initiated by stretch-induced opening of mechanosensitive calcium channels[232]. The flares are short-lived (~3 min) and their kinetics strongly suggest fast positive feedback and delayed negative feedback. Consistent with this hypothesis, the GEF responsible for Rho flares, p115-RhoGEF (also known as ARHGEF1)[233] (Fig. 4d), was previously shown to engage in direct positive feedback with RHOA-GTP via its PH domain[67]. Additionally, ROCK inhibition resulted in repeating cycles of Rho activation and inactivation[231], suggesting that ROCK-dependent delayed negative feedback is involved.

Context dependency of Rho GTPase pattern formation

Two important points emerge from consideration of these examples. First, there is no 'typical' way in which cells implement feedback during Rho GTPase signalling. For example, during yeast budding, the positive feedback is effector-dependent and involves GEF stimulation, during cytokinesis it is direct and involves GEF stimulation, and during junctional assembly it is effector target-dependent and acts through inhibition of a GAP. Further, although several of the examples of negative feedback are F-actin-dependent, this likely reflects the fact that rapid manipulation of F-actin is relatively straightforward, making it comparatively easy to test the role of actin in feedback. Certainly, there are many other mechanisms for negative feedback (Table 1). Second, simply looking at signalling network diagrams for Rho GTPase regulation such as those presented in Fig. 4 does not permit one to predict what kind of pattern will form. The diagrams for pulsed contractions in *C. elegans* and embryonic cytokinesis are identical but one produces pulsed contractions and the other wave trains throughout the cell cortex. Similarly, the mechanism for pulsed contractions in *C. elegans* embryos resembles that for actin coating but the latter produces single waves that wrap around exocytic secretory granules rather than cortical contractions. This ambiguity extends to finer levels of mechanistic detail, in that the impact on patterning of parameter manipulations such as raising or lowering feedback strength or altering the abundance of downstream GTPase targets is highly context-dependent. For example, increasing the RHOA GAP to RHOA GEF ratio in immature frog oocytes promotes a transition from pulses to waves, whereas the same manipulation in starfish oocytes reduces wave amplitude[214].

Benefits of self-organizing Rho GTPase patterns

The examples presented above represent organisms from six different phyla, prompting the question what are the evolutionary benefits that arise from self-organized Rho GTPase pattern? Some benefits of self-organization based on positive and negative feedback are well known, such as the ability to respond quickly or to filter out noise, which is likely to be important for cells to interpret and filter internal and external inputs during processes such as cell migration, where

cells must navigate complex environments[234], or plasma membrane repair, where cells must be able to respond within seconds to damage[235].

The examples suggest another benefit, namely, pattern (and thus response) variation. That is, the same core players can produce very different patterns in different systems, as in pulsed contractions in *C. elegans* embryos and cytokinesis in starfish and frog embryos which both rely on RHOA, F-actin, ECT2 and RGA-3/4 (refs. 13,214). Further, even within a single cell, engaging the same core players, considerable pattern diversity is possible. The manipulations in RBL cells demonstrate that modulation of different phosphoinositide lipids produce dramatic differences in wave properties[218] whereas in U2OS cells increasing the expression of GEF-H1 promotes formation of RHOA-GTP waves over pulses[18]. Similarly, increasing expression of RGA-3/4 against a constant level of ECT2 in immature frog oocytes produces a progression from pulses, to trains of short-lived waves, to persistent spiral waves[214], whereas the pulsed contractions in *C. elegans* embryos can be pushed towards or away from wavelike behaviour by manipulation of a RHOA effector levels[236]. And even under conditions where the same players are present in the same cell, pattern transitions can happen via redistribution of one or more of the players to a different subcellular location. For example, modelling results indicate that the transition from waves of RHOA-GTP and F-actin to a stationary cytokinetic zone of RHOA-GTP and F-actin can arise simply from the spindle-dependent concentration of ECT2 on the equatorial cortex past some critical threshold[42].

There are also less intuitive advantages that can arise from self-organized patterns such as those that produce periodic behaviour. Perhaps the best example is provided by pulsed contractions (although similar arguments would apply to activity waves). As described above, pulsed contractions based on F-actin and myosin 2 are common outcomes of Rho GTPase signalling in both developing organisms and cultured cells and, in the former, are harnessed to drive tissue morphogenesis. In many cases, such pulses are associated with 'ratcheting' in which each pulse results in a reduction of the apical domain of the cell or one side of the apical domain of the cell and subsequent pulses result in further reductions[155]. The net effect is tissue bending in a manner that minimizes competition between contracting cells — a contracting cell does not have to overcome neighbour contractions to achieve a cell shape change[237]. Similarly, empirical and modelling studies indicate that spatially and temporally heterogeneous contractile events are important during collective cell movement[238] and junctional shortening[239] in epithelial cells. Modelling work also indicates that pulsed contractions permit developing systems to maintain persistent, large-scale contractions in the face of local disconnections or breakages in the contractile network[240]. Additionally, pattern transitions induced by increasing negative feedback can, paradoxically, increase the amplitude of local RHOA-GTP waves[214] meaning that higher local contractility could be possible in the presence of a RHOA GAP than in its absence. Moreover, despite their seeming simplicity, self-organized patterns can template very complex dynamic cytoskeletal arrays that are nonetheless remarkably resilient[194,195].

Lastly, there is an additional, broader benefit of self-organization that may be particularly important for the Rho GTPases, given that they control the cell's contractile machinery — actin filaments and myosin 2. This machinery can be enormously powerful, such that when it is improperly harnessed it can literally tear the cell to pieces[241]. Yet somehow this same machinery must be employed to drive processes that require considerable precision, such as splitting the cell in half between the separating chromosomes or compressing a secretory granule following exocytosis. Furthermore, the contractile machinery must do these things in exactly the right place and time. Self-organization makes this possible, both by poising the cell to respond to diverse signals and by ensuring that the output of the contractile machinery is modulated in a manner appropriate to the task at hand.

Conclusions and future directions

The examples of self-organization of Rho GTPase signalling presented above represent the tip of the iceberg, as the selection criteria applied were stringent. Nonetheless, other examples of what are likely to be self-organized Rho GTPase patterns include frustrated phagocytosis[242,243], invadopodia[244], periodic pulses of another Rho isoform, RHOB, on internal membranous compartments[245], adhesive actin waves[246], apical constriction in *Drosophila melanogaster* embryonic epithelial cells[162], formation of microridges on the apical surface of epithelial cells[247,248], plant cell patterning by ROPs[249] and, of course, the many examples of cell migration that are associated with dynamic Rho GTPase patterns but whose control circuitry awaits characterization[250–254].

In short, the available evidence indicates that Rho GTPase self-organization is not simply a curiosity restricted to a select few cell types or situations but, rather, a fundamental feature of Rho GTPase regulation and function. Because the self-organization dictates the Rho GTPase patterns, and the Rho GTPase patterns dictate the outcome for the cortex and the cell, an emphasis on Rho GTPase self-organization is obviously warranted. That is, if we are to understand mechanistically how Rho GTPases contribute to complex cellular processes such as cytokinesis, cell migration and morphogenesis, it will no longer be enough to simply suppress their function. Rather, we will need to understand their pattern-forming mechanisms with sufficient level of mechanistic detail to permit the manipulation of these patterns.

With this view in mind, several research directions are likely to be especially important. First, increased attention to high temporal resolution study of Rho GTPase dynamics may reveal that apparently stationary patterns are actually periodic. Second, an increased emphasis on feedback circuitry and, in particular, negative feedback is likely to be fruitful. Third, continued imaging tool development for both the Rho GTPases themselves[255,256] and their regulators[257] will be essential, particularly as there is increased interest in studying Rho GTPase dynamics in more complex samples, such as tissues, which are likely to be more challenging than single cells[258–260]. Fourth, more structural and biochemical analyses of GEFs and GAPs will be needed to identify and characterize novel feedback mechanisms[84]. Finally, increased application of single molecule imaging[13] and reconstitution approaches[21,191] are likely to provide much-needed information about how, exactly, Rho GTPase cycles are executed.

Note added in proof

During the preparation of this review, two additional studies of dynamic Rho GTPase patterning were published[261,262].

Published online: 3 January 2024

References

1. Hodge, R. G. & Ridley, A. J. Regulating Rho GTPases and their regulators. *Nat. Rev. Mol. Cell Biol.* **17**, 496–510 (2016).
2. Jaffe, A. B. & Hall, A. Rho GTPases: biochemistry and biology. *Annu. Rev. Cell Dev. Biol.* **21**, 247–269 (2005).
3. Scheffzek, K. & Ahmadian, M. R. GTPase activating proteins: structural and functional insights 18 years after discovery. *Cell Mol. Life Sci.* **62**, 3014–3038 (2005).
4. Rossman, K. L., Der, C. J. & Sondek, J. GEF means go: turning on RHO GTPases with guanine nucleotide-exchange factors. *Nat. Rev. Mol. Cell Biol.* **6**, 167–180 (2005).
5. Michaud, A. et al. Cortical excitability and cell division. *Curr. Biol.* **31**, R553–R559 (2021).
6. Allen, W. E., Zicha, D., Ridley, A. J. & Jones, G. E. A role for Cdc42 in macrophage chemotaxis. *J. Cell Biol.* **141**, 1147–1157 (1998).

7. Kishi, K., Sasaki, T., Kuroda, S., Itoh, T. & Takai, Y. Regulation of cytoplasmic division of *Xenopus* embryo by rho p21 and its inhibitory GDP/GTP exchange protein (rho GDI). *J. Cell Biol.* **120**, 1187–1195 (1993).
8. Massol, P., Montcourrier, P., Guillemot, J. C. & Chavrier, P. Fc receptor-mediated phagocytosis requires CDC42 and Rac1. *EMBO J.* **17**, 6219–6229 (1998).
9. Stowers, L., Yelon, D., Berg, L. J. & Chant, J. Regulation of the polarization of T cells toward antigen-presenting cells by Ras-related GTPase CDC42. *Proc. Natl Acad. Sci. USA* **92**, 5027–5031 (1995).
10. Barrett, K., Leptin, M. & Settleman, J. The Rho GTPase and a putative RhoGEF mediate a signaling pathway for the cell shape changes in *Drosophila* gastrulation. *Cell* **91**, 905–915 (1997).
11. Howell, A. S. et al. Singularity in polarization: rewiring yeast cells to make two buds. *Cell* **139**, 731–743 (2009).
12. Das, M. et al. Oscillatory dynamics of Cdc42 GTPase in the control of polarized growth. *Science* **337**, 239–243 (2012).
13. Michaux, J. B., Robin, F. B., McFadden, W. M. & Munro, E. M. Excitable RhoA dynamics drive pulsed contractions in the early *C. elegans* embryo. *J. Cell Biol.* **217**, 4230–4252 (2018).
14. Abreu-Blanco, M. T., Verboon, J. M. & Parkhurst, S. M. Coordination of Rho family GTPase activities to orchestrate cytoskeleton responses during cell wound repair. *Curr. Biol.* **24**, 144–155 (2014).
15. Bement, W. M. et al. Activator-inhibitor coupling between Rho signalling and actin assembly makes the cell cortex an excitable medium. *Nat. Cell Biol.* **17**, 1471–1483 (2015).
16. Bischof, J. et al. A cdk1 gradient guides surface contraction waves in oocytes. *Nat. Commun.* **8**, 849 (2017).
17. Burkel, B. M., Benink, H. A., Vaughan, E. M., von Dassow, G. & Bement, W. M. A Rho GTPase signal treadmill backs a contractile array. *Dev. Cell* **23**, 384–396 (2012).
18. Graessl, M. et al. An excitable Rho GTPase signaling network generates dynamic subcellular contraction patterns. *J. Cell Biol.* **216**, 4271–4285 (2017).
19. Wu, M., Wu, X. & De Camilli, P. Calcium oscillations-coupled conversion of actin travelling waves to standing oscillations. *Proc. Natl Acad. Sci. USA* **110**, 1339–1344 (2013).
20. Xiao, S., Tong, C., Yang, Y. & Wu, M. Mitotic cortical waves predict future division sites by encoding positional and size information. *Dev. Cell* **43**, 493–506.e3 (2017).
21. Landino, J. et al. Rho and F-actin self-organize within an artificial cell cortex. *Curr. Biol.* **31**, 5613–5621.e5 (2021).
22. Ozbudak, E. M., Becskei, A. & van Oudenaarden, A. A system of counteracting feedback loops regulates Cdc42p activity during spontaneous cell polarization. *Dev. Cell* **9**, 565–571 (2005).
23. Bement, W. M., Benink, H. A. & von Dassow, G. A microtubule-dependent zone of active RhoA during cleavage plane specification. *J. Cell Biol.* **170**, 91–101 (2005).
24. Benink, H. A. & Bement, W. M. Concentric zones of active RhoA and Cdc42 around single cell wounds. *J. Cell Biol.* **168**, 429–439 (2005).
25. Sokac, A. M., Co, C., Taunton, J. & Bement, W. Cdc42-dependent actin polymerization during compensatory endocytosis in *Xenopus* eggs. *Nat. Cell Biol.* **5**, 727–732 (2003).
26. Nalbant, P., Hodgson, L., Kraynov, V., Toutchkine, A. & Hahn, K. M. Activation of endogenous Cdc42 visualized in living cells. *Science* **305**, 1615–1619 (2004).
27. Machacek, M. et al. Coordination of Rho GTPase activities during cell protrusion. *Nature* **461**, 99–103 (2009).
28. Somers, W. G. & Saint, R. A RhoGEF and Rho family GTPase-activating protein complex links the contractile ring to cortical microtubules at the onset of cytokinesis. *Dev. Cell* **4**, 29–39 (2003).
29. Bement, W. M., Miller, A. L. & von Dassow, G. Rho GTPase activity zones and transient contractile arrays. *Bioessays* **28**, 983–993 (2006).
30. Goryachev, A. B. & Pokhilko, A. V. Computational model explains high activity and rapid cycling of Rho GTPases within protein complexes. *PLoS Comput. Biol.* **2**, e172 (2006).
31. Clay, M. R. & Halloran, M. C. Rho activation is apically restricted by Arhgap1 in neural crest cells and drives epithelial-to-mesenchymal transition. *Development* **140**, 3198–3209 (2013).
32. Mason, F. M., Xie, S., Vasquez, C. G., Tworoger, M. & Martin, A. C. RhoA GTPase inhibition organizes contraction during epithelial morphogenesis. *J. Cell Biol.* **214**, 603–617 (2016).
33. Budnar, S. et al. Anillin promotes cell contractility by cyclic resetting of RhoA residence kinetics. *Dev. Cell* **49**, 894–906.e12 (2019).
34. Bendezu, F. O. et al. Spontaneous Cdc42 polarization independent of GDI-mediated extraction and actin-based trafficking. *PLoS Biol.* **13**, e1002097 (2015).
35. van Bruggen, R., Anthony, E., Fernandez-Borja, M. & Roos, D. Continuous translocation of Rac2 and the NADPH oxidase component $p67^{phox}$ during phagocytosis. *J. Biol. Chem.* **279**, 9097–9102 (2004).
36. Goryachev, A. B. & Pokhilko, A. V. Dynamics of Cdc42 network embodies a turing-type mechanism of yeast cell polarity. *FEBS Lett.* **582**, 1437–1443 (2008).
37. Tatebe, H., Nakano, K., Maximo, R. & Shiozaki, K. Pom1 DYRK regulates localization of the Rga4 GAP to ensure bipolar activation of Cdc42 in fission yeast. *Curr. Biol.* **18**, 322–330 (2008).
38. Simões, S. et al. Compartmentalisation of Rho regulators directs cell invagination during tissue morphogenesis. *Development* **133**, 4257–4267 (2006).
39. di Pietro, F. et al. Systematic analysis of RhoGEF/GAP localizations uncovers regulators of mechanosensing and junction formation during epithelial cell division. *Curr. Biol.* **33**, 858–874.e7 (2023).
40. Müller, P. M. et al. Systems analysis of RhoGEF and RhoGAP regulatory proteins reveals spatially organized RAC1 signalling from integrin adhesions. *Nat. Cell Biol.* **22**, 498–511 (2020).
41. Allard, J. & Mogilner, A. Traveling waves in actin dynamics and cell motility. *Curr. Opin. Cell Biol.* **25**, 107–115 (2013).
42. Goryachev, A. B., Leda, M., Miller, A. L., von Dassow, G. & Bement, W. M. How to make a static cytokinetic furrow out of traveling excitable waves. *Small GTPases* **7**, 65–70 (2016).
43. Landge, A. N., Jordan, B. M., Diego, X. & Müller, P. Pattern formation mechanisms of self-organizing reaction-diffusion systems. *Dev. Biol.* **460**, 2–11 (2020).
44. Mitchison, T. J. & Field, C. M. Self-organization of cellular units. *Annu. Rev. Cell Dev. Biol.* **37**, 23–41 (2021).
45. Valdez, V. A., Neahring, L., Petry, S. & Dumont, S. Mechanisms underlying spindle assembly and robustness. *Nat. Rev. Mol. Cell Biol.* **24**, 523–542 (2023).
46. Heald, R. et al. Self-organization of microtubules into bipolar spindles around artificial chromosomes in *Xenopus* egg extracts. *Nature* **382**, 420–425 (1996).
47. Collinet, C. & Lecuit, T. Programmed and self-organized flow of information during morphogenesis. *Nat. Rev. Mol. Cell Biol.* **22**, 245–265 (2021).
48. Hansen, S. D. et al. Stochastic geometry sensing and polarization in a lipid kinase–phosphatase competitive reaction. *Proc. Natl Acad. Sci. USA* **116**, 15013–15022 (2019).
49. Murray, J. D. *Mathematical Biology* 19 (Springer-Verlag, 1993).
50. Meinhardt, H. & Gierer, A. Pattern formation by local self-activation and lateral inhibition. *Bioessays* **22**, 753–760 (2000).
51. Rao, C. V., Wolf, D. M. & Arkin, A. P. Control, exploitation and tolerance of intracellular noise. *Nature* **420**, 231–237 (2002).
52. Chiou, J.-g, Moran, K. D. & Lew, D. J. How cells determine the number of polarity sites. Preprint at *bioRxiv* https://doi.org/10.1101/2020.05.21.109520 (2020).
53. Hennig, K. et al. Stick–slip dynamics of cell adhesion triggers spontaneous symmetry breaking and directional migration of mesenchymal cells on one-dimensional lines. *Sci. Adv.* **6**, eaau5670 (2020).
54. Lavrsen, K. et al. Microtubule detyrosination drives symmetry breaking to polarize cells for directed cell migration. *Proc. Natl Acad. Sci. USA* **120**, e2300322120 (2023).
55. Hoyle, R. *Pattern Formation* (Cambridge Univ. Press, 2006).
56. Cross, M. C. & Greenside, H. *Pattern Formation and Dynamics in Nonequilibrium Systems* (Cambridge Univ. Press, 2009).
57. Miller, A. L. & Bement, W. M. Regulation of cytokinesis by Rho GTPase flux. *Nat. Cell Biol.* **11**, 71–77 (2009).
58. Zheng, Y., Bender, A. & Cerione, R. A. Interactions among proteins involved in bud-site selection and bud-site assembly in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **270**, 626–630 (1995).
59. Loose, M., Fischer-Friedrich, E., Ries, J., Kruse, K. & Schwille, P. Spatial regulators for bacterial cell division self-organize into surface waves in vitro. *Science* **320**, 789–792 (2008).
60. Ramm, B., Glock, P. & Schwille, P. In vitro reconstitution of self-organizing protein patterns on supported lipid bilayers. *J. Vis. Exp.* **28**, 58139 (2018).
61. Cezanne, A., Lauer, J., Solomatina, A., Sbalzarini, I. F. & Zerial, M. A non-linear system patterns Rab5 GTPase on the membrane. *eLife* **9**, e54434 (2020).
62. Goryachev, A. B. & Leda, M. Autoactivation of small GTPases by the GEF-effector positive feedback modules. *F1000Res* **8**, https://doi.org/10.12688/f1000research.20003.1 (2019).
63. Jackson, C. L. GEF–effector interactions. *Cell Logist.* **4**, e943616 (2014).
64. Horiuchi, H. et al. A novel Rab5 GDP/GTP exchange factor complexed to Rabaptin-5 links nucleotide exchange to effector recruitment and function. *Cell* **90**, 1149–1159 (1997).
65. Grosshans, B. L., Ortiz, D. & Novick, P. Rabs and their effectors: achieving specificity in membrane traffic. *Proc. Natl Acad. Sci. USA* **103**, 11821–11827 (2006).
66. Chen, Z. et al. Activated RhoA binds to the pleckstrin homology (PH) domain of PDZ-RhoGEF, a potential site for autoregulation. *J. Biol. Chem.* **285**, 21070–21081 (2010).
67. Medina, F. et al. Activated RhoA is a positive feedback regulator of the Lbc family of Rho guanine nucleotide exchange factor proteins. *J. Biol. Chem.* **288**, 11325–11333 (2013).
68. Chen, M. et al. Structure and regulation of human epithelial cell transforming 2 protein. *Proc. Natl Acad. Sci. USA* **117**, 1027–1035 (2020).
69. Lin, Q., Yang, W., Baird, D., Feng, Q. & Cerione, R. A. Identification of a DOCK180-related guanine nucleotide exchange factor that is capable of mediating a positive feedback activation of Cdc42. *J. Biol. Chem.* **281**, 35253–35262 (2006).
70. Bose, I. et al. Assembly of scaffold-mediated complexes containing Cdc42p, the exchange factor Cdc24p, and the effector Cla4p required for cell cycle-regulated phosphorylation of Cdc24p. *J. Biol. Chem.* **276**, 7176–7186 (2001).
71. Butty, A. C. et al. A positive feedback loop stabilizes the guanine-nucleotide exchange factor Cdc24 at sites of polarization. *EMBO J.* **21**, 1565–1576 (2002).
72. Hussain, N. K. et al. Endocytic protein intersectin-l regulates actin assembly via Cdc42 and N-WASP. *Nat. Cell Biol.* **3**, 927–932 (2001).
73. Lamas, I., Weber, N. & Martin, S. G. Activation of Cdc42 GTPase upon CRY2-induced cortical recruitment is antagonized by GAPs in fission yeast. *Cells* **9**, 2089 (2020).
74. McGavin, M. K. et al. The intersectin 2 adaptor links Wiskott Aldrich syndrome protein (WASp)-mediated actin polymerization to T cell antigen receptor endocytosis. *J. Exp. Med.* **194**, 1777–1787 (2001).
75. Castro-Castro, A. et al. Coronin 1A promotes a cytoskeletal-based feedback loop that facilitates Rac1 translocation and activation. *EMBO J.* **30**, 3913–3927 (2011).
76. Segal, D., Zaritsky, A., Schejter, E. D. & Shilo, B. Z. Feedback inhibition of actin on Rho mediates content release from large secretory vesicles. *J. Cell Biol.* **217**, 1815–1826 (2018).
77. Nguyen, T. T. et al. PLEKHG3 enhances polarized cell migration by activating actin filaments at the cell front. *Proc. Natl Acad. Sci. USA* **113**, 10091–10096 (2016).
78. DerMardirossian, C., Schnelzer, A. & Bokoch, G. M. Phosphorylation of RhoGDI by Pak1 mediates dissociation of Rac GTPase. *Mol. Cell* **15**, 117–127 (2004).

79. Kitzing, T. M. et al. Positive feedback between Dia1, LARG, and RhoA regulates cell morphology and invasion. *Genes. Dev.* **21**, 1478–1483 (2007).
80. Priya, R. et al. Feedback regulation through myosin II confers robustness on RhoA signalling at E-cadherin junctions. *Nat. Cell Biol.* **17**, 1282–1293 (2015).
81. Barrows, D. et al. p21-activated kinases (PAKs) mediate the phosphorylation of PREX2 protein to initiate feedback inhibition of Rac1 GTPase. *J. Biol. Chem.* **290**, 28915–28931 (2015).
82. Radu, M. et al. ArhGAP15, a Rac-specific GTPase-activating protein, plays a dual role in inhibiting small GTPase signaling. *J. Biol. Chem.* **288**, 21117–21125 (2013).
83. Dibus, M., Brábek, J. & Rösel, D. A screen for PKN3 substrates reveals an activating phosphorylation of ARHGAP18. *Int J. Mol. Sci.* **21**, 7769 (2020).
84. Diring, J., Mouilleron, S., McDonald, N. Q. & Treisman, R. RPEL-family rhoGAPs link Rac/Cdc42 GTP loading to G-actin availability. *Nat. Cell Biol.* **21**, 845–855 (2019).
85. Lee, C. S., Choi, C. K., Shin, E. Y., Schwartz, M. A. & Kim, E. G. Myosin II directly binds and inhibits Dbl family guanine nucleotide exchange factors: a possible link to Rho family GTPases. *J. Cell Biol.* **190**, 663–674 (2010).
86. Premkumar, L. et al. Structural basis of membrane targeting by the Dock180 family of Rho family guanine exchange factors (Rho-GEFs). *J. Biol. Chem.* **285**, 13211–13222 (2010).
87. Kunisaki, Y. et al. DOCK2 is a Rac activator that regulates motility and polarity during neutrophil chemotaxis. *J. Cell Biol.* **174**, 647–652 (2006).
88. Kanai, A. et al. Identification of DOCK4 and its splicing variant as PIP3 binding proteins. *IUBMB Life* **60**, 467–472 (2008).
89. Fritsch, R. et al. RAS and RHO families of GTPases directly regulate distinct phosphoinositide 3-kinase isoforms. *Cell* **153**, 1050–1063 (2013).
90. Zheng, Y., Bagrodia, S. & Cerione, R. A. Activation of phosphoinositide 3-kinase activity by Cdc42Hs binding to p85. *J. Biol. Chem.* **269**, 18727–18730 (1994).
91. Goryachev, A. B. & Leda, M. Many roads to symmetry breaking: molecular mechanisms and theoretical models of yeast cell polarity. *Mol. Biol. Cell* **28**, 370–380 (2017).
92. Cherfils, J. & Zeghouf, M. Regulation of small GTPases by GEFs, GAPs, and GDIs. *Physiol. Rev.* **93**, 269–309 (2013).
93. Hennig, A., Markwart, R., Esparza-Franco, M. A., Ladds, G. & Rubio, I. Ras activation revisited: role of GEF and GAP systems. *Biol. Chem.* **396**, 831–848 (2015).
94. Arkowitz, R. A. & Bassilana, M. Polarized growth in fungi: symmetry breaking and hyphal formation. *Semin. Cell Dev. Biol.* **22**, 806–815 (2011).
95. Martin, S. G. & Arkowitz, R. A. Cell polarization in budding and fission yeasts. *FEMS Microbiol. Rev.* **38**, 228–253 (2014).
96. Bi, E. & Park, H. O. Cell polarization and cytokinesis in budding yeast. *Genetics* **191**, 347–387 (2012).
97. Chiou, J. G., Balasubramanian, M. K. & Lew, D. J. Cell polarity in yeast. *Annu. Rev. Cell Dev. Biol.* **33**, 77–101 (2017).
98. Richman, T. J., Sawyer, M. M. & Johnson, D. I. *Saccharomyces cerevisiae* Cdc42p localizes to cellular membranes and clusters at sites of polarized growth. *Eukaryot. Cell* **1**, 458–468 (2002).
99. Adams, A. E., Johnson, D. I., Longnecker, R. M., Sloat, B. F. & Pringle, J. R. CDC42 and CDC43, two additional genes involved in budding and the establishment of cell polarity in the yeast *Saccharomyces cerevisiae*. *J. Cell Biol.* **111**, 131–142 (1990).
100. Johnson, D. I. & Pringle, J. R. Molecular characterization of CDC42, a *Saccharomyces cerevisiae* gene involved in the development of cell polarity. *J. Cell Biol.* **111**, 143–152 (1990).
101. Wedlich-Soldner, R., Wai, S. C., Schmidt, T. & Li, R. Robust cell polarity is a dynamic state established by coupling transport and GTPase signaling. *J. Cell Biol.* **166**, 889–900 (2004).
102. Tong, Z. et al. Adjacent positioning of cellular structures enabled by a Cdc42 GTPase-activating protein-mediated zone of inhibition. *J. Cell Biol.* **179**, 1375–1384 (2007).
103. Okada, S., Lee, M. E., Bi, E. & Park, H. O. Probing Cdc42 polarization dynamics in budding yeast using a biosensor. *Methods Enzymol.* **589**, 171–190 (2017).
104. Lichius, A. et al. CDC-42 and RAC-1 regulate opposite chemotropisms in *Neurospora crassa*. *J. Cell Sci.* **127**, 1953–1965 (2014).
105. Okada, S. et al. Daughter cell identity emerges from the interplay of Cdc42, septins, and exocytosis. *Dev. Cell* **26**, 148–161 (2013).
106. Caudron, F. & Barral, Y. Septins and the lateral compartmentalization of eukaryotic membranes. *Dev. Cell* **16**, 493–506 (2009).
107. Virag, A., Lee, M. P., Si, H. & Harris, S. D. Regulation of hyphal morphogenesis by Cdc42 and Rac1 homologues in *Aspergillus nidulans*. *Mol. Microbiol.* **66**, 1579–1596 (2007).
108. Böhmer, C., Ripp, C. & Bölker, M. The germinal centre kinase Don3 triggers the dynamic rearrangement of higher-order septin structures during cytokinesis in *Ustilago maydis*. *Mol. Microbiol.* **74**, 1484–1496 (2009).
109. Kayano, Y., Tanaka, A. & Takemoto, D. Two closely related Rho GTPases, Cdc42 and RacA, of the en-dophytic fungus *Epichloë festucae* have contrasting roles for ROS production and symbiotic infection synchronized with the host plant. *PLoS Pathog.* **14**, e1006840 (2018).
110. Pan, X., Pérez-Henríquez, P., Van Norman, J. M. & Yang, Z. Membrane nanodomains: dynamic nanobuilding blocks of polarized cell growth. *Plant. Physiol.* **193**, 83–97 (2023).
111. Li, E. et al. Signaling network controlling ROP-mediated tip growth in *Arabidopsis* and beyond. *Plant. Commun.* **4**, 100451 (2023).
112. Ou, H. & Yi, P. ROP GTPase-dependent polarity establishment during tip growth in plants. *N. phytol.* **236**, 49–57 (2022).
113. Mulvey, H. & Dolan, L. RHO GTPase of plants regulates polarized cell growth and cell division orientation during morphogenesis. *Curr. Biol.* **33**, 2897–2911.e6 (2023).
114. Drubin, D. G. Development of cell polarity in budding yeast. *Cell* **65**, 1093–1096 (1991).
115. Park, H. O. & Bi, E. Central roles of small GTPases in the development of cell polarity in yeast and beyond. *Microbiol. Mol. Biol. Rev.* **71**, 48–96 (2007).
116. Ayscough, K. R. et al. High rates of actin filament turnover in budding yeast and roles for actin in establishment and maintenance of cell polarity revealed using the actin inhibitor latrunculin-A. *J. Cell Biol.* **137**, 399–416 (1997).
117. Butty, A. C., Pryciak, P. M., Huang, L. S., Herskowitz, I. & Peter, M. The role of Far1p in linking the heterotrimeric G protein to polarity establishment proteins during yeast mating. *Science* **282**, 1511–1516 (1998).
118. Li, R. & Bowerman, B. Symmetry breaking in biology. *Cold Spring Harb. Perspect. Biol.* **2**, a003475 (2010).
119. Irazoqui, J. E., Gladfelter, A. S. & Lew, D. J. Scaffold-mediated symmetry breaking by Cdc42p. *Nat. Cell Biol.* **5**, 1062–1070 (2003).
120. Kozubowski, L. et al. Symmetry-breaking polarization driven by a Cdc42p GEF–PAK complex. *Curr. Biol.* **18**, 1719–1726 (2008).
121. Woods, B., Kuo, C. C., Wu, C. F., Zyla, T. R. & Lew, D. J. Polarity establishment requires localized activation of Cdc42. *J. Cell Biol.* **211**, 19–26 (2015).
122. Witte, K., Strickland, D. & Glotzer, M. Cell cycle entry triggers a switch between two modes of Cdc42 activation during yeast polarization. *eLife* **6**, e26722 (2017).
123. Chang, E. C. et al. Cooperative interaction of *S. pombe* proteins required for mating and morphogenesis. *Cell* **79**, 131–141 (1994).
124. Lamas, I., Merlini, L., Vještica, A., Vincenzetti, V. & Martin, S. G. Optogenetics reveals Cdc42 local activation by scaffold-mediated positive feedback and Ras GTPase. *PLoS Biol.* **18**, e3000600 (2020).
125. Altschuler, S. J., Angenent, S. B., Wang, Y. & Wu, L. F. On the spontaneous emergence of cell polarity. *Nature* **454**, 886–889 (2008).
126. Wedlich-Soldner, R., Altschuler, S., Wu, L. & Li, R. Spontaneous cell polarization through actomyosin-based delivery of the Cdc42 GTPase. *Science* **299**, 1231–1235 (2003).
127. Wu, C. F., Savage, N. S. & Lew, D. J. Interaction between bud-site selection and polarity-establishment machineries in budding yeast. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **368**, 20130006 (2013).
128. Wu, C. F. et al. Role of competition between polarity sites in establishing a unique front. *eLife* **4**, e11611 (2015).
129. Otsuji, M. et al. A mass conserved reaction-diffusion system captures properties of cell polarity. *PLoS Comput. Biol.* **3**, e108 (2007).
130. Chiou, J. G. et al. Principles that govern competition or co-existence in Rho-GTPase driven polarization. *PLoS Comput. Biol.* **14**, e1006095 (2018).
131. Brauns, F., Halatek, J. & Frey, E. Phase-space geometry of mass-conserving reaction-diffusion dynamics. *Phys. Rev. X* **10**, 041036 (2020).
132. Verschueren, N. & Champneys, A. A model for cell polarization without mass conservation. *SIAM J. Appl. Dyn. Syst.* **16**, 1797–1830 (2017).
133. Goryachev, A. B. & Leda, M. Compete or coexist? Why the same mechanisms of symmetry breaking can yield distinct outcomes. *Cells* **9**, 2011 (2020).
134. Mori, Y., Jilkine, A. & Edelstein-Keshet, L. Wave-pinning and cell polarity from a bistable reaction-diffusion system. *Biophys. J.* **94**, 3684–3697 (2008).
135. Smith, S. E. et al. Independence of symmetry breaking on Bem1-mediated autocatalytic activation of Cdc42. *J. Cell Biol.* **202**, 1091–1106 (2013).
136. Klunder, B., Freisinger, T., Wedlich-Soldner, R. & Frey, E. GDI-mediated cell polarization in yeast provides precise spatial and temporal control of Cdc42 signaling. *PLoS Comput. Biol.* **9**, e1003396 (2013).
137. Freisinger, T. et al. Establishment of a robust single axis of cell polarity by coupling multiple positive feedback loops. *Nat. Commun.* **4**, 1807 (2013).
138. Jose, M., Tollis, S., Nair, D., Sibarita, J. B. & McCusker, D. Robust polarity establishment occurs via an endocytosis-based cortical corralling mechanism. *J. Cell Biol.* **200**, 407–418 (2013).
139. Giese, W., Eigel, M., Westerheide, S., Engwer, C. & Klipp, E. Influence of cell shape, inhomogeneities and diffusion barriers in cell polarization models. *Phys. Biol.* **12**, 066014 (2015).
140. Lee, M. E., Lo, W. C., Miller, K. E., Chou, C. S. & Park, H. O. Regulation of Cdc42 polarization by the Rsr1 GTPase and Rga1, a Cdc42 GTPase-activating protein, in budding yeast. *J. Cell Sci.* **128**, 2106–2117 (2015).
141. Lo, W. C., Lee, M. E., Narayan, M., Chou, C. S. & Park, H. O. Polarization of diploid daughter cells directed by spatial cues and GTP hydrolysis of Cdc42 budding yeast. *PLoS ONE* **8**, e56665 (2013).
142. Bonazzi, D. et al. Actin-based transport adapts polarity domain size to local cellular curvature. *Curr. Biol.* **25**, 2677–2683 (2015).
143. Bonazzi, D. et al. Symmetry breaking in spore germination relies on an interplay between polar cap stability and spore wall mechanics. *Dev. Cell* **28**, 534–546 (2014).
144. Daalman, W. K., Sweep, E. & Laan, L. A tractable physical model for the yeast polarity predicts epistasis and fitness. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **378**, 20220044 (2023).
145. Kuo, C. C. et al. Inhibitory GEF phosphorylation provides negative feedback in the yeast polarity circuit. *Curr. Biol.* **24**, 753–759 (2014).

146. Dyer, J. M. et al. Tracking shallow chemical gradients by actin-driven wandering of the polarization site. *Curr. Biol.* **23**, 32–41 (2013).
147. Howell, A. S. et al. Negative feedback enhances robustness in the yeast polarity establishment circuit. *Cell* **149**, 322–333 (2012).
148. Khalili, B., Merlini, L., Vincenzetti, V., Martin, S. G. & Vavylonis, D. Exploration and stabilization of Ras1 mating zone: a mechanism with positive and negative feedbacks. *PLoS Comput. Biol.* **14**, e1006317 (2018).
149. Ghose, D. & Lew, D. Mechanistic insights into actin-driven polarity site movement in yeast. *Mol. Biol. Cell* **31**, 1085–1102 (2020).
150. Layton, A. T. et al. Modeling vesicle traffic reveals unexpected consequences for Cdc42p-mediated polarity establishment. *Curr. Biol.* **21**, 184–194 (2011).
151. Watson, L. J., Rossi, G. & Brennwald, P. Quantitative analysis of membrane trafficking in regulation of Cdc42 polarity. *Traffic* **15**, 1330–1343 (2014).
152. Gerganova, V. et al. Cell patterning by secretion-induced plasma membrane flows. *Sci. Adv.* **7**, eabg6718 (2021).
153. Khalili, B., Lovelace, H. D., Rutkowski, D. M., Holz, D. & Vavylonis, D. Fission yeast polarization: modeling Cdc42 oscillations, symmetry breaking, and zones of activation and inhibition. *Cells* **9**, 1769 (2020).
154. Pino, M. R. et al. Cdc42 GTPase-activating proteins (GAPs) regulate generational inheritance of cell polarity and cell shape in fission yeast. *Mol. Biol. Cell* **32**, ar14 (2021).
155. Coravos, J. S., Mason, F. M. & Martin, A. C. Actomyosin pulsing in tissue integrity maintenance during morphogenesis. *Trends Cell Biol.* **27**, 276–283 (2017).
156. Munjal, A., Philippe, J. M., Munro, E. & Lecuit, T. A self-organized biomechanical network drives shape changes during tissue morphogenesis. *Nature* **524**, 351–355 (2015).
157. Munjal, A. & Lecuit, T. Actomyosin networks and tissue morphogenesis. *Development* **141**, 1789–1793 (2014).
158. Coravos, J. S. & Martin, A. C. Apical sarcomere-like actomyosin contracts nonmuscle *Drosophila* epithelial cells. *Dev. Cell* **39**, 346–358 (2016).
159. Kim, H. Y. & Davidson, L. A. Punctuated actin contractions during convergent extension and their permissive regulation by the non-canonical Wnt-signaling pathway. *J. Cell Sci.* **124**, 635–646 (2011).
160. Munro, E., Nance, J. & Priess, J. R. Cortical flows powered by asymmetrical contraction transport PAR proteins to establish and maintain anterior-posterior polarity in the early *C. elegans* embryo. *Dev. Cell* **7**, 413–424 (2004).
161. Costache, V. et al. Rapid assembly of a polar network architecture drives efficient actomyosin contractility. *Cell Rep.* **39**, 110868 (2022).
162. Mason, F. M., Tworoger, M. & Martin, A. C. Apical domain polarization localizes actin–myosin activity to drive ratchet-like apical constriction. *Nat. Cell Biol.* **15**, 926–936 (2013).
163. Nishikawa, M., Naganathan, S. R., Jülicher, F. & Grill, S. W. Controlling contractile instabilities in the actomyosin cortex. *eLife* **6**, e19595 (2017).
164. Schmutz, C., Stevens, J. & Spang, A. Functions of the novel RhoGAP proteins RGA-3 and RGA-4 in the germ line and in the early embryo of *C. elegans*. *Development* **134**, 3495–3505 (2007).
165. Baird, M. A. et al. Local pulsatile contractions are an intrinsic property of the myosin 2A motor in the cortical cytoskeleton of adherent cells. *Mol. Biol. Cell* **28**, 240–251 (2017).
166. Beach, J. R. et al. Actin dynamics and competition for myosin monomer govern the sequential amplification of myosin filaments. *Nat. Cell Biol.* **19**, 85–93 (2017).
167. Lehtimäki, J. I., Rajakylä, E. K., Tojkander, S. & Lappalainen, P. Generation of stress fibers through myosin-driven reorganization of the actin cortex. *eLife* **10**, e60710 (2021).
168. Kumari, A. et al. Actomyosin-driven force patterning controls endocytosis at the immune synapse. *Nat. Commun.* **10**, 2870 (2019).
169. Kamps, D. et al. Optogenetic tuning reveals Rho amplification-dependent dynamics of a cell contraction signal network. *Cell Rep.* **33**, 108467 (2020).
170. Chen, X., Venkatachalapathy, M., Dehmelt, L. & Wu, Y. W. Multidirectional activity control of cellular processes by a versatile chemo-optogenetic approach. *Angew. Chem. Int. Ed. Engl.* **57**, 11993–11997 (2018).
171. Beta, C., Edelstein-Keshet, L., Gov, N. & Yochelis, A. From actin waves to mechanism and back: how theory aids biological understanding. *eLife* **12**, e87181 (2023).
172. Sokac, A. M. & Bement, W. M. Kiss-and-coat and compartment mixing: coupling exocytosis to signal generation and local actin assembly. *Mol. Biol. Cell* **17**, 1495–1502 (2006).
173. Nemoto, T., Kojima, T., Oshima, A., Bito, H. & Kasai, H. Stabilization of exocytosis by dynamic F-actin coating of zymogen granules in pancreatic acini. *J. Biol. Chem.* **279**, 37544–37550 (2004).
174. Rousso, T., Schejter, E. D. & Shilo, B. Z. Orchestrated content release from *Drosophila* glue-protein vesicles by a contractile actomyosin network. *Nat. Cell Biol.* **18**, 181–190 (2016).
175. Ma, W. et al. Arp2/3 nucleates F-actin coating of fusing insulin granules in pancreatic β cells to control insulin secretion. *J. Cell Sci.* **133**, jcs236794 (2020).
176. Miklavc, P., Wittekindt, O. H., Felder, E. & Dietl, P. Ca^{2+}-dependent actin coating of lamellar bodies after exocytotic fusion: a prerequisite for content release or kiss-and-run. *Ann. N. Y. Acad. Sci.* **1152**, 43–52 (2009).
177. Nightingale, T. D. et al. Actomyosin II contractility expels von Willebrand factor from Weibel–Palade bodies during exocytosis. *J. Cell Biol.* **194**, 613–629 (2011).
178. Yu, H. Y. & Bement, W. M. Control of local actin assembly by membrane fusion-dependent compartment mixing. *Nat. Cell Biol.* **9**, 149–159 (2007).
179. Yu, H. Y. & Bement, W. M. Multiple myosins are required to coordinate actin assembly with coat compression during compensatory endocytosis. *Mol. Biol. Cell* **18**, 4096–4105 (2007).
180. Miklavc, P. et al. Actin coating and compression of fused secretory vesicles are essential for surfactant secretion—a role for Rho, formins and myosin II. *J. Cell Sci.* **125**, 2765–2774 (2012).
181. Tran, D. T., Masedunskas, A., Weigert, R. & Ten Hagen, K. G. Arp2/3-mediated F-actin formation controls regulated exocytosis in vivo. *Nat. Commun.* **6**, 10098 (2015).
182. Mietkowska, M., Schuberth, C., Wedlich-Söldner, R. & Gerke, V. Actin dynamics during Ca^{2+}-dependent exocytosis of endothelial Weibel–Palade bodies. *Biochim. Biophys. Acta Mol. Cell Res.* **1866**, 1218–1229 (2019).
183. Sokac, A. M., Schietroma, C., Gundersen, C. B. & Bement, W. M. Myosin-1c couples assembling actin to membranes to drive compensatory endocytosis. *Dev. Cell* **11**, 629–640 (2006).
184. Kamalesh, K. et al. Exocytosis by vesicle crumpling maintains apical membrane homeostasis during exocrine secretion. *Dev. Cell* **56**, 1603–1616.e6 (2021).
185. Miklavc, P. et al. Actin depolymerisation and crosslinking join forces with myosin II to contract actin coats on fused secretory vesicles. *J. Cell Sci.* **128**, 1193–1203 (2015).
186. Kono, K., Saeki, Y., Yoshida, S., Tanaka, K. & Pellman, D. Proteasomal degradation resolves competition between cell polarization and cellular wound healing. *Cell* **150**, 151–164 (2012).
187. Xu, J. et al. Redox-sensitive CDC-42 clustering promotes wound closure in *C. elegans*. *Cell Rep.* **37**, 110040 (2021).
188. Horn, A. et al. Mitochondrial redox signaling enables repair of injured skeletal muscle cells. *Sci. Signal.* **10**, eaaj1978 (2017).
189. Moe, A. M., Golding, A. E. & Bement, W. M. Cell healing: calcium, repair and regeneration. *Semin. Cell Dev. Biol.* **45**, 18–23 (2015).
190. Verboon, J. M. & Parkhurst, S. M. Rho family GTPases bring a familiar ring to cell wound repair. *Small GTPases* **6**, 1–7 (2015).
191. Golding, A. E., Visco, I., Bieling, P. & Bement, W. M. Extraction of active RhoGTPases by RhoGDI regulates spatiotemporal patterning of RhoGTPases. *eLife* **8**, e50471 (2019).
192. Vaughan, E. M., Miller, A. L., Yu, H. Y. & Bement, W. M. Control of local Rho GTPase crosstalk by Abr. *Curr. Biol.* **21**, 270–277 (2011).
193. Nakamura, M., Verboon, J. M. & Parkhurst, S. M. Prepatterning by RhoGEFs governs Rho GTPase spatiotemporal dynamics during wound repair. *J. Cell Biol.* **216**, 3959–3969 (2017).
194. Mandato, C. A. & Bement, W. M. Contraction and polymerization cooperate to assemble and close actomyosin rings around *Xenopus* oocyte wounds. *J. Cell Biol.* **154**, 785–797 (2001).
195. Hui, J., Nakamura, M., Dubrulle, J. & Parkhurst, S. M. Coordinated efforts of different actin filament populations are needed for optimal cell wound repair. *Mol. Biol. Cell* **34**, ar15 (2023).
196. Abreu-Blanco, M. T., Verboon, J. M. & Parkhurst, S. M. Cell wound repair in *Drosophila* occurs through three distinct phases of membrane and cytoskeletal remodeling. *J. Cell Biol.* **193**, 455–464 (2011).
197. Holmes, W. R., Golding, A. E., Bement, W. M. & Edelstein-Keshet, L. A mathematical model of GTPase pattern formation during single-cell wound repair. *Interface Focus.* **6**, 20160032 (2016).
198. Simon, C. M., Vaughan, E. M., Bement, W. M. & Edelstein-Keshet, L. Pattern formation of Rho GTPases in single cell wound healing. *Mol. Biol. Cell* **24**, 421–432 (2013).
199. Chuang, T. H. et al. Abr and Bcr are multifunctional regulators of the Rho GTP-binding protein family. *Proc. Natl Acad. Sci. USA* **92**, 10282–10286 (1995).
200. Davenport, N. R., Sonnemann, K. J., Eliceiri, K. W. & Bement, W. M. Membrane dynamics during cellular wound repair. *Mol. Biol. Cell* **27**, 2272–2285 (2016).
201. Vaughan, E. M. et al. Lipid domain-dependent regulation of single-cell wound repair. *Mol. Biol. Cell* **25**, 1867–1876 (2014).
202. Moe, A. et al. Cross-talk-dependent cortical patterning of Rho GTPases during cell repair. *Mol. Biol. Cell* **32**, 1417–1432 (2021).
203. Yüce, O., Piekny, A. & Glotzer, M. An ECT2–centralspindlin complex regulates the localization and function of RhoA. *J. Cell Biol.* **170**, 571–582 (2005).
204. Wagner, E. & Glotzer, M. Local RhoA activation induces cytokinetic furrows independent of spindle position and cell cycle stage. *J. Cell Biol.* **213**, 641–649 (2016).
205. Bastos, R. N., Penate, X., Bates, M., Hammond, D. & Barr, F. A. CYK4 inhibits Rac1-dependent PAK1 and ARHGEF7 effector pathways during cytokinesis. *J. Cell Biol.* **198**, 865–880 (2012).
206. Su, K. C., Takaki, T. & Petronczki, M. Targeting of the RhoGEF Ect2 to the equatorial membrane controls cleavage furrow formation during cytokinesis. *Dev. Cell* **21**, 1104–1115 (2011).
207. Nishimura, Y. & Yonemura, S. Centralspindlin regulates ECT2 and RhoA accumulation at the equatorial cortex during cytokinesis. *J. Cell Sci.* **119**, 104–114 (2006).
208. Jordan, S. N. & Canman, J. C. Rho GTPases in animal cell cytokinesis: an occupation by the one percent. *Cytoskeleton* **69**, 919–930 (2012).
209. Zhuravlev, Y. et al. CYK-4 regulates Rac, but not Rho, during cytokinesis. *Mol. Biol. Cell* **28**, 1258–1270 (2017).
210. Canman, J. C. et al. Inhibition of Rac by the GAP activity of centralspindlin is essential for cytokinesis. *Science* **322**, 1543–1546 (2008).
211. Zhang, D. & Glotzer, M. The RhoGAP activity of CYK-4/MgcRacGAP functions non-canonically by promoting RhoA activation during cytokinesis. *eLife* **4**, e08898 (2015).

212. Loria, A., Longhini, K. M. & Glotzer, M. The RhoGAP domain of CYK-4 has an essential role in RhoA activation. *Curr. Biol.* **22**, 213–219 (2012).
213. Swider, Z. T. et al. Cell cycle and developmental control of cortical excitability in *Xenopus laevis*. *Mol. Biol. Cell* **33**, ar73 (2022).
214. Michaud, A. et al. A versatile cortical pattern-forming circuit based on Rho, F-actin, Ect2, and RGA-3/4. *J. Cell Biol.* **221**, e202203017 (2022).
215. Zanin, E. et al. A conserved RhoGAP limits M phase contractility and coordinates with microtubule asters to confine RhoA during cytokinesis. *Dev. Cell* **26**, 496–510 (2013).
216. Bell, K. R. et al. Novel cytokinetic ring components drive negative feedback in cortical contractility. *Mol. Biol. Cell* **31**, 1623–1636 (2020).
217. Krendel, M., Zenke, F. T. & Bokoch, G. M. Nucleotide exchange factor GEF-H1 mediates cross-talk between microtubules and the actin cytoskeleton. *Nat. Cell Biol.* **4**, 294–301 (2002).
218. Tong, C. S., Xu, X. J. & Wu, M. Periodicity, mixed-mode oscillations, and multiple timescale in a phosphoinositide–Rho GTPase network. *Cell Rep.* **42**, 112857 (2023).
219. Scott, D. W., Tolbert, C. E. & Burridge, K. Tension on JAM-A activates RhoA via GEF-H1 and p115 RhoGEF. *Mol. Biol. Cell* **27**, 1420–1430 (2016).
220. Scholze, M. J. et al. PI(4,5)P(2) forms dynamic cortical structures and directs actin distribution as well as polarity in *Caenorhabditis elegans* embryos. *Development* **145**, dev164988 (2018).
221. Reyes, C. C. et al. Anillin regulates cell–cell junction integrity by organizing junctional accumulation of Rho-GTP and actomyosin. *Curr. Biol.* **24**, 1263–1270 (2014).
222. Terry, S. J. et al. Spatially restricted activation of RhoA signalling at epithelial junctions by p114RhoGEF drives junction formation and morphogenesis. *Nat. Cell Biol.* **13**, 159–166 (2011).
223. Ratheesh, A., Priya, R. & Yap, A. S. Coordinating Rho and Rac: the regulation of Rho GTPase signaling and cadherin junctions. *Prog. Mol. Biol. Transl. Sci.* **116**, 49–68 (2013).
224. Varadarajan, S., Stephenson, R. E. & Miller, A. L. Multiscale dynamics of tight junction remodeling. *J. Cell Sci.* **132**, jcs229286 (2019).
225. Citi, S., Guerrera, D., Spadaro, D. & Shah, J. Epithelial junctions and Rho family GTPases: the zonular signalosome. *Small GTPases* **5**, 1–15 (2014).
226. Arnold, T. R., Stephenson, R. E. & Miller, A. L. Rho GTPases and actomyosin: partners in regulating epithelial cell–cell junction structure and function. *Exp. Cell Res.* **358**, 20–30 (2017).
227. Ratheesh, A. et al. Centralspindlin and α-catenin regulate Rho signalling at the epithelial zonula adherens. *Nat. Cell Biol.* **14**, 818–828 (2012).
228. Breznau, E. B., Semack, A. C., Higashi, T. & Miller, A. L. MgcRacGAP restricts active RhoA at the cytokinetic furrow and both RhoA and Rac1 at cell–cell junctions in epithelial cells. *Mol. Biol. Cell* **26**, 2439–2455 (2015).
229. Arnold, T. R. et al. Anillin regulates epithelial cell mechanics by structuring the medial–apical actomyosin network. *eLife* **8**, e39062 (2019).
230. Priya, R. et al. Bistable front dynamics in a contractile medium: travelling wave fronts and cortical advection define stable zones of RhoA signaling at epithelial adherens junctions. *PLoS Comput. Biol.* **13**, e1005411 (2017).
231. Stephenson, R. E. et al. Rho flares repair local tight junction leaks. *Dev. Cell* **48**, 445–459. e5 (2019).
232. Varadarajan, S. et al. Mechanosensitive calcium flashes promote sustained RhoA activation during tight junction remodeling. *J. Cell Biol.* **221**, e202102107 (2022).
233. Chumki, S. A., van den Goor, L. M., Hall, B. N. & Miller, A. L. p115RhoGEF activates RhoA to support tight junction maintenance and remodeling. *Mol. Biol. Cell* **33**, ar136 (2022).
234. Weiner, O. D., Marganski, W. A., Wu, L. F., Altschuler, S. J. & Kirschner, M. W. An actin-based wave generator organizes cell motility. *PLoS Biol.* **5**, e221 (2007).
235. Sonnemann, K. J. & Bement, W. M. Wound repair: toward understanding and integration of single-cell and multicellular wound responses. *Annu. Rev. Cell Dev. Biol.* **27**, 237–263 (2011).
236. Yao, B., Donoughe, S., Michaux, J. & Munro, E. Modulating RhoA effectors induces transitions to oscillatory and more wavelike RhoA dynamics in *Caenorhabditis elegans* zygotes. *Mol. Biol. Cell* **33**, ar58 (2022).
237. Xie, S. & Martin, A. C. Intracellular signalling and intercellular coupling coordinate heterogeneous contractile events to facilitate tissue folding. *Nat. Commun.* **6**, 7161 (2015).
238. Zulueta-Coarasa, T. & Fernandez-Gonzalez, R. Dynamic force patterns promote collective cell movements during embryonic wound repair. *Nat. Phys.* **14**, 750–758 (2018).
239. Cavanaugh, K. E., Chmiel, T. A. & Gardel, M. L. Caveolae spelunking: exploring a new modality in tensional homeostasis. *Dev. Cell* **54**, 3–5 (2020).
240. Mak, M., Zaman, M. H., Kamm, R. D. & Kim, T. Interplay of active processes modulates tension and drives phase transition in self-renewing, motor-driven cytoskeletal networks. *Nat. Commun.* **7**, 10323 (2016).
241. Su, K. C., Bement, W. M., Petronczki, M. & von Dassow, G. An astral simulacrum of the central spindle accounts for normal, spindle-less, and anucleate cytokinesis in echinoderm embryos. *Mol. Biol. Cell* **25**, 4049–4062 (2014).
242. Poirier, M. B., Fiorino, C., Rajasekar, T. K. & Harrison, R. E. F-Actin flashes on phagosomes mechanically deform contents for efficient digestion in macrophages. *J. Cell Sci.* **133**, jcs239384 (2020).
243. Herron, J. C. et al. Spatial models of pattern formation during phagocytosis. *PLoS Comput. Biol.* **18**, e1010092 (2022).
244. Kreider-Letterman, G. et al. ARHGAP17 regulates the spatiotemporal activity of Cdc42 at invadopodia. *J. Cell Biol.* **222**, e202207020 (2023).
245. Reinhard, N. R. et al. Spatiotemporal analysis of RhoA/B/C activation in primary human endothelial cells. *Sci. Rep.* **6**, 25502 (2016).
246. Case, L. B. & Waterman, C. M. Adhesive F-actin waves: a novel integrin-mediated adhesion complex coupled to ventral actin polymerization. *PLoS ONE* **6**, e26631 (2011).
247. van Loon, A. P., Erofeev, I. S., Goryachev, A. B. & Sagasti, A. Stochastic contraction of myosin minifilaments drives evolution of microridge protrusion patterns in epithelial cells. *Mol. Biol. Cell* **32**, 1501–1513 (2021).
248. van Loon, A. P., Erofeev, I. S., Maryshev, I. V., Goryachev, A. B. & Sagasti, A. Cortical contraction drives the 3D patterning of epithelial cell surfaces. *J. Cell Biol.* **219**, e201904144 (2020).
249. Sternberg, H. et al. Formation of self-organizing functionally distinct Rho of plants domains involves a reduced mobile population. *Plant. Physiol.* **187**, 2485–2508 (2021).
250. Fritz, R. D. & Pertz, O. The dynamics of spatio-temporal Rho GTPase signaling: formation of signaling patterns. *F1000Res* **5**, https://doi.org/10.12688/f1000research.7370.1 (2016).
251. Bolado-Carrancio, A. et al. Periodic propagating waves coordinate RhoGTPase network dynamics at the leading and trailing edges during cell migration. *eLife* **9**, e58165 (2020).
252. Yang, H. W., Collins, S. R. & Meyer, T. Locally excitable Cdc42 signals steer cells during chemotaxis. *Nat. Cell Biol.* **18**, 191–201 (2016).
253. Martin, K. et al. Spatio-temporal co-ordination of RhoA, Rac1 and Cdc42 activation during prototypical edge protrusion and retraction dynamics. *Sci. Rep.* **6**, 21901 (2016).
254. Huang, C. H., Tang, M., Shi, C., Iglesias, P. A. & Devreotes, P. N. An excitable signal integrator couples to an idling cytoskeletal oscillator to drive cell migration. *Nat. Cell Biol.* **15**, 1307–1316 (2013).
255. Mahlandt, E. K., Kreider-Letterman, G., Chertkova, A. O., Garcia-Mata, R. & Goedhart, J. Cell-based optimization and characterization of genetically encoded location-based biosensors for Cdc42 or Rac activity. *J. Cell Sci.* **136**, jcs260802 (2023).
256. Mahlandt, E. K. et al. Visualizing endogenous Rho activity with an improved localization-based, genetically encoded biosensor. *J. Cell Sci.* **134**, jcs258823 (2021).
257. Azoitei, M. L. et al. Spatiotemporal dynamics of GEF-H1 activation controlled by microtubule- and Src-mediated pathways. *J. Cell Biol.* **218**, 3077–3097 (2019).
258. Ichikawa, T., Stuckenholz, C. & Davidson, L. A. Non-junctional role of Cadherin3 in cell migration and contact inhibition of locomotion via domain-dependent, opposing regulation of Rac1. *Sci. Rep.* **10**, 17326 (2020).
259. Floerchinger, A. et al. Optimizing metastatic-cascade-dependent Rac1 targeting in breast cancer: guidance using optical window intravital FRET imaging. *Cell Rep.* **36**, 109689 (2021).
260. Gupta, S. et al. Enhanced RhoA signalling stabilizes E-cadherin in migrating epithelial monolayers. *J. Cell Sci.* **134**, jcs258767 (2021).
261. Nanda, S. et al. Rho GTPase activity crosstalk mediated by Arhgef11 and Arhgef12 coordinates cell protrusion-retraction cycles. *Nat. Commun.* **14**, 8356 (2023).
262. Patwardhan, R. et al. Cdc42 activity in the trailing edge is required for persistent directional migration of keratinocytes. *Mol Biol Cell.* https://doi.org/10.1091/mbc.E23-08-0318 (2024).
263. Dada, O., Gutowski, S., Brautigam, C. A., Chen, Z. & Sternweis, P. C. Direct regulation of p190RhoGEF by activated Rho and Rac GTPases. *J. Struct. Biol.* **202**, 13–24 (2018).
264. Chen, Z. et al. Activation of p115-RhoGEF requires direct association of Gα13 and the Dbl homology domain. *J. Biol. Chem.* **287**, 25490–25500 (2012).
265. Baird, D., Feng, Q. & Cerione, R. A. The Cool-2/α-Pix protein mediates a Cdc42–Rac signaling cascade. *Curr. Biol.* **15**, 1–10 (2005).
266. Bellanger, J. M. et al. The Rac1- and RhoG-specific GEF domain of Trio targets filamin to remodel cytoskeletal actin. *Nat. Cell Biol.* **2**, 888–892 (2000).
267. Kim, K., Hou, P., Gorski, J. L. & Cooper, J. A. Effect of Fgd1 on cortactin in Arp2/3 complex-mediated actin assembly. *Biochemistry* **43**, 2422–2427 (2004).
268. Nayal, A. et al. Paxillin phosphorylation at Ser273 localizes a GIT1–PIX–PAK complex and regulates adhesion and protrusion dynamics. *J. Cell Biol.* **173**, 587–589 (2006).
269. Ten Klooster, J. P. et al. Interaction between Tiam1 and the Arp2/3 complex links activation of Rac to actin polymerization. *Biochem. J.* **397**, 39–45 (2006).
270. Lee, C. C., Huang, C. C. & Hsu, K. S. The phospholipid-binding protein SESTD1 negatively regulates dendritic spine density by interfering with Rac1–Trio8 signaling pathway. *Sci. Rep.* **5**, 13250 (2015).
271. Barrows, D., He, J. Z. & Parsons, R. PREX1 protein function is negatively regulated downstream of receptor tyrosine kinase activation by p21-activated kinases (PAKs). *J. Biol. Chem.* **291**, 20042–20054 (2016).
272. Fauchereau, F. et al. The RhoGAP activity of OPHN1, a new F-actin-binding protein, is negatively controlled by its amino-terminal domain. *Mol. Cell. Neurosci.* **23**, 574–586 (2003).
273. Schlam, D. et al. Phosphoinositide 3-kinase enables phagocytosis of large particles by terminating actin assembly through Rac/Cdc42 GTPase-activating proteins. *Nat. Commun.* **6**, 8623 (2015).
274. Michaelson, D. et al. Differential localization of Rho GTPases in live cells: regulation by hypervariable regions and RhoGDI binding. *J. Cell Biol.* **152**, 111–126 (2001).

275. Yonemura, S., Hirao-Minakuchi, K. & Nishimura, Y. Rho localization in cells and tissues. *Exp. Cell Res.* **295**, 300–314 (2004).
276. Lu, M. S. & Drubin, D. G. Cdc42 GTPase regulates ESCRTs in nuclear envelope sealing and ER remodeling. *J. Cell Biol.* **219**, e201910119 (2020).
277. Boulter, E. et al. Regulation of Rho GTPase crosstalk, degradation and activity by RhoGDI1. *Nat. Cell Biol.* **12**, 477–483 (2010).
278. Yoshizaki, H. et al. Activity of Rho-family GTPases during cell division as visualized with FRET-based probes. *J. Cell Biol.* **162**, 223–232 (2003).
279. Kraynov, V. S. et al. Localized Rac activation dynamics visualized in living cells. *Science* **290**, 333–337 (2000).
280. Fritz, R. D. et al. A versatile toolkit to produce sensitive FRET biosensors to visualize signaling in time and space. *Sci. Signal.* **6**, rs12 (2013).

Acknowledgements
The authors thank colleagues who generously provided unpublished images: J. Michaux and E. Munro, University of Chicago; C. Tong and M. Wu, Yale University; A. Michaud, Promega Corp.; M. Graessl, P. Nalbant and L. Dehmelt, University of Duisburg and Technical University of Dortmund; and L. Hoachlander-Hobby, University of Wisconsin–Madison. W.M.B. acknowledges funding from the National Institutes of Health (NIH RO1 GM052932) and the National Science Foundation (NSF 2132606), A.L.M. acknowledges funding from the National Institutes of Health (NIH R01 GM112794) and the National Science Foundation (NSF 1615338), and A.B.G acknowledges funding from the Biotechnology and Biological Sciences Research Council (BB/W013614/1) and the Leverhulme Trust (RPG-2020-220). To the memory of Robert K. Bement, 1932–2023: 'Er ist geflohen, als wir ihn nicht beobachteten'.

Author contributions
The authors contributed equally to all aspects of the article.

Competing interests
The authors declare no competing interests.

Additional information
Peer review information *Nature Reviews Molecular Cell Biology* thanks the anonymous reviewers for their contribution to the peer review of this work.

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