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2016 Phys. Biol. 13 046001

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Analysis of a minimal Rho-GTPase circuit regulating cell shape

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Keywords: Rac–Rho, cell shape, ameboid/mesenchymal motility, local perturbation analysis

Abstract

Networks of Rho-family GTPases regulate eukaryotic cell polarization and motility by controlling assembly and contraction of the cytoskeleton. The mutually inhibitory Rac–Rho circuit is emerging as a central, regulatory hub that can affect the shape and motility phenotype of eukaryotic cells. Recent experimental manipulation of the amounts of Rac and Rho or their regulators (guanine nucleotide-exchange factors, GTPase-activating proteins, guanine nucleotide dissociation inhibitors) have been shown to bias the prevalence of these different states and promote transitions between them. Here we show that part of this data can be understood in terms of inherent Rac–Rho mutually inhibitory dynamics. We analyze a spatio-temporal mathematical model of Rac–Rho dynamics to produce a detailed set of predictions of how parameters such as GTPase rates of activation and total amounts affect cell decisions (such as Rho-dominated contraction, Rac-dominated spreading, and spatially segregated Rac–Rho polarization). We find that in some parameter regimes, a cell can take on any of these three fates depending on its environment or stimuli. We also predict how experimental manipulations (corresponding to parameter variations) can affect cell shapes observed. Our methods are based on local perturbation analysis (a kind of nonlinear stability analysis), and an approximation of nonlinear feedback by sharp switches. We compare the Rac–Rho model to an even simpler single-GTPase (‘wave-pinning’) model and demonstrate that the overall behavior is inherent to GTPase properties, rather than stemming solely from network topology.

1. Introduction

Eukaryotic cell shape, polarity, and migration are governed by complex signaling networks with many hundreds of key interacting components. Deciphering these networks, and understanding how their numerous components interact to regulate cell behavior remains a grand challenge in cell biology. At the same time, in recent years a view has emerged that small subsets of these huge networks form central processing units that determine broad aspects of cell behavior, whereas other components assist in fine-tuning the responses of the cell to a wide range of stimuli. A case in point is the current view that small circuits of Rho-family GTPases orchestrate the morphology and motility of a eukaryotic cell. In particular, recent attention has focused on the circuit consisting of Rac and Rho, members of this GTPase family, whose interactions and downstream effects are being successfully dissected by a combination of experimental and theoretical approaches. Here we examine this simple circuit and analyze its dynamics in space and time.

Rho-family GTPases regulate cell morphology and migration by controlling the cytoskeleton. Rac promotes the assembly and branching of actin filaments, which leads to formation and protrusion of lamellipodia. In contrast, Rho promotes (via Rho kinase, ROCK) myosin light chain phosphorylation and actomyosin contraction, which leads to lamellipodial retraction and contraction of parts or all of the cell. Both Rac and Rho have several forms and play a range of other functions in various cell types, but here we focus on their most commonly observed roles.

GTPases cycle between active forms that are membrane bound and inactive cytosolic forms that are sequestered by guanine nucleotide dissociation
inhibitors (GDIs). Transitions between these states are accelerated by guanine nucleotide-exchange factors (GEFs) that activate the GTPases, and GTPase-activating proteins (GAPs) that inactivate them [1]. Evolution has wired numerous layers of control over each of these regulators, including cross-connections between active GTPases and GEFs, GAPs, GDIs, as well as gene transcription factors [2]. Importantly, Rac and Rho crosstalk has been shown to include antagonistic interactions. For example, Rac1 activates p21-activated kinases (PAKs) that, in turn phosphorylate GEF-H1, a GEF for RhoA. These and other Rac–Rho interactions are reviewed in [2].

The activities of GTPases such as Rac and Rho can be modulated in numerous ways. Cell type and gene activity can affect the expression level (total amount) of a GTPase, and the GEFs, GAPs, and GDIs that regulate it. Stimuli that funnel into activation of one GTPase can affect all others that are wired to it in the regulatory cross-talk via cycles of phosphorylation or other protein modifications. Experimental manipulation by RNA interference (RNAi) [3–5], molecular constructs [6], or inhibitors [7] can be used to explore what happens when either Rac or Rho is up- or down-regulated.

As a result of this growing body of experimental research, a clearer picture has emerged about how manipulating Rac and Rho GTPases affects downstream behaviors such as levels of actin and stress fibers, cell speed, cell shape [4], and multicellular responses in scratch-wound assays [7]. For example, cells that have high levels of Rac and little Rho are typically flat and spread out, whereas those that are enriched in Rho at the expense of Rac tend to be rounded and contracted (shapes 7 versus 1 in [4].) There is also evidence for mutually segregated zones of Rac and Rho in some cells and conditions [8–11], reviewed in [2, 12]. Hence, an intermediate state with both Rac and Rho in distinct cell localizations often takes on a polarized morphology, with a Rac-dominated expanding lamellipodial leading edge and a Rho-dominated contracted trailing edge (shape 5 in [4]). Here we adopt the caricature shown in figure 1(a) with three broad categories for cell behavior (i.e., contracted, spread, and polarized states).

Intriguingly, the levels of Rac and Rho in a cell have been shown to influence phenotypic cell motility. Rac dominated cells tend to exhibit slower, mesenchymal motility. Rho-dominated cells on the other hand exhibit a more rapidly migrating ameboid phenotype, with rounded cell shape [5, 7, 13, 14]. In cancer cells, such modes of motility appear to be interconvertible, and closely dependent on the interactions of Rac and Rho [15]. Moreover, blocking either Rac or Rho experimentally by one of several pathways tunes the resultant cell behavior along a range of mesenchymal–ameboid phenotype [4, 7, 15].

The emerging interest in GTPase dynamics, and in particular, Rac–Rho mutual inhibition has motivated quantitative analysis, based on mathematical models such as dynamical systems, partial differential equations, and computations (reviewed in [16–20]). Models for cell polarization with small circuits [21–23] or with greater level of detail [24–26], including abstract examples [27–29], or those linked directly to experiments [6, 30, 31] have appeared in recent years. Models focusing on Rac–Rho interactions in particular include [7, 14]. Many of these are based on limited computational exploration of parameter space, or simplifications that allow for mathematical insights,
such as assumptions of spatial uniformity \[7, 14\], Boolean interactions \[4\], or a simplified circuit \[32\]. In most cases, a full characterization of the regimes of spatio-temporal behavior predicted by such models remains challenging because partial differential equations that govern reaction–diffusion systems such as biochemical networks are notoriously hard to analyze.

Here we analyze the behavior of the Rac–Rho circuit (‘model 2’) shown in figure 1(b), side by side with an even simpler single-GTPase module (‘model 1’). We ask whether one or both of these GTPase circuits can generate the three basic cellular responses analogous to spreading, contraction, and/or polarization where GTPase activity is either uniformly high, low, or spatially polarized. Using new approximation methods, we compare properties of the two models and map out parameter regimes corresponding to each of these outcomes. We also investigate how intracellular or environmental changes could drive transitions between different phenotypes. Our reason for including model 1 in this investigation are two fold. First, it allows for a pedagogical introduction to the techniques of our paper. Second and more importantly, its comparison with model 2 allows us to elucidate the respective influence of the cycling dynamics of GTPases themselves versus the cross-talk interactions between them.

‘Model 1’ (the so-called ‘wave-pinning (WP)’ circuit) is one of the simplest spatial models of GTPase dynamics. Rigorous analysis of that model (using full bifurcation analysis \[32\], as well as reductions \[33, 34\]) predates this paper, but several aspects remain to be discussed. ‘Model 2’ is the Rac–Rho mutually-inhibitory circuit. In contrast to previous models \[4, 7, 14\], our treatment here includes the spatial distributions of Rac and Rho activities within a cell. A similar setup is found in \[35\], whereas \[4\] consider two cellular compartments in which Rac and Rho can act.

Using a sharp-switch approximation of nonlinearities, as well as local perturbation analysis (LPA), as described in section 3, we uncover new aspects, not previously recognized, of the behavior of each model. Specifically, we determine conditions on the parameters that lead to the three distinct outcomes: spreading (associated with uniformly high Rac and low Rho activity), contraction (high Rho and low Rac), or polarity (mutually exclusive spatially distributed Rac and Rho activities). We then explore how cells could transition between these behaviors.

We find two surprising results. First, the parameter regime that corresponds to bistability (associated with coexistence of spread and contracted states) is embedded in a considerably larger regime where the polarized state is possible. This means that a single parameter-set could, in principle, be consistent with any of the three cell states. A second curious result is that the essential structure of parameter space is qualitatively similar in the two models, suggesting that properties of GTPases (even at a level lower than their cross-talk) are responsible for some of the fundamental features of the behavior. We discuss the biological implications of these results in a concluding section.

### 2. Models

Models 1 and 2 share several common features. Both consist of reaction–diffusion equations, with non-linear feedback terms and with very different rates of diffusion stemming from the membrane versus cytosolic residence of the active versus inactive GTPase forms, respectively \[36, 37\]. Second, in both cases, it is reasonable to assume that the total amount of a given GTPase is approximately constant, since GTPase activation/inactivation is on the order of seconds, whereas gene expression and synthesis of protein is on the timescale of hour(s). As we shall see, these properties are essential in shaping the behavior of the circuits.

We track the active (\(G\)) (membrane) and inactive (\(G_i\)) (cytosolic) GTPase states. The spatial domain is a 1D slice along the diameter of the cell, scaled to unit length. A typical equation for the level of active GTPase is of the form

\[
\frac{\partial G}{\partial t} = \text{[Rate of activation]} \cdot G_i - \text{[Rate of inactivation]} \cdot G + \text{Diffusion.}
\]

A similar equation (with opposite signs for the first two terms) holds for the inactive GTPase. Feedback and crosstalk between GTPases can affect either rates of activation (via GEFs) or inactivation (via GAPs). When this is formulated as a system of PDEs, we obtain

\[
\begin{align*}
\frac{\partial G}{\partial t} &= A(G) \cdot G_i - I \cdot G + D_\Delta G, \\
\frac{\partial G_i}{\partial t} &= -A(G) \cdot G_i + I \cdot G + D_i \Delta G_i, \\
x &\in [0, 1]
\end{align*}
\]

(2.1a)

From here on, we will assume a constant rate of inactivation \(I = \delta\). By scaling time in terms of \(1/\delta\) (active GTPase residence time) we can set \(I = 1\) (see supplementary material). Nonlinear feedbacks and crosstalk interactions (which are essential for non-trivial dynamics) could be encoded in either \(A\) or \(I\), either of which could lead to similar results (not shown). We assume these nonlinearities mathematically reside in \(A\), which is akin to assuming feedback through GEFs that activate GTPases. As mentioned previously and discussed in section 2.1, the structure of these equations encodes conservation of total GTPase.

We scale distance by the cell diameter so that the domain is \(0 \leq x \leq 1\) with impermeable cell ends (no flux boundaries at \(x = 0, 1\)) and assume proteins can diffuse across the cell. However, the rate of diffusion of active GTPase (\(D\)) is far smaller than the rate of
diffusion of the inactive protein \((D)\), as previously noted. This diffusion disparity and the aforementioned conservation are critical aspects of this system and determine the emergent properties of the model.

**Model 1: single GTPase—positive feedback.** After non-dimensionalizing (see supplementary material) the equations representing model 1 are equation (2.1a) with

\[
A(G) = \left( b + \gamma \frac{G^n}{1 + G^n} \right), \quad I = 1.
\]  

(2.1b)

The activation term is comprised of a basal rate \(b\) of activation and a term that depends on feedback of the GTPase on its own activation, with \(\gamma\) the magnitude of that feedback. The level of GTPase has been scaled by an \(EC_{50}\) value (level of \(G\) that produces a 50% feedback response, denoted \(G_0\) in the supplementary material).

Here, the Hill function increases with the protein concentrations are scaled by their \(EC_{50}\) values, as discussed in the supplementary material.

2.1. Conservation of total GTPase

It is evident that the components \(G, G_i, R, R_i, \rho, \rho_i\) in equations ((2.1a), (2.1b)) and ((2.2a), (2.2b), (2.2c)) satisfy conservation statements, since each component merely exchanges between an active and an inactive form. Hence

\[
\int_0^1 (G(x) + G_i(x)) \, dx = G_T
\]

(2.3)

and similarly for \(R\) and \(\rho\), with two conservation parameters \(R_T\) and \(\rho_T\). As pointed out in [29, 32], this conservation property is an important feature on which much of the analysis will be based.

Note that \(G_T, R_T, \rho_T\) represent dimensionless mean GTPase concentrations in the cell (obtained by dividing the total amounts by a characteristic concentration \(G_0\) and by the constant domain size \(L\); see SM for further detail). We later discuss the (dimension-carrying) total amount of GTPase (typically in units of nM, for example), which is simply

\[
\text{Total GTPase} = L \cdot G_0 \cdot G_T.
\]

(2.4)

For fixed \(L\), the parameter \(G_T\) is a good ‘surrogate’ for the total GTPase. In particular, to link our results to experimental manipulations of GTPase amounts, we will associate increased or decreased total GTPase amounts with an increase/decrease in these parameters. Later, we will comment on the effect of changing cell size where it is important to distinguish between total amounts and mean concentrations in the cell.

3. Methods

Our goal is to investigate the full scope of dynamics of models 1 and 2, mapping out parameter regimes where distinct dynamics occur. We use two approximations that facilitate analytical results. First, we analyze the spatially homogeneous ‘well-mixed’ variant of the models, utilizing a sharp-switch approximation for simplification. (See [38] for a similar idea in a larger network.) In this limit, steady state solutions can be written down in closed form, making bifurcation analysis transparent.

Second, we apply a relatively new method, the LPA to determine how and under what conditions these systems would respond to spatial stimuli (figure 1(d)). LPA has proven to be a powerful approximation.
method that guides an understanding of spatial dynamics [33, 34, 39–42]. It harnesses existing ordinary differential equation (ODE) bifurcation software (and in the case of this paper, simple steady state analysis of ODEs) to characterize nonlinear regimes of behavior where polarization can occur.

3.1. Sharp switch limit
We approximate GTPase feedback terms in the activation rate functions by sharp switches (Hill coefficient \( n \to \infty \), figure 1(c)). This produces tractable piecewise-linear models. For model 1, the switch turns ‘on’ once \( G \) is large enough, so

\[
A_H(G) = (b + \gamma H (G - 1)) = \begin{cases} 
    b, & G \leq 1, \\
    b + \gamma, & G > 1, 
\end{cases}
\]

where \( H \) is the Heaviside step function. Similarly, in model 2, \( A^R \), \( A^L \) are approximated by switches that turn off when \( \rho, R > 1 \).

\[
A^R_H(\rho) = [b_R + \gamma_R H (1 - \rho)], \\
A^L_H(R) = [b_L + \gamma_L H (1 - R)].
\]

This simplification leads to analytic results for regimes of behavior of both models, as shown in subsequent sections.

3.2. Local perturbation analysis
We seek to determine how each of these models responds to a spatially heterogenous perturbation such as a chemical gradient. This is a difficult task for general RD equations and perturbations. In order to simplify matters and rapidly map the response regimes for these models, we utilize the ‘LPA’, an approximation method first described in [43] with additional details in [23, 39–41] and validation in [33, 34]. Details of the method are included in the supplementary material. Briefly, the idea is to write down a set of ODEs that track the activation level of a narrow, spatially localized pulse of active GTPase in a background of uniform active and inactive GTPase. The advantage is that we can then use simple ODE bifurcation software to gain an appreciation of the parameter space structure of the models.

3.3. Verification of approximations
To test the accuracy of the ‘sharp switch’ approximation, we analyze the same models with smooth Hill functions (finite \( n \) case). Here we rely on the numerical bifurcation package Matcont [44] since closed form solutions are no longer available. To test the predictions of LPA, we run numerical simulation of the full PDE versions of the models in the distinct parameter regimes.

4. Results
To map the behavior of models 1 and 2 in parameter space, we first apply a well-mixed and LPA analysis to the sharp switch limits of both. Subsequently we do the same for the finite ‘\( n \)’ limit and simulate the PDE’s at selected parameter values to verify results. We carry out these analyzes for models 1 and 2 in parallel to aid comparison.

4.1. Sharp-switch approximation
Figure 2 shows a fully analytic parameter space mapping of model 1 (panel a) and model 2 (panel b) in the sharp-switch limit. Blue boundaries represent boundaries between different well mixed regimes whereas red borders indicate the results of LPA that predict borders of regimes of polarization. In both cases, the results are found analytically by writing down steady states (in terms of parameters) in closed form (see SM).

4.1.1. Model 1
In figure 2(a), the horizontal axis is \( G_T \) (surrogate for total GTPase) and the vertical axis is the magnitude of the feedback parameter \( \gamma \). Solving the well mixed equations for model 1 (see SM) we find two possible stable steady states

\[
G_1 = \frac{b}{1 + b} G_T, \quad G_2 = \frac{b + \gamma}{1 + b + \gamma} G_T.
\]

Since \( G_1 < G_2 \), these represent states with low (\( G_1 \)) and high (\( G_2 \)) GTPase activation, respectively. Existence of these steady states depends on parameters, and we can write down explicit equations for the borders of the parameter regimes where one or another steady state is present (blue curves in figure 2(a), see caption for equations). To the right of both blue curves, \( G_T \) is high and only the high-activity steady state \( G_2 \) is possible (regime I). To the left of both, \( G_T \) is low and only the low activity state \( G_1 \) exists (regime III). Between the curves, both \( G_1 \) and \( G_2 \) are stable (regime II, bistability).

If \( G_T < 1 \), the level of GTPase is insufficient to trigger feedback, so bistability is not possible (though polarity is still possible, as indicated by the red shading). A second important observation is that when there is no basal GTPase activation rate (\( b = 0 \)), there is a degeneracy: the bistable regime (II) overtakes regime (I) and the system is bistable for all values of \( G_T > 1 \) (provided \( \gamma > 0 \), otherwise no feedback is present). In that limit, the steady states are \( G_1 = 0 \) and \( G_2 = \gamma / (1 + \gamma) \cdot G_T \).

To determine where in parameter space polarity is possible, we apply LPA (as detailed in the SM). We thereby determine the red borders enclosing the polarity region in figure 2(a). The LPA analysis establishes that a spatial stimulus can provoke a spatial response in any cell whose parameters fall inside of the red borders of the parameter plane in figure 2(a). Numerical
4.1.2. Model 2

When the same techniques are applied to model 2, we find four possible well-mixed regimes of behavior. In the simple case that \( b_R = b_g = 0 \) steady states are \((R_s, \rho) = (0, \rho_g), (R_s, 0), \) or \((R_s, \rho_g)\) where

\[
R_s = \frac{7R}{1 + \gamma_R} R_T, \quad \rho_g = \frac{\gamma_0}{1 + \gamma_\rho} \rho_T, \quad (4.2)
\]

Analogous steady state expressions in the \( b_R \neq 0, b_g \neq 0 \) case are more complicated and omitted here for brevity. Figure 2(b) shows four well-mixed regimes, separated by gray borders.

(I) \( \rho \) dominated \((R_s < 1, \rho_g > 1)\): there is a single steady state at \((\bar{R}, \bar{\rho}) = (0, \rho_g)\).

(II) Bistable \((R_s > 1 \quad \text{and} \quad \rho_g > 1)\), bounded by blue lines: there are two stable and one unstable steady states.

(III) Mixed \((R_s < 1 \quad \text{and} \quad \rho_g < 1)\): there is a coexistence state where both \( R \) and \( \rho \) are non-zero, \((\bar{R}, \bar{\rho}) = (R_s, \rho_g)\).

(IV) \( \bar{R} \) dominated \((R_s > 1, \rho_g < 1)\): there is a single steady state at \((R_s, 0)\).

Applying LPA to model 2 with the sharp switch approximation reveals the red borders displayed in figure 2(b) (details in SM). These results show that, similar to model 1, the polarity regime again completely encompasses the bistable regime and extends beyond it.

4.2. Verification of regimes with smooth Hill function activation rates

In section 4.1, we obtained analytic results using the sharp-switch approximations of the GTPase rates of activation. Here we compare to results for the original models with smooth Hill functions \((n = 4\) case). Since we can no longer write steady states in closed form, we apply numerical continuation to both the spatially homogeneous and LPA systems to detect and map the boundaries between different steady state regimes. Results in figures 3(a) and (b) are directly comparable to those in figures 2(a) and (b).

4.2.1. Model 1

The polarity regime enclosing a bistable regime is present in model 1 with smooth Hill function feedback, as shown in figure 3(a). To make correspondence with previous work, note that the border of the polarity regime (red curve) is analogous to the V-shaped border in the \( K_\epsilon \) parameter plane of the figure 4.2(a) in [32]. In that work, \( K \) represented the total GTPase and \( \epsilon^2 = (1/\eta) \cdot (D/L^2) \) with \( 1/\eta \) a timescale of GTPase kinetics that is related to our parameter \( 1/\gamma \) in figure 3(a). These results confirm
the parameter space structure determined in figure 2(a) for model 1.

4.2.2. Model 2

Figure 3(b) is the corresponding parameter plot for smooth Hill function terms in model 2 with $b_R = b_p = 0$. The smooth version of the model leads to curved, rather than straight borders for bistable regimes but has a structure comparable to that of figure 2(b). The bistable regime in this panel closely resembles that of figure 2(B) in [7], where a more detailed but spatially homogeneous Rac–Rho model was considered. Numerical continuation of the LPA equations produces the red borders for the polarizable regime (see SM for details.) We find yet another regime, inside the dashed black loop, where the system is linearly unstable and noise would spontaneously generate polarity. The dashed gray line in figure 3(b) subdivides the bistable regime into Rac versus Rho dominated states. We also considered model 2 with nonzero basal activation rates, $b_R = b_p = 0.2, 0.3$. Results (figure 3(c) and figure S4) indicate that as $b$ increases, the bistable regime shrinks and then disappears entirely, leaving only the polarizable regime.

Overall, the results indicate that the sharp switch limit and analytic results obtained in that limit provided a reasonable qualitative approximation of the parameter space structure of the model with smooth Hill functions feedback terms. Further, we see that the polarity regime completely encompasses the bistable regime and extends beyond it. This illustrates two points, both consistent with model 1. First, polarity is a more prominent feature of this system than bistability. Second, as with model 1, polarity can occur even when the underlying kinetics are bistable. This particularly surprising feature will be discussed in more detail in section 5.1.

4.2.3. Confirmation of results with PDE simulation
Recall that the LPA analysis (resulting in the polarity regimes discussed above) is an approximation that assumes $D \to 0$, $D_0 \to \infty$. Hence, we asked how well these results compare with full solutions of the models with finite diffusion. To answer this question, we numerically simulated the original model PDEs (with finite $n$, $D$, $D_0$) for each model, testing for the presence of predicted regimes for several parameter settings. In particular, we probed the regime in which polarity and bistability coexist, using a variety of initial conditions to elicit one and then another of the multiple outcomes predicted above.

Figure 3(d) displays the three distinct steady state solutions obtained for model 1 in the polarity + bistability regime (parameter set corresponding to black dot in figure 3(a)). We find both low and high uniform activity states (gray) as well as polarized state (green).
Supplementary movie 1 illustrates the full time-dependent behavior of the polarized solution: a local region of high activity sweeps into the domain, depleting the inactive substrate (Gi), causing the wave to slow and eventually halt. A variety of parameter sets from within the polarity region were simulated (not shown), confirming the presence of polarity throughout the regime indicated in figure 3(a).

We also tested a variety of initial conditions and perturbations. In the supplementary material (Movies 1–4), we illustrate the response of a model 1 cell in the bistable regime (parameter set ’d’ in figure 3(a)) to graded stimuli. We simulate the system until it settles at a low steady state and subsequently apply varying perturbations of the form \( S(x) = A + Mx \) directly to the activated GTPase concentration profile \( G(x, t) \) at time \( t = 0 \) (these generate graded initial conditions). At \( t = 0 \), we also reduce the inactive form (uniformly in space) to preserve the total amount of GTPase in the domain. We ran four in silico experiments, keeping the slope of the gradient, \( M \) constant, but increasing its baseline \( A \). As shown in corresponding movies, the cell responds in one of three distinct behaviors: low or high uniform activity or polarization.

We similarly compared the predictions for model 2 by simulating the original Rac–Rho PDEs (2.2a) numerically at the points marked d–f in figure 3(b) (SM Movie 5). Results (figures 3(e), (f)) confirm that stable spatial patterns are present where predicted, including inside the bistable regime. As before, this demonstrates the coexistence of multiple cellular behaviors: Rac dominated, Rho dominated, and polarized.

5. Conclusions

As shown in this paper, Rac–Rho mutual antagonism (model 2) produces any of three behaviors in the appropriate regimes; the cell can be uniformly dominated by Rho or Rac, or polarized, with spatially distinct zones of Rac or Rho activation. A single GTPase with positive feedback (model 1) produces similar behavior: uniformly high or uniformly low GTPase activity, or polarization in which GTPase activity is concentrated at one end (’front’ or ’back’) of the cell. Using a convenient model simplification and new methods, we have mapped the location of these distinct regimes in parameter space, uncovering further complexity that was previously overlooked [23, 32, 33]. One caveat is that the borders of the polarity regimes (red curves, figures 2 and 3) are only precise if rates of diffusion are very dissimilar, with \( D \to 0 \) and \( D_i \to \infty \). These parameter regimes shrink in the finite diffusion regime. Nonetheless, detailed numerical simulations (and comparison with [32]) confirm that the predicted structure of the parameter space is qualitatively correct, allowing us to propose how transitions between these behaviors might occur.

5.1. A surprising link between bistability, wave propagation, and polarity

Our results have mathematical implications. Several interesting distinctions are found when we compare our GTPase models to a classical bistable reaction–diffusion system, such as the well-studied example

\[
\frac{\partial u}{\partial t} = u(u - 1)(a - u) + D \Delta u. \tag{5.1}
\]

It was shown nearly 40 years ago [45] that the bistable RD equation (5.1) sustains traveling waves that propagate with a constant velocity (figure 4(a) and SM movie 6). Furthermore, the shape of such waves asymptotically approaches the two spatially homogeneous steady states \( u = 0, 1 \) as \( z \to \pm \infty \) (in the moving frame of the wave), with a wavefront that smoothly interpolates between these. It has also been shown that for a wide class of bistable systems (i.e. monotone systems [46]), stable heterogenous steady states are impossible [47, 48]. Thus, the long-standing belief has been that in the presence of bistability, diffusion-driven waves sweep across the domain, causing one homogenous steady state to replace another. It has been previously shown that with proper assumptions, bistable systems can generate stable patterned states. In [49] for example, permeable
boundary conditions coupled with bistability give rise to stable heterogenous states (through a global, gradient transport driven mechanism). Nonetheless, historically, bistability in reaction diffusion systems has been viewed as incompatible with spatial pattern formation.

Interestingly, this is not the case in our models 1 and 2 where, as shown in this paper, bistability coexists with stable spatially polarized pattern. Furthermore, the types of wave-like solutions in these and related WP systems [24, 37, 40, 50], are substantially different. A perturbation initiates a transiently propagating wave that decelerates to create a stable, polarized state (figure 4(b) and SM movie 1). Observe that the leading and trailing edges of the wave change over the time, in contrast with the traveling wave solutions of the classical equation (5.1).

Coexistence of WP (polarization) behavior with bistability is surprising. This finding is one of the key results in our paper, not previously described in analyses of WP [29, 32]. A second key finding is that the polarity regime completely encloses (and hence dominates over) the bistable regime. This leads to an apparent paradox. If the system is in the bistable regime, and responds to some perturbation, why would the wave triggered not propagate through the whole domain?

The resolution to this apparent paradox requires us to consider the influence of the type of perturbation or initialization of the response. As already discussed, at a fixed set of parameters (e.g. ‘d’ in figure 3(a)), the model cell responds to distinct perturbations with one of three distinct behaviors: low or high uniform activity or polarization. Each of these end states can be viewed as attractors in function space (or in biological terms, a cell’s ‘shape space’) with the type of perturbation determining which basin of attraction the system ends up in. Broadly speaking, this decision depends on the portion of the cell in which some threshold GTPase activity level is exceeded. Too small a region fails to excite the response and the system is drawn back to the pre-perturbed state. Too large a region activates GTPase so strongly that the cell ends up fully activated. If an intermediate portion is excited, WP kinetics dominate and polarization results.

5.2. Biological implications and comparison with experiments
We first stress that our analysis has focused on simplicity and thorough understanding of the basic behavior, which comes at the expense of intricate biological detail. Hence, we have considered only GEF-mediated feedbacks and no self-amplification in the Rac–Rho model. Such additional interactions are surely present biologically, and could be analyzed with similar methods in the future. Such interactions introduce many more parameters, and more exotic possible behaviors. With this in mind, we nevertheless make several biological predictions.

In models 1 and 2, adjusting the mean GTPase concentration ($G_T$), the strength of feedback ($\gamma$), and basal activation rates ($b$) all influence cellular responses to stimuli. A first conclusion is that the basal activation rate ($b$) has substantial but subtle influence. In both models, $b$ is associated with a linear contribution to the activation rate, that is, a contribution proportional to the amount of inactive GTPase. Our results demonstrate that when $b$ increases, the sizes of all sensitivity regimes quickly shrink in parameter space. We can understand this based on the fact that at large $b$, linear components of the rate of activation dominate nonlinear feedback contributions that generate interesting spatial dynamics. Thus, polarity and other behaviors are present only when basal activation strength is sufficiently low.

Both sufficiently strong feedback magnitude ($\gamma$) and sufficiently high GTPase concentrations ($G_T$) are required for bistability and for polarization in both models. This follows from a similar line of reasoning. The former provides the nonlinear feedback to generate dynamics while the latter provides a pool of inactive GTPase to be activated. Both should be large enough to enable stimuli to overcome feedback thresholds. However, while increasing $\gamma$ expands these parameter regimes, flooding the system with an excessive total amount of GTPase (which is the same as increasing the mean level $G_T$ at fixed $L$) overwhelms the system, constitutively activating the same feedbacks so that they never turn off. This also eliminates the interesting range of spatial dynamics. In conclusion, distinct parameters have qualitatively different ranges corresponding to a polarization response. The basal activation strength should not be too large (0 < $b$ < $\tilde{b}$ for some $\tilde{b}$). The feedback strength must be sufficiently large ($\gamma < \gamma_c$ for some minimal $\gamma_c$). Moreover, GTPase levels must be neither too low nor too high ($G_T^{low} < G_T < G_T^{high}$). Similar qualitative results hold for model 2 modulo the fact that more states are possible. For example, if $R_T > \rho_T$ (respectively $\rho_T > R_T$), the system will become uniformly Rac (respectively Rho) activated.

In view of discussions in the literature (e.g. [32, 51, 52]) we also comment on the effect of cell size $L$. Recall that in our models, cell size was normalized to 1 in the dimensionless formulation of the equations, and assumed to be fixed for a given cell. Unlike the full PDE bifurcation analysis in [32], LPA bifurcation analysis fails to detect diffusion-driven length scales as it relies on the limits of asymptotically large or small rates of diffusion (see [34]). Nevertheless, from equation (2.4) we see that each bifurcation diagram for $G_T$, $R_T$, $\rho_T$ could be easily reinterpreted in terms of cell size. Since $[G_T = \text{total GTPase}/(L \cdot G_0)]$, increasing $G_T$ is equivalent to decreasing $L$ or increasing total GTPase. For a full discussion of the effects of GTPase dilution in cells that grow or deform, see our analysis in [40]. Mutations or experimental manipulations could also affect cell size. In principle, mutations
that increase the cell size, $L$, could have one of several effects: (a) lowering $G_T$ if the total GTPases is preserved in the mutant form. This could clearly affect polarizability, as bifurcation plots for $G_T$ attest. (b) Preserving mean GTPase concentration $G_T$ (e.g. if more ribosomes concurrently create a higher total GTPase amount in the cell). In the latter case, the change in $L$ would not affect the cell’s behavior. (c) Some compromise between these extremes, in which case there would be a shift in cell behavior, though less significant than in case (a).

Biologically, a key result of our paper is that a given cell type, with a fixed set of biochemical parameters (amounts of GTPases, rates of activation and inactivation, etc.), can be prodded into a variety of responses depending on the stimulus it receives: a stimulus that activates either Rho or Rac strongly enough in a large enough portion of the cell would lead to a uniformly high level of one or the other, whereas a localized stimulus or appropriate gradient would evoke a polarized response. The mechnano-chemical prodding of cells crawling over one another in a dense population (e.g. in [4]) could provide such perturbations, as could other internal signaling circuits inside a cell with stochastic inputs to the Rac–Rho circuit. Thus this coexistence of states could partly explain the heterogeneity of cell shapes observed experimentally in control cell populations [53, 54] as well as spontaneous transitions between shapes in such control populations.

How do these results relate to experiments in which Rac or Rho is manipulated? The Bakal group [4] utilized RNAi and gene over-expression to vary the total amount of a GTPase such as Rac or Rho (among other variables) and quantify transitions between cell shapes. As one example, they found that over-expressing Rho drove cells to a contractile, rounded morphology, whereas over-expression of Rac produced flat, spread-out cells. This fits well with our predictions for a typical parameter plane configuration (figure 5, solid arrows). In the experiments by the Kholodenko group described in [7], the level of a PAK inhibitor, IPA-3 was gradually increased, releasing the inhibition of Rho by Rac This experimental manipulation corresponds to making Rho less sensitive to Rac inhibition, which can be captured by an increase in the ‘IC$_{50}$’ inhibition parameter ($R_{50}$, level of Rac needed to inhibit rho activation by 50%, see SM). In the supplementary material, we show that this is equivalent to decreasing the (scaled) parameter $R_T$ and corresponds to horizontal motion along the dashed arrow in figure 5.

We end our discussion with the following more philosophical points. Comparison of the two models highlights their qualitatively similar parameter space structure: both have a bistable regime encompassed in a dominant polarization regime. Furthermore, some of these features appear to be universal in GTPase circuits we have studied elsewhere [23, 34, 40], suggesting that many of these are intrinsic properties of GTPases themselves. In light of this, it is interesting to note that there are a number of common structures shared by the two models that are independent of network topology. First, both encode activation/inactivation dynamics of GTPases with a relatively constant total GTPase pool on the timescale of interest. Second, both account for the different diffusivity of membrane versus cytosolic GTPases. Coupled with previous observations that these two properties are critical to GTPase function [29, 32], our results indicate that, in part, the repertoire of GTPase mediated behaviors that cells can exhibit (spread, contract, or polarize) may be intrinsically linked to protein characteristics, independent of the topological connectivity of GTPase networks themselves. We also note that the Rac–Rho motif studied here is similar to other biochemical regulatory systems such as the PI3K—PTEN system [55] or the PAR (‘partitioning-defective’ proteins) system that regulates polarity of C. elegans embryos [56] and epithelial sheets [57], so similar ideas may apply more widely.

Of course, models 1 and 2 will not give rise to the same diversity of cellular behaviors. Spreading and contraction for example require different sets of machinery to remodel the actin cytoskeleton. Spreading requires recruitment of Wave/WASP/Arp2/3 (which are downstream of Rac) and other regulators of branched actin growth. Contraction on the other hand requires recruitment of myosin (which is downstream of Rho). Thus model 1 cannot simultaneously account for both since both Rac and Rho are required. Nonetheless, our results indicate the mathematical structure underlying responses in models 1 and 2 are very similar, and that structure is encoded in the behavior of GTPases themselves rather than their crosstalk.

![Figure 5](image-url)
Viewed through the lens of evolution, this makes sense: it is likely that the properties of GTPases arose in life-forms earlier than the intricate web of crosstalk and GTPase specialization. Moreover, details of GTPase networks differ across cell types. Hence, it speaks to an evolutionary advantage if such key properties as cell polarization are ‘hardwired’ into individual components and preserved in networks, rather than being predicated on a detailed and possibly fallible network topology. Viewed in this light, the overall manner in which GTPases respond to a stimulus (whether they exhibit a whole cell or polarized response, or even if they respond at all) could be a function of GTPase properties themselves, while the overarching crosstalk topology would serve to refine that response, direct traffic, and ensure that each GTPase ends up at the appropriate location within the cell.

Acknowledgments

We thank Andre Levchenko and Jinseok Park for valuable discussions that motivated our interest in the Rac–Rho system. WRH was funded by NSF Grants DMS 1562078 and SES 1556325. LEK was funded by an NSERC discovery grant.

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