A mathematical model of GTPase pattern formation during single-cell wound repair

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Rho GTPases are regulatory proteins whose patterns on the surface of a cell affect cell polarization, cell motility and repair of single-cell wounds. The stereotypical patterns formed by two such proteins, Rho and Cdc42, around laser-injured frog oocytes permit experimental analysis of GTPase activation, inactivation, segregation and crosstalk. Here, we review the development and analysis of a spatial model of GTPase dynamics that describe the formation of concentric zones of Rho and Cdc42 activity around wounds, and describe how this model has provided insights into the roles of the GTPase effector molecules protein kinase C (PKCβ and PKCδ) and guanosine nucleotide dissociation inhibitor (GDI) in the wound response. We further demonstrate how the use of a ‘sharp switch’ model approximation in combination with bifurcation analysis can aid mapping the model behaviour in parameter space (approximate results confirmed with numerical simulation methods). Using these methods in combination with experimental manipulation of PKC activity (PKC overexpression (OE) and dominant negative conditions), we have shown that: (i) PKCβ most probably acts by enhancing existing positive feedbacks (from Rho to itself via the guanosine nucleotide exchange factor domain of Abr, and from Cdc42 to itself), (ii) PKCδ most probably increases basal rates of inactivation (or possibly decreases basal rates of activation) of Rho and Cdc42, and (iii) the graded distribution of PKCδ and its effect on initial Rho activity accounts for inversion of zones in a fraction (20%) of PKCδ OE cells. Finally, we speculate that GDIs (which sequester GTPases) may have a critical role in defining the spatial domain, where the wound response may occur. This paper provides a more thorough exposition of the methods of analysis used in the investigation, whereas previous work on this topic was addressed to biologists and abbreviated such discussion.

1. Introduction

Biological pattern formation has been studied mathematically since the seminal work of Alan Turing in 1952 [1]. Originally concerned with macroscopic patterns in morphogenesis, his work and that of others showed that chemical prepatterns can engender regular placement of body parts, leaves on plants, bristles or other periodic biological structures [2]. As early as 1972, it was proposed by Gierer & Meinhardt [3] that related mechanisms of short-range activation and long-range inhibition could also explain intracellular patterns and account for polarity of cells, a notion that was expanded more than 25 years later in a groundbreaking paper by Meinhardt [4]. Over the past two decades, research on chemical patterns and chemical polarization in cells has blossomed, providing fertile grounds for both experimental and theoretical contributions.

Broadly speaking, this field has addressed two challenges: the first has been to identify the molecular players that participate in the pattern-forming system in cell polarity and to experimentally demonstrate the existence of hypothesized ‘local excitation, global inhibition’ [5–7] or generalized Turing interactions [8]. A second line of inquiry has been to use phenomenological observations of
By virtue of their positive and negative interactions with one another [15], Rho GTPases form a network with spatial pattern-forming properties [16]. Both the dynamics of and the spatial distributions of Rho proteins are key to their ability to regulate facets of cell behaviour [17]. The interplay of Rho proteins, their GEFs, GAPs, GDIs and other effectors are subject of intense biological study, though it is often challenging to untangle the network of crosstalk and downstream effects. An important challenge is to use the observed spatial pattern of GTPase activity to elucidate the underlying interactions responsible for that organization. Here, we report on one example of such experiment–theory research.

Single-cell wounds have proved to be a useful model system in which to study Rho GTPase signalling. Upon wounding, the surface of the frog egg (Xenopus oocyte) undergoes a stereotypical response involving Rho GTPases (figure 1): Rho and Cdc42 are quickly activated near the wound, and segregate within tens of seconds into concentric rings of activity (hereon termed ‘zones’). The fluorescence intensity is high within the zones and low (background) elsewhere. The Cdc42 zone surrounds the Rho zone, which in turn surrounds the wound edge [18,19]. This spatial pattern results in the recruitment of actin filaments and myosin-2, which assemble into a contractile ring that closes over the site of damage [19,20]. An important participant in this process is the dual GEF–GAP Abr which activates Rho and inactivates Cdc42 and thereby promotes zone segregation [21,22]. Because the location and timing of wounds are controlled by the investigator, wound elicited GTPase activation provides a powerful alternative to more complex models of network interaction analysis such as those provided by cell motility or cell division.
Figure 2. Schematic diagram of the GTPase signalling network. The GTPases Rho and Cdc42 in their active (GTP-bound) and inactive (GDP-bound) forms. Horizontal black arrows represent GTPase (in)activation by unspecified GAPs and GEFs. Rho enhances the activity of the GEF–GAP Abr, which then inactivates Cdc42, and activates both Rho and (to a weak extent) Cdc42. Thick vertical blue arrows depict plausible effects of PKCβ on basal or feedback rates of (in)activation. PKCη influence (not shown) is opposite that of PKCβ. Yellow arrows denote sequestration of inactive GTPases by GDI. The mathematical model allows us to distinguish between competing hypotheses for the actions of the PKCs and for the role of GDI in defining a playing field for pattern formation to occur.

Mathematical modelling has helped to focus a number of questions that experiment and theory can address in this system. Such questions include the following:

1. What initiates patterning?
2. What causes the Rho and Cdc42 zones to segregate?
3. What prevents GTPase zones from spreading outward?
4. How can we understand the roles of other players that damp or accentuate one or another zone or background intensity?

In previous work, we addressed (2–4) in the context of a PDE model for Rho, Abr and Cdc42 [23]. A schematic of the basic system is shown in figure 2. More recently, there has been evidence for lipid-dependent regulation of the wounds [24]. Of particular interest has been the involvement of the antagonistic protein kinases eta and beta (PKC β and η) [9,25]. The former activates Rho and Cdc42, whereas the latter inhibits them, but the exact mechanism is unclear. This led to several additional questions, such as: How do PKCs eta and beta affect the system? One puzzling observation is that PKCη overexpression (OE) dampens the Rho zone in approximately 80% of cells, but in the remaining approximately 20%, it causes the Cdc42 and Rho zones to trade places (zone inversion). We asked how this could be explained.

2. Description of models

In order to explore the spatio-temporal interactions based on the schematic in figure 2, we formulated a set of PDEs for the key signalling components that direct the wound response, Rho, Cdc42 and Abr. We take \( x = 0 \) at the wound edge, as shown in figure 1 and model the period of time 48–78 s post-wounding, when the patterns emerge (\( t = 0 \) corresponds to 48 s post-wounding). Earlier events such as calcium-dependent initiation are not in the scope of this model.

For a typical GTPase, let \( G(x, t) \) be the concentration of active GTPase on the cell membrane at position \( x \) and time \( t \), and let \( G_i \) be the concentration of inactive GTPase in the cytosol (available to be activated). Rho GTPases are activated by GEFs, and inactivated and sequestered by GAPs and GDIs, respectively. The general dynamics of the active GTPases are encoded in model equations of the form

\[
\frac{\partial G}{\partial t} = \text{(Activation rate)} \cdot G_i - \text{(Inactivation rate)} \cdot G + D\Delta G,
\]

where

\[
\text{Activation rate} = b_c + F(G_1, G_2, \ldots),
\]

\[
\text{Inactivation rate} = d_c + H(G_1, G_2, \ldots).
\]

Here \( D\Delta C \) is diffusion of active GTPase in the plasma membrane. Terms such as \( b_c, d_c \) depict basal rates while the remaining terms account for feedbacks and crosstalk between various GTPases and their regulators. For example, Abr acts as a GEF for Rho and GAP/GEF for Cdc42 (figure 2), and so appears in the model equations. In addition to basal activation and inactivation rates, some form of feedback stemming from GTPase crosstalk in either one or both of the above is required to account for coexistence of low background activity levels throughout the egg surface as well as high activity levels within the ‘zones’ next to the wound. (Restated mathematically, the model has to be sufficiently nonlinear to display bistable behaviour.) Typically, such feedbacks are represented by sigmoidal Hill functions (for \( F, H \), a commonplace assumption in models of signalling circuits in the cell. Such terms depict the fact that the activation or inactivation of one GTPase by another is nonlinear and saturating. While feedbacks are likely present in both activation and inactivation kinetics, in practice, it is not possible to disentangle the two when the underlying molecular mechanisms are unknown. We thus arbitrarily choose \( H \) to be constant, and assume linearity in the activation term \( F \). Similar behaviour is obtained if \( F \) is constant and \( H \) is a decreasing Hill function [25].

We define \( R(x, t) \) and \( C(x, t) \) as the levels of Rho and Cdc42 activity at a distance \( x \) from the wound edge at time \( t \), and \( A(x, t) \) as the level of Abr activity. The equations that track these variables are then:

\[
\frac{\partial R}{\partial t} = \left(b_R + \gamma_{AR}\frac{A^R}{K_{R}^R + A^R}\right)R_i - \delta_R R + D\Delta R,
\]

\[
\frac{\partial C}{\partial t} = \left(b_C + \gamma_{AC} \cdot A + \gamma_{C} \frac{C^C}{K_{C}^C + C^C}\right)C_i - \left(\delta_C + \delta_{AC} \cdot A\right)C + D\Delta C,
\]

\[
\frac{\partial A}{\partial t} = \gamma_{RA} \cdot R - \delta_A \cdot A + D\Delta A,
\]

where \( D\Delta C, D\Delta R \) and \( D\Delta A \) represent diffusion of active GTPases and of Abr in the plasma membrane. We use no-flux boundary conditions at \( x = 0, \infty \) and step-function initial conditions (shown as dashed lines in figures 3e,f and 4d).

In equations (2.1a,b), \( b_R \) and \( b_C \) are the basal rates of Rho and Cdc42 activation, respectively. The Hill function \((\gamma_{AR}A^R/[K_{R}^R + A^R])\) is the rate of activation of Rho by the Abr-GEF motif (with typically \( n = 6 \)). As Abr acts both as GAP and GEF for Cdc42, we include terms \((\gamma_{AC} \cdot A)\) for the Abr-GEF activation, and \((\delta_{AC} \cdot A)\) for the Abr-GAP inactivation rates of Cdc42. We also model positive feedback of Cdc42 on its own activation rate \((\gamma_{C}C^C/[K_{C}^C + C^C])\). Beyond that, we cannot experimentally decompose all distinct components of the inactivation pathways. Hence we have used...
the notation \( d_R \) and \( d_C \) to represent their aggregate actions. Such parameters could in principle be decomposed into specific terms such as

\[
\begin{align*}
d_R &= d_{Rb} + d_{Rc} \cdot \eta + d_{GR} \cdot G, \\
d_C &= d_{Cb} + d_{Cc} \cdot \eta + d_{GC} \cdot G,
\end{align*}
\]

(2.1d)

where we have explicitly separated terms for basal rates of inactivation (\( d_{Rb} \) and \( d_{Cb} \)), inactivation due to PKC\( \gamma \)-mediated pathways (\( d_{Rc}, d_{Cc} \)), and sequestration of GTPases by the Rho-GDI (\( d_{GR}, d_{GC} \)). (See discussion of these effects later on.)

The values, meanings and units of all parameters are given in table 1. It is worth commenting on several simplifications used in constructing the model. (i) The wound is small (\( \approx 10 \mu m \)) relative to the whole cell (\( \approx 1000 \mu m \)) so that the pattern that forms does not deplete the cellular GTPase reserves. Furthermore, diffusion in the bulk, where inactive forms reside, can rapidly replenish their local levels. Hence we can consider the concentrations of inactive GTPases (\( R, C \)) as roughly constant. Note that this is in distinction with cell polarization patterns, where we have argued that depletion of the inactive GTPases plays a role in stabilizing a polar pattern [26,27]. (ii) Since GDIs are also cytosolic, at first, we will consider their concentration as spatially uniform by the same argument. This is refined in §3.5. (iii) The full problem in two dimensions has radial symmetry and also entails a transport term due to flow of material towards the wound edge. However, for the purpose of addressing the pattern-forming system and understanding crosstalk, we can study the model with a static edge and one-dimensional spatial GTPase profiles. See [23] for a fuller treatment of the two-dimensional model and the contracting wound edge.

3. Results

Wound healing is a transient phenomenon, where zones of regulatory activity form, move inward with closure of the wound, and are finally extinguished. While this is a dynamic process, steady-state analysis provides important information about the process. In particular, the formation of ‘zones’ of high activities near the wound implies model bistability, since these zones coexist with a low activity background.

From figure 2, it is apparent that the model can be decomposed into two subsystems: a Rho–Abr and a Cdc42 subsystem. Abr-GEF/GAP activity mediates positive feedback from Rho to its own activation, and negative feedback from Rho to Cdc42. Together, these actions of Abr account for formation and enhancement of the Rho zone and its exclusion of Cdc42 activity. However, the Rho–Abr subsystem receives no direct inputs from Cdc42 in the model. Analysing each subsystem on its own allows us to determine how each operates individually. We used a combination of qualitative methods and simulations to accomplish this.

3.1. Basic model

A basic Rho–Abr–Cdc42 model with two-dimensional spatial geometry (and radial coordinates with respect to wound centre), developed by Simon et al. [23], led to several conclusions. First, a positive feedback loop from Cdc42 to its own activation is essential for Cdc42 zone formation (\( \gamma_C \neq 0 \)). Second, the basic interactions shown in figure 2, together with Hill activation kinetics leads to spontaneous separation of the Rho and Cdc42 zones. Third, the model is predictive and explanatory of several manipulations and mutants, including OE of Abr, GEF-dead and GAP-dead Abr, and Rho inactivation (by C3 eco-transferase injection). The existence, stability and magnitudes of multiple steady states in model variants explain how such manipulations lead to the experimentally observed changes. Finally, two wounds at various distances apart lead
to overlapping patterns in two-dimensional model simulations that agree with experimental observations. In short, experiments and model concur in most broad qualitative features of the pattern formation.

3.2. Deciphering the effects of GTPase modulators

**PKCβ and PKCγ**

In more recent experiments, it has emerged that protein kinase C beta and eta (PKCβ, PKCγ) affect GTPase patterns after cell-wounding. The PKCs are recruited to wounds quickly by calcium and the lipid diacylglycerol (DAG) (PKCβ) or DAG alone (PKCγ). Spatial localizations of PKCs are however different [24]. PKCβ overlaps the Rho and Cdc42 zones where it enhances both GTPase activities, whereas PKCγ is restricted to a narrow region near the wound edge where it inhibits both. We asked whether PKCs act by damping or accelerating the existing basal rates or by amplifying existing feedbacks. We used mathematical modelling to address this issue in [9].

Upon introducing new players to the model, we could either choose to expand the model to include new (dynamic) variables, or we could treat these players as effective parameter variations. The former comes at the expense of adding complexity to the model, while the latter could miss some important dynamics. We chose the latter more parsimonious approach which proves easier to analyse while providing useful insights. This raises the question of which components of the system (and therefore which parameters) are targeted by the PKCs. To address this, we used data for OE and dominant negative (DN) manipulations of both PKCs in combination with modelling to identify the likely PKC targets.

To do so, we consider regimes where the Rho–Abr subsystem is bistable, to account for coexistence of zones and background states. We then examined bifurcations with respect to $b_R$, $b_C$, $d_R$, $d_C$, $\gamma_{AR}$ and $\gamma_C$, all of which are potential PKCβ/ PKCγ targets, paying particular attention to the qualitative and semi-quantitative changes in both background (low steady state) and zone intensities (high steady state) as these parameters are varied. Importantly, we used the following observations:

(O1) For PKCβ OE, both Rho and Cdc42 zones are significantly brighter while background activity is the same as in the control; similarly, for PKCβ DN, both Rho and Cdc42 zones are suppressed, with unchanged background activity.

(O2) For PKCγ DN, both Rho and Cdc42 zone intensities are the same as control, whereas both their background activities are higher.

(O3) For PKCγ OE, 80% of cells show a loss of the Rho zone, but the Cdc42 zone intensity and localization remain similar to control.

(O4) For PKCγ OE, 20% of the cells retain both zones, but the Rho zone is outside of the Cdc42 zone. This latter puzzling behaviour is termed ‘zone inversion’.

Details of the analysis is concentrated in §3.3. We examined the following hypotheses:

(H1) PKCβ enhances basal GEF activity ($b_R$, $b_C$).

(H2) PKCβ enhances feedback strength ($\gamma_{AR}$ and $\gamma_C$).

(H3) PKCγ either increases GTPase inactivation ($d_R$, $d_C$) or suppresses GTPase basal activation ($b_R$, $b_C$).

(H4) PKCγ influences feedback strengths ($\gamma_{AR}$, $\gamma_C$).

Several conclusions are summarized below. Upon analysing the dependence of both zone brightness and background GTPase activation levels with respect to parameters, we

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**Table 1. Synopsis of the basic ‘control’ (WT) parameter values used in simulations for our model.** All parameters are based on the original model by Simon et al. [23], with the exception of parameters influenced by PKCγ, $d_{RC}$ and $d_{RB}$. Owing to lack of relevant data, parameters associated with the influence of GDI on Rho ($d_{gr}$) and Cdc42 ($d_{gc}$) (introduced for illustrative purposes only in equations (2.1d)) are folded into $d_R$ and $d_C$, respectively. See text for more details.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Meaning</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>$b_R$</td>
<td>basal Rho activation rate</td>
<td>0.009</td>
<td>s$^{-1}$</td>
</tr>
<tr>
<td>$\gamma_{AR}$</td>
<td>Abr-induced Rho feedback strength</td>
<td>0.023</td>
<td>s$^{-1}$</td>
</tr>
<tr>
<td>$K_R$</td>
<td>Abr level for (1/2)-max feedback to Rho</td>
<td>0.0082</td>
<td>$\mu$mol</td>
</tr>
<tr>
<td>$R$</td>
<td>amount of inactive Rho</td>
<td>3.1</td>
<td>$\mu$mol</td>
</tr>
<tr>
<td>$d_R$</td>
<td>Rho basal inactivation rate</td>
<td>1.1</td>
<td>s$^{-1}$</td>
</tr>
<tr>
<td>$d_{AR}$</td>
<td>PKCγ-induced Rho inactivation rate</td>
<td>1</td>
<td>s$^{-1}$ $\mu$mol$^{-1}$</td>
</tr>
<tr>
<td>$b_C$</td>
<td>basal Cdc42 activation rate</td>
<td>0.0015</td>
<td>s$^{-1}$</td>
</tr>
<tr>
<td>$\gamma_C$</td>
<td>Cdc42 positive feedback strength</td>
<td>2</td>
<td>s$^{-1}$ $\mu$mol$^{-1}$</td>
</tr>
<tr>
<td>$\gamma_C$</td>
<td>Cdc42 positive feedback strength</td>
<td>0.031</td>
<td>s$^{-1}$</td>
</tr>
<tr>
<td>$K_C$</td>
<td>Cdc42 level for (1/2)-max feedback</td>
<td>0.055</td>
<td>$\mu$mol</td>
</tr>
<tr>
<td>$C$</td>
<td>amount of inactive Cdc42</td>
<td>2.4</td>
<td>$\mu$mol</td>
</tr>
<tr>
<td>$d_C$</td>
<td>Cdc42 basal inactivation rate</td>
<td>0.47</td>
<td>s$^{-1}$</td>
</tr>
<tr>
<td>$d_{AC}$</td>
<td>PKCγ-induced Cdc42 inactivation rate</td>
<td>1</td>
<td>s$^{-1}$ $\mu$mol$^{-1}$</td>
</tr>
<tr>
<td>$d_{BC}$</td>
<td>Abr-GAP mediated Cdc42 inactivation rate</td>
<td>127.4</td>
<td>s$^{-1}$ $\mu$mol$^{-1}$</td>
</tr>
<tr>
<td>$\gamma_{AB}$</td>
<td>feedback from Rho to Abr activation rate</td>
<td>0.1</td>
<td>s$^{-1}$</td>
</tr>
<tr>
<td>$d_A$</td>
<td>Abr inactivation/decay rate</td>
<td>0.67</td>
<td>s$^{-1}$</td>
</tr>
<tr>
<td>$D$</td>
<td>protein diffusion coefficient along membrane</td>
<td>0.1</td>
<td>$\mu$m s$^{-2}$</td>
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</table>
These values represent, respectively, the low-background Rho activity level, and the high activity level inside the Rho ‘zone’ adjacent to the wound. (An unstable steady state separates these two states.) Formulae (3.1) produce bifurcation diagrams for Rho activity (R) with respect to parameters. In figure 3a,b, we plot steady-state R against basal (bR) and feedback (γAR) activations. (Comparison with the numerical continuation of the original well-mixed model, figure 3c,d, verifies that the ‘sharp switch’ approximation is reasonable.) We can also identify parametric criteria for bistability, namely

\[
b_1 < b_R < b_2 \quad \text{where} \quad b_1 = b_2 - \gamma_{AR}, \quad b_2 = \frac{d_A d_R K_R}{\gamma_{RA}} \frac{R_A}{R_i}
\]

\[
d_1 < d_R < d_2 \quad \text{where} \quad d_1 = \frac{b_R K_{RA} R_i}{d_A K_R}, \quad d_2 = \frac{(b_R + \gamma_{AR}) \gamma_{RA} R_i}{d_A K_R}, \quad \gamma_1 < \gamma_{AR} < \gamma_2 \quad \text{where} \quad \gamma_1 = \frac{d_A d_R K_R}{\gamma_{RA}} - b_R, \quad \gamma_2 = \infty.
\]

(3.2)

Figure 3a,b indicates that zone brightness (upper branches) increases with both bR and \(\gamma_{AR}\). By contrast, background levels (lower branches) are sensitive to the basal activation rate bR, but not to the feedback strength \(\gamma_{AR}\). (These conclusions are confirmed by the numerical continuation of the full well-mixed model shown in figure 3c,f.)

Based on Observation (O1), we take this result to mean that the effect of PKCβ OE is consistent with an increase in the feedback parameter \(\gamma_{AR}\), rather than the basal activation rate bR. Similar analysis of the Cdc42 subsystem (omitted for brevity) combined with Observation (O1) for Cdc42 leads to similar results. We conclude that PKCβ acts on both Rho and Cdc42 feedback parameters—increasing those feedback strengths \(\gamma_{AR}, \gamma_{C}\) for PKCβ OE and damping it for PKCγ DN. We do not rule out other pathways, nor can we identify molecular details for the feedback mechanism in the case of Cdc42.

The full model PDEs were simulated numerically for the parameter set in table 1. Profiles of Rho (green), Cdc42 (red) and Abr (black) are shown in figure 3e for the control and figure 3f for PKCβ OE. These spatial profiles are in good agreement with observed experimental data.

3.4. Zone inversion reveals a potential role for PKCγ
In opposition to PKCβ, PKCγ either
(I) promotes inactivation or
(II) suppresses the activation of Rho and/or Cdc42.
We explored the possibilities that it acts by depressing feedback parameters $\gamma_{AR}, \gamma_c$, or by increasing basal inactivation represented by $d_R$ and $d_c$. From previous analysis (figure 3b, d), we found that varying $\gamma_{AR}$ leads to significant changes in zone brightness but no change in background intensity, which is inconsistent with Observations (O2)–(O3). Hence we explored the second possibility, that PKC$_{\eta}$ affects both inactivation rates $d_R$ and $d_c$ in equation (2.1d).

As before, we used the sharp switch model approximation (figure 4a) and numerical confirmation (figure 4b) to assess the influence of inactivation rates on Rho steady states. The model predicts that as inactivation strength decreases (e.g. from the hypothesized effect of PKC$_{\eta}$ DN), both zone intensity and background intensity increase. Increased background intensity is consistent with observation (O2). While zone intensity appears to be highly sensitive to the inactivation rate near $d_R = 0$, in practice, we expect that basal components of the inactivation rate in equation (2.1d) bound the relevant parameter range away from zero. It is reasonable to assume that the relevant range of net inactivation rate resides beyond the grey band in figure 4a. Thus while our analysis points to an increase in zone intensity for PKC$_{\eta}$ DN, the change would be fairly small and would be similar in magnitude to the increase in background. Hence our model predictions are consistent with the hypothesis that PKC$_{\eta}$ negatively regulates GTPases by promoting their inactivation. (Similar conclusions would be found in a model where PKC$_{\eta}$ suppresses basal activation levels, omitted here.)

Up to this point, we have considered PKC$_{\eta}$ to be spatially homogeneous. Observations (O3)–(O4) however require us to revise this and take a more sophisticated view of how its spatial distribution influences zone formation. Imaging indicates that PKC$_{\eta}$ is spatially graded with the highest concentration near the wound edge [24]. To determine how this spatial distribution might influence zone formation, we analysed the joint dependence of steady states for Rho (green) and Cdc42 (red) on the parameter $\eta$ (figure 4c). Here $\eta$ represents the level of PKC$_{\eta}$ and $d_{\eta}$, its proportional contribution to inactivation of the two GTPases as per equation (2.1d). We set $G = 0$, which is equivalent to folding the GDI-mediated inactivation terms into the basal terms ($d_R$ and $d_c$, respectively).

With $\eta$ as the bifurcation parameter (figure 4c), we found three regimes (right to left): (1) at high $\eta$, the Rho/Abr and Cdc42 subsystems have a single, low, background steady-state level and neither Rho nor Cdc42 zone forms, (2) at intermediate $\eta$ only the Cdc42 zone can form (only Cdc42 is bistable) and (3) at lower $\eta$, both Cdc42 and Rho zones are possible. In such a case, we would speculate that the control wound pattern is entirely contained within regime (3), permitting the formation of both zones. While a graded distribution is not required to account for zone formation in control cells, it is needed to explain why in PKC$_{\eta}$ OE, the remaining Cdc42 zone is no closer to the wound than in control cells (O3). If PKC$_{\eta}$ were homogeneous, and the OE manipulation effectively pushed the region near the wound into regime (2), the Cdc42 zone would be expected to form at the wound edge, inconsistent with observations. We thus hypothesize that the graded distribution stratifies the spatial domain so that near the wound edge, the system is in regime (1) and at intermediate distances it is in regime (2) (Grey bar under figure 4c.) In this scenario, no zone could form adjacent to the wound, but rather, a Cdc42 zone could form at intermediate distances (as it does in control cells), consistent with (O3).

While a graded PKC$_{\eta}$ distribution can account for Cdc42 zone localization in PKC$_{\eta}$ OE cells, its effect on inactivation rates alone does not explain zone inversion. In particular, it does not explain why the Rho zone is significantly further from the wound edge. The formation of this zone does imply that such distant regions of the wound repair field are in the Rho-permissive regime (3). What though drives the formation of this distant Rho zone in PKC$_{\eta}$ OE cells, why does it only occur in 20% of cases, and why does it not appear in control cells?

The answer emerges from closer inspection of fluorescence images. In PKC$_{\eta}$ OE, we find a broad halo of elevated Rho activity outside the Cdc42 zone, even in the 80% of cells with no inversion [9]. This observation supports the idea that PKC$_{\eta}$ OE not only affects GTase inactivation rates, but also broadens the ‘initial conditions’ that set up Rho activity further from the wound. It then also follows that whether the zones invert (20% of cases) or not (remaining 80% of cells) simply depends on whether elevated Rho activity breaches thresholds required to initiate a Rho activity zone. In other words, while all PKC$_{\eta}$ OE cells have broad Rho-permissive regions, some cells stochastically form this distant Rho zone, while others do not.

To test the hypothesis that the spatially graded distribution of PKC$_{\eta}$ along with this broader halo of initial Rho activity is responsible for Observations (O3) and (O4), we numerically simulated the spatial PDEs with a graded PKC$_{\eta}$ distribution (grey curve, figure 4d) that stratifies the spatial domain across regimes (1)–(3). If the initial Rho distribution is broader than in controls, a zone inversion occurs, consistent with (O3). When the initial Rho distribution is narrower (as in control simulations, e.g. figure 3e), only the Cdc42 zone forms (results not shown) consistent with (O3). Combined, these results point to a scenario where PKC$_{\eta}$ sculpts the spatial profile of the Rho and Cdc42 zones, and these distant Rho zones result from both PKC$_{\eta}$ elevation and the secondary effects on initial Rho localization that coincide with OE.

3.5. Creating a ‘playing field’: possible role of guanosine nucleotide dissociation inhibitors

It is natural to ask what prevents the activity zones of GTases from spreading laterally outwards, away from the wound, along the surface of the cell. Restated, we ask what defines the playing field within which the pattern formation takes place. As previously noted, reserves of inactive Rho and Cdc42 are not globally depleted when the wound-induced zones form. Indeed, multiple wounds made within just a few micrometres from each other elicit the same rapid formation of concentric Rho and Cdc42 zones [23]. Thus, depletion cannot be responsible for spatially limiting the extent of these zones as proposed in cell polarization involving the same GTases [26–29]. What then is responsible for spatially limiting these zones of activity?

The idea we explore is that local conditions affect the availability of inactive GTase in the periphery, for example, by rapid sequestration. GDIs are proteins that sequester GTases and limit their activation by GEFs. The activity of GDIs is, in turn, modulated by at least two types of mechanisms: phosphorylation by various kinases including protein kinases C, and by the so-called GDI displacement factors (GDFs). Phosphorylation can reduce the affinity of GDIs for Rho, Rac and Cdc42, depending on the kinase in question and the site(s) of
phosphorylation [30,31]. GDFs, which include PIP2 and members of the ERM (ezrin, radixin and moesin) family, have been reported to promote the release of GTPases by GDI in the presence of GEFs (e.g. [32,33]). Given that both PKCs and PIP2 concentrate around wounds, overlapping the regions occupied by the GTPase zones [24] (figure 5) it is reasonable to postulate that playing field establishment might reflect local antagonism of GDI. That is, the net effect of having relatively high local PKC activity and local accumulation of PIP2 or other GDFs could locally depress sequestration of GTPases by GDI and thus increase levels of available inactive GTPases near the wound.

As an initial test of these ideas, we simply asked how the availability of inactive GTPases, represented by the parameters $R_i$, $C_i$ in the model, influence zone formation. Restated, we asked over what range of values of these parameters is bistability (the hallmark of coexistence of zones and background levels) possible. In figure 5, we show model bifurcation plots with respect to $R_i$ in both ‘sharp switch’ and continuous model variants (figure 5a,b, respectively). We find bistability only at intermediate values of $R_i$ (and similarly for $C_i$, not shown).

We can interpret these results in terms of the GDI playing field hypothesis. For uninjured cells, the sequestering activity of GDI is spatially uniform and relatively strong, sequestering Cdc42 and Rho to such an extent that insufficient GTPase is available to initiate zone formation. Thus, while the GTPases are present in sufficient quantity throughout the cell, GDI sequestration reduces the available GTPase levels $R_i$, $C_i$ (leftmost regime, figure 5a,b) and prevents zone formation. Upon wounding, the recruitment of PKCs and PIP2 results in local suppression of GDI’s sequestering activity and commensurate increases in $R_i$, $C_i$, making the wound region permissive to zone formation (middle regime, figure 5a,b). Since PKCs and GDFs are membrane-bound and concentrated near the wound, they do not alter the normal low levels of $R_i$ and $C_i$ in other regions of the cell, preventing the spread of the zones further afield. Hence, localized suppression of GDI activity in the model is consistent with higher levels of $R_i$, $C_i$ near the wound, and with the idea that GDI helps to define a local playing field in which the patterns could form.

More detailed models and future experiments could be adapted to explore the link between GDI and the playing field hypothesis. Here we speculate that local recruitment of factors such as GDFs and PKCβ to the membrane near the wound (figure 5c), together with characteristic unbinding rates $\alpha$, and diffusion rates $D$ generate local gradient(s) of permissive factors that decay away from the wound edge with an effective length scale of $\sqrt{D/\alpha}$. This could define the spatial extent of the playing field. The 10 μm size of the region of zone formation is consistent with common diffusion length scales. Hence, it seems reasonable to suggest that wounding leads to the local release of sequestered GTPases that can then be activated and that participate in the healing process.

4. Discussion

In this review, we demonstrated how an interdisciplinary approach, using both biological experiments and mathematical modelling, improved our understanding of the mechanisms at play in single-cell wound patterning by Rho GTPases. Much like the canonical polarity response preceding chemotaxis [34,35], cell wound repair requires proper localization of small Rho GTPases (Cdc42 and Rho in this case) to direct cytoskeleton assembly and contraction. The approximate analytical and numerical methods described here, in combination with biological experiments [18,22,24,36] have been essential to understanding the molecular mechanisms regulating the repair response.

Based on results here and in previous investigations [9,23], we posit that wounding induces a multi-layered response where (1) local modulation of GDI activity generates a region near the wound (the so-called playing field) permissive to GTPase activation, (2) within that region, interactions between Rho, Cdc42 and Abr generate spatially patterned regions of activity and (3) PKCs modulate and fine-tune that activity. The first aspect of this hypothesis is based on the observations that (I) once initiated, GTPase zones do not spread away from the wound, (II) GDI modulation would provide a mechanism for controlling the system’s capacity for bistability (and hence zone formation), and (III) multiple factors (PKCβ, ezrin, PIP2)
which are known to weaken GDI’s suppressive effects (at least in other systems) are recruited to the cell membrane near the wound.

The interactions between Rho, Cdc42 and Abr were fleshed out by comparing a collection of models, each encoding different hypothesized interactions, to observations from experiments manipulating Abr and Rho activities [23]. In particular, this modelling predicted that positive feedback of Cdc42 onto its own activation is essential to the process. Finally, our modelling [9] in combination with experimental manipulations [24] reveal specific roles of two GTPase effectors, PKCβ and PKCγ. Based on semi-quantitative observations of how background and zone intensities change when these effectors are overexpressed (OE) or depressed (DN), we were able to rule out some plausible mechanisms. In the present case, we showed that PKCβ is unlikely to act on basal rates of activation, and more likely acts by enhancing both the positive feedback from Rho to itself via Abr and the auto-catalytic Cdc42 feedback. Furthermore, we were able to show that, as a result of being spatially graded, PKCγ differentially affects Rho and Cdc42 at different distances from the wound, and that this is critical to account for the inversion of the Cdc42 and Rho zones observed in some PKC γ OE treatments.

The single-cell wound response is a dynamic process occurring over the course of minutes, with zones interacting and moving due to wound closure. Even so, the analysis of steady states herein provide insights into what drives the initial patterning. Using our one-dimensional spatial representation, we have demonstrated that it led us to reject some plausible hypotheses for the molecular mechanisms governing that patterning. From a mathematical perspective, the sharp switch approximation adopted here facilitated a more thorough exploration of parameter space, generating predictions that were confirmed with full numerical simulations. Future refinements of this modelling will be aimed at more fully understanding the role of GDIIs and their phosphorylation, and identification of other effectors that modulate the GTPases during the repair process. At the same time, having mapped out regimes of behaviour, our model is ready for more sophisticated simulation methods in two and three dimensions that could describe the spherical cell geometry in a more accurate spatial representation.

References


