Model supports mechanics-driven regulation of the Rho GTPase signaling pathway for collective cell polarization

Introduction.
Cohesive movement of cells crucial in wound healing, and positioning and shaping of tissue and organ development. To initiate movement, cells need to break their internal symmetry and establish a front and a rear. How this is achieved in individual cells has been well-studied: (1) chemical segregation of Rho GTPases (Rac/Cdc42 at the front of the cell, while Rho at the rear of the cell) in budding yeast, etc. and (2) cytoskeletal-driven (branched/Arp2/3 lamellipodia at the front of the cell, while bundled actomyosin network at the rear of the cell) in keratocytes. This process can happen spontaneously or in the presence of an external stimulus. In most cells (e.g. neutrophils) both chemical segregation and cytoskeletal re-arrangement are believed to be necessary for polarity and re-polarization in new direction of an external stimulus.

In groups of cells, the question of how collective polarity is established is less well-understood. Pivotal discoveries in epithelial layers and neural crest cell migration have shaped our current understanding of collective polarity maintenance (cite). But the effects of both Rho and Rac signaling are seemingly contradictory across the literature, promoting both cell-cell junction strengthening and breakdown. Furthermore, impressive examples such as one cell takes a while to polarize, but two cells not only decide to move in the same direction but do so quicker (find citations, maybe in Ciona? Anecdotal galvanotaxis from Alex). **In the case of spontaneous co-polarization, cells must collectively agree on the same polarity axis, thus for example not forming protrusive fronts towards each other.** But how are these choices mitigated and negotiate across the cell-cell junction? This construct presents a further constraint -- Rac/Rho GTPases (and cytoskeletal networks) need to spatially segregate within a cell while considering its neighbors own internal arrangement.

Here, we leverage theoretical setting to investigate how Rac/Rho chemical segregation and cytoskeletal dynamics could be negotiated across the cell-cell junction to improve/ensure a co-polarity axis. We choose a model with four considerations: (1) can robustly polarize both spontaneously and in a stimulus-dependent manner in individual cells, (2) grounded in established literature of polarity, (3) incorporates both cytoskeletal dynamics and Rho GTPases signaling, and (4) minimal in assumptions. Within this framework we test all, known to us, existing hypotheses regarding the effects of both Rho GTPase signaling. We find that for spontaneous co-polarization, mechanical interaction is needed in two possible mechanisms: (1) cytoskeletal dynamics (i.e., pulling/pushing forces at the cell-cell junction) or (2) differential Rac and/or Rho signaling. In the presence of an external stimulus available to one cell, two other possible mechanisms emerge – Rac/Rho antagonism up-regulated at the cell-cell junction, Rac up-regulation or Rho down-regulation at the cell-cell junction.

Taken together, our theoretical results posit that mechanical forces transmitted at the cell-cell junction may regulate either cytoskeletal dynamics or small GTPases differentially as downstream effectors. For example, contractile forces in one cell may be transmitted via cadherins or adherent junctions to neighboring cells to stimulate Rho or Rac activities.

Figure 1. Schematic + single cell results

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Model.

Rac/Rho mutual inhibition: Mackay, J. L. & Kumar, S. Simultaneous and independent tuning of RhoA and Rac1 activity with orthogonally inducible promoters. Integr. Biol. (U. Kingd.) 6, 885–894 (2014).

Arp2/3 lamellipodia give rise to stress-activated RhoA recruitment: Yamada, S. & Nelson, W. J. Localized zones of Rho and Rac activities drive initiation and expansion of epithelial cell-cell adhesion. J. Cell Biol. 178, 517–527 (2007).

Results.

Large number of possibilities: For example, if we only consider parameters for binding/unbinding of Rac/Rho and up-regulation of branched/bundled network formation that is 6 parameters with 3 choices (default, up, high up): 3^6 = 729 and then across two cells (729C2) = 265,356. Does not include more complicated schemes like presence of bundled causes Rac up-regulation in a concentration-dependent manner.

Figure 2. Large param sweep with zoomed in regions-of-interest and model schematics?

1) We scan literature and identify possible mechanisms as well as a handful of other speculated mechanisms. Exploratory search of about 10% of the parameter space.
In the absence of an external stimulus (spontaneous polarization), we find ~25% chance of agreeing on the same polarity axis. Sometimes worse. This is improved in only 2 pathways:

- **Rac/Rho differentially regulated across the cell boundary.**
  (either Rac up /Rho up, Rac down/Rho down, Rac up/Rac down, or Rho up/Rho down)
  - Independent
  - Can this be RELATED TO BRANCHED/BUNDLED CONCENTRATIONS?
- **Branched/Bundled differentially regulated across the cell boundary.**
  - Independent
  - Dependent on pushing/pulling forces from the other cell.

2) What if now we expand our definition of co-polarity direction based on mechanical models and include leader-to-follower arrangement? All previous pathways plus two new ones:

- **Differential Rac up-regulation across the cells**
- **Bundled -> Rac & branched -> Rho**

Figure 3. Same as Fig.2 but explain leader-to-follower arrangement and show the interaction schematics (param sweeps in SI).

3) What happens in the presence of an external signal perceived by one cell?

- **Same as above**, and
- **Increased Rac/Rho antagonism**
- **Differential Rho down-regulation across the cells**

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Figure 4.

4) Lastly, is there something special about 2 vs 3,4, or 5 cells? ~~Alternative ending, dynamic cadherin formation? But which rules to include for cadherin formation? I don't like this option.~~ Timing? Does the time to polarization decrease with more than 1 cell?

Figure 5. Summary figure

Discussion.

Predictions:

Local protrusive activity away from the cell-cell site should slow down/arrest co-polarization.

Optogenetic constructs for mechanical stimulation