**Introduction**.

Cohesive movement of cells is crucial in wound healing, and positioning and shaping of tissue and organ development.

Polarization of cells has been demonstrated as a critical event in evolutionary biology. At the onset of collective migration, stationary cells must undergo a drastic intracellular reorganization to acquire the required migratory phenotype with a front-to-back polarity due to an asymmetric distribution of proteins, lipids, and other molecules (cite). For example, in Ciona, Rho GTPases… (cite). In the epithelium monolayer, cells at the wound edge receive a distinct cue from the cell-free space, and their polarity is mostly parallel to the direction of migration, but cells deep within the monolayer are surrounded by neighbors on all sides, which makes the polarity establishment a non-trivial problem (cite).

Discoveries in epithelial layer (Rao and Zaidel-Bar, 2016), neural crest cell migration in chick embryo (Shellard/Szabo and Mayor, 2016), and border cell migration in the fly egg chamber (cite) have shaped our current understanding of collective polarity maintenance. But the effects of both Rho and Rac signaling are seemingly contradictory across the literature, promoting both cell-cell junction strengthening and breakdown (Vivek

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.

To study we use the construct of (1) Rho GTPase signaling and (2) cytoskeletal rearrangement because we want to do an exhaustive search of the implicated mechanisms for co-polarization.

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: primarily through (1) localization of proteins and lipids at the cell membrane (PAR system, Wnt system, Scribble complex, and Rho GTPases system), or, in some cell types such as fast-moving fish epithelial keratocytes, (2) mechanical forces and actin flows can drive the rearrangement of the actin cytoskeleton. This process can happen spontaneously or in the presence of an external stimulus. Although cell polarity can emerge from systems that are either biochemical or mechanical, in many cases cell polarity depends on the interplay between the two. One such example, are neutrophils where both chemical segregation and cytoskeletal re-arrangement are believed to be necessary for polarity and re-polarization in new direction of an external stimulus.

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 way in individual cells, (2) grounded in established literature of cell 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 GTPases. We find that for spontaneous co-polarization, mechanical interaction is needed through two possible pathways: (1) force-driven cytoskeletal rearrangement (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 of the cells, 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.

A. Schematic representation of one cell with polarity markers. The abundance of the polarity protein marker at the two sites shown with Rho and Rac.

B. Schematic representation of the molecular interactions of the polarity model.

C. Results for single cell with spontaneous polarization and re-polarization (show signal in B)

D. Possible outcomes for two cells.

E. Schematic representation of the molecular interactions of the 2-cell polarity model.

**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).

**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.

A. Choose an interaction model

B. Perform simulations (x100)

C/D. Identify successful outcomes (formula?)

E. Possible dynamics (labels of co-polarized, etc.)

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**

*Williams, Donoughe, Munro, Horne-Badovinac. eLife 2022 Fat2 locally (sub-micron scale puncta) enrich the WAVE complex in corresponding puncta just across the leding-trailing cell-cell interface creating stable regions of protrusive activity in each cell. Cadherin Fat2 localizes to the trailing edge of each cell and promotes the formation of F-actin-rich protrusions at the leading edge of the cell behind.*

* **Bundled -> Rac & branched -> Rho**

*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).*

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**

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?

Transition (with elevated Rac unbinding rates between 10-100) from L/F and parallel co-polarity axes to distribution of effort? (CIL/COA

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.