Review

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Exploratory cell dynamics: a sense of touch for cells?

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Abstract: Cells need to process multifaceted external cues to steer their dynamic behavior. To efficiently perform this task, cells implement several exploratory mechanisms to actively sample their environment. In particular, cells can use exploratory actin-based cell protrusions and contractions to engage and squeeze the environment and to actively probe its chemical and mechanical properties. Multiple excitable signal networks were identified that can generate local activity pulses to control these exploratory processes. Such excitable signal networks offer particularly efficient mechanisms to process chemical or mechanical signals to steer dynamic cell behavior, such as directional migration, tissue morphogenesis and cell fate decisions.

Keywords: Cdc42; protrusion-retraction cycles; Rac1; Ras; RhoA; subcellular contraction pulses.

Introduction

Dynamic changes in cell morphology are central to many biological processes. For example, during embryonic development, dynamic rearrangements between cells shape the layer structure of tissues and organs. In adults, coordinated reorganization of the cellular morphology drives directional cell migration during the immune response or wound repair. On the cellular level, dynamic shape changes result from the concerted action of multiple cytoskeleton-associated processes that control cell protrusion, cell contraction and cell adhesion. However, how these processes are coordinated in space and time is still poorly understood.

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Exploratory cell dynamics

Migrating cells are often described as highly polarized with a clearly defined protruding front and retracting back. However, *in vivo* and *in vitro*, various distinct modes of migration exist that vary in the spatial and temporal dynamics of protrusion and retraction (Petrie and Yamada, 2016; Te Boekhorst et al., 2016). For example, amoeboid *Dictyostelium* cells can generate transient protrusions and retractions towards all directions (Bosgraaf and Van Haastert, 2009a). In contrast, keratocyte-like migration is characterized by a stable protruding front and a stable retracting back (Figure 1) (Cooper and Schliwa, 1986). Several cell types, including fibroblasts, migrate with an intermediate degree of protrusion and retraction dynamics. These cells still generate a polarized front and back, however, the front region is characterized by transient periods of local protrusions and retractions (Abercrombie et al., 1970; Bear et al., 2002; Giannone et al., 2004; Krause and Gautreau, 2014). In central and back regions, contractile forces predominate that can pull the cell center forward, and detach the back (Lauffenburger and Horwitz, 1996) (Figure 1).

In the cell front, protrusion retraction cycles are manifested from opposing dynamic forces. Actin polymerization at the leading edge of the cell generates a force that can push the cell forward, while contractile actomyosin structures at the base of protrusions oppose this pushing force. Depending on the relative contribution and the geometric arrangement of these forces, cells either continuously protrude or retract within a subcellular region, or generate cycles of protrusions and retraction (Lim et al., 2010). Depending on environmental conditions and cellular state, cycles between protrusive and contractile forces can vary widely in their period and spatial organization, and can manifest as clearly discernable events, for example, during *Dictyostelium* chemotaxis (Andrew and Insall, 2007) and growth cone

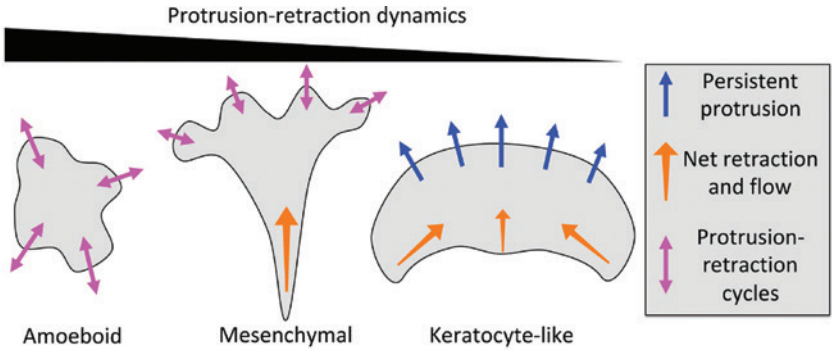

Figure 1: Spatio-temporal organization of cell protrusion and retraction.
Schematic representations of distinct cell migration modes that are observed on two-dimensional (2D) glass surfaces, and that differ in their extent of local persistence of cell protrusion and cell retraction phases. Migration modes in three dimensions and their comparison to 2D migration were recently reviewed elsewhere (Petrie and Yamada, 2016; Te Boekhorst et al., 2016).

advance (Goldberg and Burmeister, 1986; Dent et al., 2011), or as a rapid fluctuations during membrane ruffling (Giannone et al., 2004; Krause and Gautreau, 2014).

Such protrusion-retraction cycles were suggested to play an exploratory role during directional cell migration. Early work by Günther Gerisch and colleagues suggested that protruding pseudopodia in *Dictyostelium* serve as sensors to probe the cellular environment during chemotaxis (Gerisch et al., 1974). In the original hypothesis, it was proposed that cells generate pseudopodia towards random directions in the absence of chemoattractant, and that directional cell migration results from selective reinforcement of pseudopods that extend in the direction of increasing chemoattractant concentrations (Figure 2A).

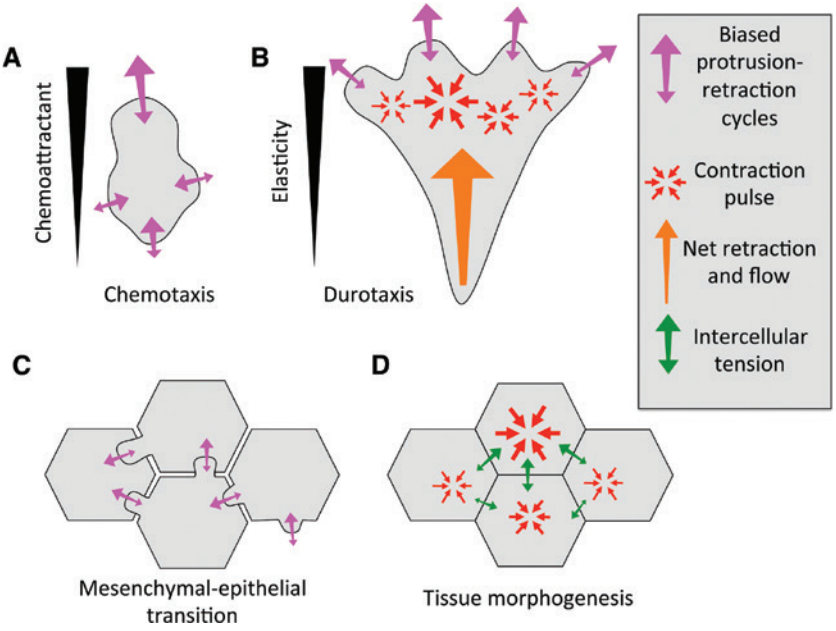

Figure 2: Exploratory cell shape changes.
Illustrations for the role of exploratory cell shape changes at single (A, B) and multicellular (C, D) levels. (A) Cells migrating via the amoeboid mode generate transient protrusions towards all directions. However, protrusions are biased over retraction in cell regions that encounter increased concentrations of chemoattractant. (B) Cells migrating via the mesenchymal mode generate protrusion-retraction cycles at the front and predominantly retract at the back. Local contraction pulses in central cell regions could play an additional role in the sensing of elastic properties and in generating a contractile bias across the cell axis. (C) During the formation of epithelial monolayers, protrusion-retraction cycles facilitate the formation of cell-cell contacts. (D) During tissue morphogenesis, transient contraction pulses play an important role in the reorganization of tissue geometry. Coordinated rearrangements of junctions between neighboring cells suggest intercellular communication via chemical or mechanical signals.

More recent work refined this hypothesis in several ways: the original idea by Gerisch and colleagues was inspired by the finding that bacteria cannot measure local differences in chemoattractant concentration across their small dimensions. Instead, they use an adaptive sensory mechanism that measures temporal changes in attractant concentration during active bacterial movement towards or away from the gradient (Berg and Brown, 1972; Macnab and Koshland, 1972). However, in subsequent studies, it was found that the larger *Dictyostelium* cells mainly use spatial differences of chemoattractant between the front and rear to perform chemotaxis (Mato et al., 1975). Furthermore, in contrast to the original hypothesis by Gerisch et al., the direction of newly formed pseudopodia in the absence of chemoattractant is not as random as originally proposed. For example, after starvation, *Dictyostelium* cells generate new protrusions by splitting existing ones (Andrew and Insall, 2007). This leads to more persistent migration (Bosgraaf and Van Haastert, 2009b), thereby increasing their exploratory radius. Thus, in this state, *Dictyostelium* cells migrate via a more persistently polarized mode. Nevertheless, the location of splitting pseudopodia is biased by a chemoattractant gradient (Bosgraaf and Van Haastert, 2009a), thereby enabling an exploratory mechanism for chemotaxis. Similarly, during chemotaxis of primary human dendritic cells (DC) and mouse fibroblasts, alternating phases of small protrusions and retractions are biased towards increasing chemoattractant concentrations (Arrieumerlou and Meyer, 2005) and thereby can play an exploratory role in directed cell migration.

Another example for exploratory cell dynamics are intermittent pulling forces that are transmitted by focal adhesions onto the extracellular matrix (Plotnikov et al., 2012). Similar to the idea of cell protrusion-retraction cycles that explore extracellular cues such as chemoattractant gradients, the repeated pulling on the ECM that was called 'tugging', was interpreted as a mechanism that locally probes the stiffness of the cell matrix (Plotnikov et al., 2012; Plotnikov and Waterman, 2013). The force fluctuations at focal adhesions could have several origins. For example, actomyosin-based stress fibers could transmit periodic local changes in contraction force to associated focal adhesions. Alternatively, local periodic changes in actin mechanics or in the coupling between stress fibers and focal adhesions could lead to the observed dynamic tugging forces (Plotnikov and Waterman, 2013).

Interestingly, local pulsatile contractions were specifically observed in cells that express the Myosin-IIa motor (Baird et al., 2017). More recently, a mechanism was proposed that can generate subcellular pulses of local cell contraction (Graessl et al., 2017), which could thereby be a source for subcellular contraction force dynamics (Figure 2B). Interestingly, these local contraction pulses were modulated by substrate elasticity and are therefore suitable to play an exploratory role in sensing the mechanical properties of the extracellular matrix on subcellular scales (Graessl et al., 2017). Such a mechanism might also be suitable to polarize contraction forces that pull the cell center forward towards a stiffness gradient during mesenchymal cell migration (Figure 2B).

Exploratory cell dynamics not only seem to play a role in the behavior of single cells, but are also relevant for multicellular systems. First, the initial formation of cell-cell junctions is guided by exploratory cell protrusion-retraction cycles (McNeill et al., 1993; Ehrlich et al., 2002) similar to individual, migrating cells (Figure 2C). Furthermore, the dynamic reorganization of cell-cell contacts during development is controlled by pulses in contractile forces (Kasza and Zallen, 2011; Coravos et al., 2017), comparable to the repeated tugging observed in individual cells (Plotnikov et al., 2012). For example, periodic apical constriction of ventral furrow cells during *Drosophila* gastrulation is driven by pulsed contractions of the actomyosin network on the apical cell surface that pull the cell surface via linked adherence junction sites (Martin et al., 2009, 2010). Similarly, repeated rounds of constriction and relaxation of epithelial cells of the amnioserosa during dorsal closure were shown to coincide with and driven by periodic accumulation of actin and myosin-II in individual cells (Blanchard et al., 2010; David et al., 2010). In principle, these periodic pulses could be controlled in a cell autonomous fashion. However, during germ band extension in *Drosophila*, neighboring cells rearrange their junctions in a highly correlated manner via cell contraction pulses (Collinet et al., 2015), suggesting that such processes might be coordinated by intercellular communication (Figure 2D). This communication could be mediated by the sensing of chemical or mechanical signals, for example, via adhesion-associated proteins such as α-catenin (Yao et al., 2014). Together with their apparently stochastic nature, contraction pulses might play an exploratory role to locally probe the mechanical property of surrounding cells (Figure 2D).

Mechanisms that control exploratory cell dynamics: role of positive and negative feedback

The question now arises, how exploratory cell dynamics described in the previous chapter arise. Simple

linear signal pathways that act downstream of receptor activation could trigger changes in cytoskeletal dynamics and thereby induce cell shape changes. However, more complex cell dynamics, such as protrusion-retraction cycles and intracellular or multicellular contraction pulses require positive and/or negative feedback regulation (Tyson et al., 2003). Multiple signal systems associated with cell morphodynamics were proposed to be controlled by positive and negative feedback regulation, including Rho and Ras GTPases, phosphoinositides and cytoskeletal effectors.

Rho GTPases are well characterized as master regulators of cytoskeletal dynamics and thereby play an important role in controlling cell dynamics (Hodge and Ridley, 2016). Similar to other members of the Ras superfamily, Rho GTPases can switch between an active, GTP-bound and an inactive, GDP-bound conformation. Guanine nucleotide exchange factors (GEFs) facilitate the exchange of bound GDP to GTP and thereby switch Rho GTPases into the active conformation. GTPase activating proteins (GAPs) stimulate GTP hydrolysis to induce the inactive conformation. In addition, RhoGDIs can solubilize GTPases in the cytosol, thereby providing additional control of GTPase function. The three most prominent members of the Rho GTPase family: RhoA, Rac1 and Cdc42, were studied for decades, revealing their roles in stimulating cell protrusion (Rac1 and Cdc42) and cell contraction (RhoA), respectively (Ridley, 2015). Classically, their activation mechanisms are described as linear pathways, in which the local activity state of upstream regulators would define the spatio-temporal activity of the GTPase and their downstream effectors to drive dynamic cell shape changes. However, in this model, the activity of the upstream regulators needs to be organized in space and time to reflect these cell shape changes.

To resolve this problem, early models of the spatio-temporal regulation of Rho GTPases focused on a proposed antagonistic nature between protrusion and contraction signals. Most prominently, positive feedback of Rac1 and/or Cdc42 in protrusion signaling (Wittmann et al., 2003; Aoki et al., 2005; Frantz et al., 2007; Ming et al., 2007) as well as mutual inhibition between protrusive Rac1/Cdc42 and contractile RhoA signals (Sander et al., 1999; Ohta et al., 2006; Rosenfeldt et al., 2006; Sanz-Moreno et al., 2008) were proposed to establish polarization between front and back signals during cell migration (Guilluy et al., 2011). However, the mechanisms that mediate this crosstalk are still not fully understood.

In addition, other signaling components might also play an important role in the spatio-temporal regulation of protrusion and contraction signaling. For example, protrusions are also regulated by phosphoinositide-3-kinase (PI3K) signaling (reviewed in Cain and Ridley, 2009) and Ras-related GTPases, including R-Ras (Wozniak et al., 2005), K-Ras (Yip et al., 2007), H-Ras (Shin et al., 2005) and Rap1a (Arthur et al., 2004). Interestingly, these signaling systems were proposed to amplify each other via positive feedback (Sasaki et al., 2007; Fivaz et al., 2008; Huang et al., 2013). For example, PI3K is a well-established effector of Ras activity (Rodriguez-Viciana et al., 1994). In addition, inhibition of PI3K leads to a decrease in Ras activity, suggesting that PI3K can also activate Ras by an as yet unknown mechanism (Sasaki et al., 2007). Together, Ras and PI3K constitute a positive feedback loop in *Dictyostelium* cells in which their activities can amplify each other (Sasaki et al., 2007). In some systems, an additional negative feedback is coupled to the protrusion generating positive feedback. For example, in *Dictyostelium*, this negative feedback was proposed to act via PKBs (Miao et al., 2017) (Figure 3A), or via yet unidentified downstream regulators that associate with the actin cytoskeleton (van Haastert et al., 2017).

Similarly, the contraction generating RhoA activity is regulated via positive and negative feedback, however, the apparent mechanism of this regulation differs between studies. In *Drosophila* germband extension, advective myosin-generated flow and actomyosin concentration were suggested to mediate positive and negative feedback, respectively (Munjal et al., 2015) (Figure 3B). More recently, a distinct mechanism for negative feedback via C-GAP was proposed to operate during apical construction in *Drosophila* (Mason et al., 2016). In *Caenorhabditis elegans*, *Xenopus* and starfish oocytes, the GEF Ect2 was proposed to stimulate positive feedback, and negative feedback was either found to be dependent (Bement et al., 2015) or independent (Nishikawa et al., 2017) on filamentous actin (Figure 3B).

In contrast, in adherent mammalian cells, the localization of the GEF Ect2 anti-correlates with active Rho and thus does not appear to mediate positive feedback amplification of Rho activity (Graessl et al., 2017). Earlier biochemical studies showed that a distinct GEF family, the Lbc-type GEFs, selectively interact with active RhoA suggesting that this interaction can mediate positive feedback amplification of Rho activity (Medina et al., 2013). Indeed, the localization of the two Lbc-family GEFs, GEF-H1 and LARG, closely correlate with Rho activity in space and time, suggesting that Rho can recruit these activators to local sites of increased activity to drive positive feedback amplification (Graessl et al., 2017) (Figure 3B). The negative feedback can be mediated via multiple pathways in adherent cells, which include the inhibition of GEFs by

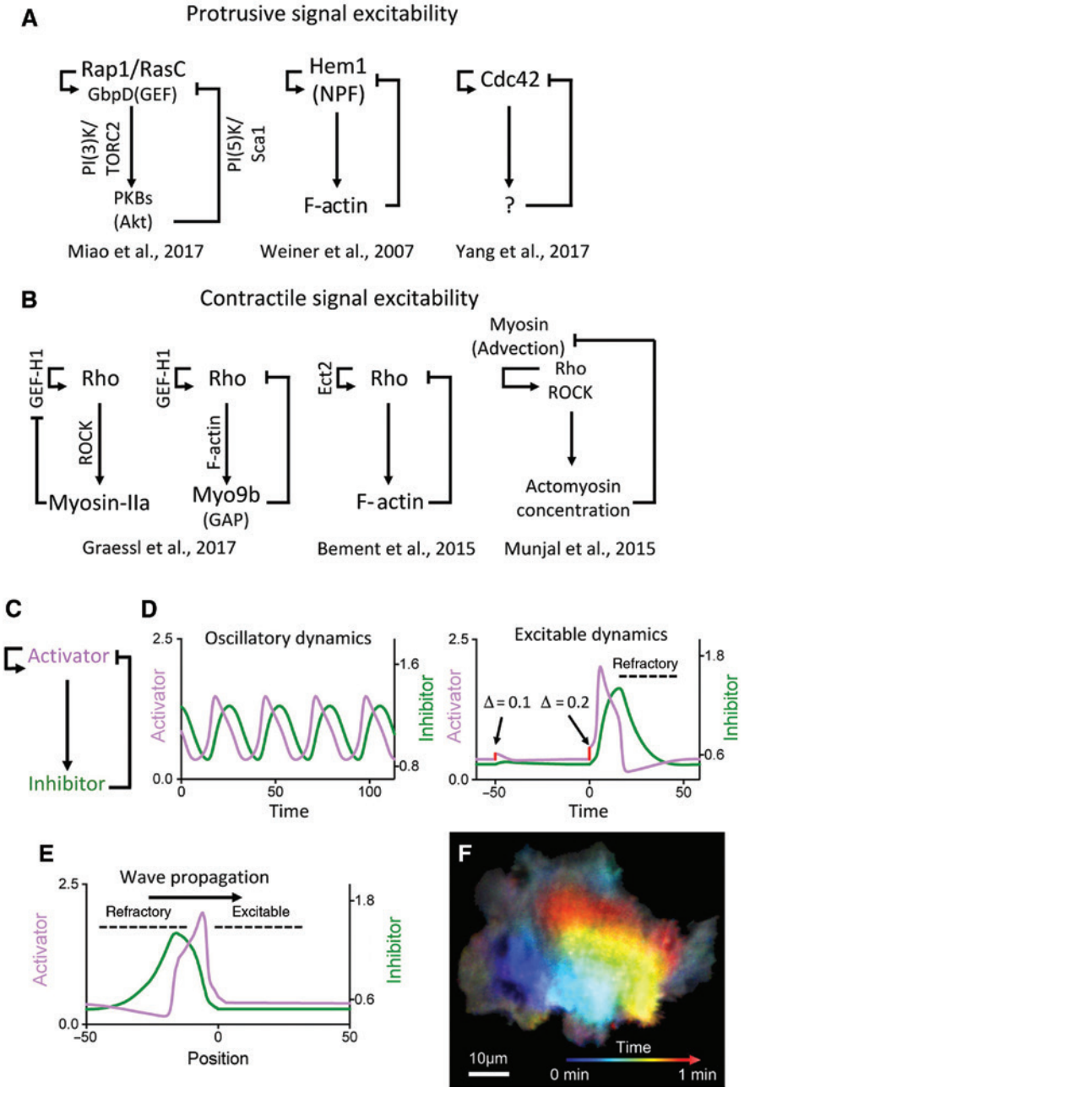

Figure 3: Proposed signal networks that generate cell protrusion or contraction excitability.
(A, B) Influence diagrams that summarize positive and negative feedback control and proposed feedback mediators of cell protrusion and cell contraction regulators. (A) Signal networks proposed to generate excitable cell protrusion dynamics by controlling the activity state of Ras superfamily GTPases, including Rap1, RasC, the Rho GTPase Cdc42 or nucleation promoting factors (NPFs) for actin polymerization. (B) Signal networks proposed to generate excitable cell contraction dynamics by controlling the active form of the GTPase Rho (i.e. one or more members of the closely related subfamily that includes RhoA, RhoB and RhoC). (C) Schematic representation of a generalized activator-inhibitor network. (D) Simulations of activator and inhibitor dynamics using a mathematical model for an activator-inhibitor signal network described by Tyson et al. (2003). Depending on the biochemical parameters (here: the concentration of the positive feedback mediator), the system can be in distinct dynamic states. In the excitable state (right), a sub-threshold perturbation of the activator ($\Delta = 0.1$) has little effect. In contrast, a super-threshold perturbation ($\Delta = 0.2$) triggers a response in the activator that is much larger than the perturbation itself. After the activator response, the system becomes transiently refractory to a second stimulus due to the increased inhibitor activity. (E) Schematic representation of directional wave propagation in space, based on activator-inhibitor excitability. Directionality is a consequence of the asymmetric segregation of excitable and refractory regions. (F) Rho activity wave propagation in a U2OS cell. Rho activity was measured via TIRF microscopy imaging of effector domain recruitment to the plasma membrane (Graessl et al., 2017). The temporal evolution of a single activity wave is represented by colors (propagation from blue to red). The simulations shown in panels (D) and (E) were performed using MATLAB.

myosin-II (Lee et al., 2010; Graessl et al., 2017) and the inhibition of Rho by the actin-associated GAP Myo9b (van den Boom et al., 2007; Graessl et al., 2017) (Figure 3B).

Coupled positive and negative feedback: excitability in cell protrusion and cell contraction signaling

Interestingly, the mechanisms described above are not based on direct crosstalk between Rho GTPases, but instead show that individual Rho or Ras-type GTPases themselves can be regulated by coupled positive and negative feedback loops. These networks (Figure 3A and B) all represent so-called activator-inhibitor networks, in which the 'activator' is amplified by positive feedback. The 'activator' is also inhibited by negative feedback, which is mediated by the 'inhibitor' (Figure 3C). Depending on the properties of the biochemical reactions, such systems are known to generate oscillatory or excitable system dynamics (Murray, 2002, 2003; Tyson et al., 2003) (Figure 3D) similar to action potential trains or spontaneous individual activity spikes in neurons. In the absence of stimulatory inputs, excitable systems are in a stable, resting steady state (Murray, 2002). However, a stimulation of the activator above a certain threshold can get rapidly amplified via the positive feedback, and subsequently become inactivated by a slower negative feedback (Figure 3D). After going though a refractory state, in which the increased inhibitor activity suppresses another amplification event, the system equilibrates again in the stable steady state. In space, such systems can generate an excitable medium that can propagate activity waves via coupling these reactions with diffusion, similar to the propagation of an action potential along an axon (Murray, 2003; Iglesias and Devreotes, 2012). Here, the refractory state generates a spatial asymmetry in excitability to ensure directionality of wave propagation (Figure 3E and F). In contrast to excitable systems, oscillatory systems do not require a trigger, but rather generate recurrent, time-shifted activity peaks or propagating waves of the activator and inhibitor spontaneously (Figure 3D) (Murray, 2003).

By linking such an activator-inhibitor system to cytoskeletal effectors, their oscillatory and excitable system dynamics could in principle lead to recurring, oscillatory or spontaneous *de novo* formation of protrusions or contractions. Based on theoretical analyses, an important condition for excitable behavior is a substantially faster positive feedback compared to the inhibitory negative feedback (Murray, 2002). The proposed association of negative feedback with relatively slow actin polymerization or actomyosin contraction kinetics could offer a mechanism for this separation of time scales (Radde, 2008).

Interestingly, manipulation of mediators of positive feedback can accentuate the typical dynamic behavior that is observed in excitable and oscillatory systems (Graessl et al., 2017; Miao et al., 2017), leading to robust pulses and activity wave propagation inside individual cells. Recently, Cdc42 activity was also shown to be excitable during chemotaxis in neutrophil-like PLB-985 cells, and this behavior was stimulated by actin depolymerization (Yang et al., 2016). However, the molecular mechanisms that implement the positive and negative feedback in the Cdc42 system are currently not known. Thus, multiple individual excitable signal network modules can generate local activity patterns of protrusion and contraction signals, such as local pulses or propagating waves (Bement et al., 2015; Yang et al., 2016; Graessl et al., 2017; Miao et al., 2017).

How these distinct excitable networks are interconnected is still poorly understood. One potential link might be PI3K (Figure 4). Indeed, PI3K activity itself can also generate pulses and propagating waves that are typical for excitable and oscillatory systems (Asano et al., 2008; Arai et al., 2010; Xiong et al., 2010, 2016; Wu et al., 2013), presumably due to its mutual activation with Ras

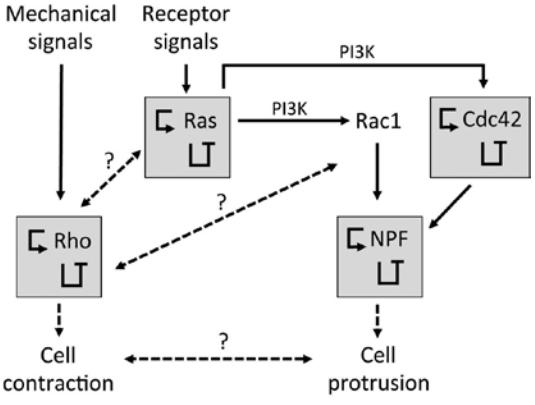

Figure 4: Potential interfaces between excitable signal modules. Influence diagram for proposed interactions between excitable signal network modules, environmental mechanical and growth factor receptor signals and associated cellular functions. Excitable signal network modules are summarized by positive and negative feedback control acting on the central regulators Ras, Cdc42, Rho and NPFs. Interactions that are well supported in the literature are shown as solid lines, interactions that are still ambiguous are shown as dotted lines.

(Sasaki et al., 2007; Miao et al., 2017). PI3K can also activate Rac1 and Cdc42 (Han et al., 1998; Benard et al., 1999; Fleming et al., 2000), and this crosstalk was suggested to couple Ras excitability to cytoskeletal and cell protrusion dynamics (Huang et al., 2013) (Figure 4).

Interestingly, in addition to excitable signal networks, cytoskeletal effectors also show excitable dynamics by themselves (Vicker, 2002; Bretschneider et al., 2004; Weiner et al., 2007; Case and Waterman, 2011). For example, nucleation promoting factors (NPFs) such as Hem1, that are activated downstream of Rac1 form a distinct, but coupled excitable system (Weiner et al., 2007; Huang et al., 2013) (Figures 3A and 4). Furthermore, excitable PI3K signaling is coupled to waves of endocytic activity (Yang et al., 2017), which were proposed to play a role in defining the cleavage furrow site in adherent, dividing cells (Xiao et al., 2017). Rho excitability might also be coupled to Ras, Rac1 and Cdc42 activity. However, due to numerous potential links, the nature of this crosstalk is less clear (Guilluy et al., 2011).

Integration of exploratory morphodynamics with chemical or mechanical cues to direct cell function

The question then arises, what the functional role for excitable cell protrusion and cell contraction signal networks might be and how they are linked to mechanical and receptor signals (Figure 4). One commonality could be that excitable signal networks can generate an active sensing process, in which cells use either exploratory protrusions or exploratory contractions to probe these environmental signals. This active process might be very similar to our own sense of touch such as when we actively reach out, touch and squeeze objects with our hands to probe their mechanical properties. Our ability to navigate through complex environments in the dark would be much less effective if we would only rely on passive tactile inputs.

We also strongly rely on our memory, on the most basic level on the short-term memory regarding the current and directly preceding actions. Similarly, cells can also utilize memory to coordinate dynamic exploratory processes. For example, cells can interpret temporal changes in stimuli by coupling their rapid immediate response to processes that persist for longer time periods. Such a mechanism was proposed to enable migration of *Dictyostelium* towards the source of propagating chemoattractant waves, by coupling immediate cell protrusion on wave arrival at the front with persistent inhibition of protrusion, while the wave traverses the cell body towards its rear (Skoge et al., 2014). Relatively long-lived cytoskeletal structures could form the basis of such a cellular short-term memory (Prentice-Mott et al., 2016).

Ultimately, more complex and yet poorly understood processing mechanisms in our brains integrate sensory inputs and our goals to make a decision where we want to go. In the case of cells, the feedback mechanisms by which excitability of cell protrusion and cell contraction is achieved are now better understood. However, it is still unclear, how these excitable signal networks are biased by chemical or mechanical cues and modulated by cellular short-term memory to convert exploratory cell dynamics into directed migration.

In the case of cell protrusion signals, it was suggested that extracellular chemoattractant gradients could locally lower the threshold for excitability (Xiong et al., 2010; Iglesias and Devreotes, 2012). Such systems, which were called biased excitable networks (BEN), generate more frequent pulses of protrusive signals at the cell front compared to the back (Iglesias and Devreotes, 2012). The local decrease in the excitability threshold could be achieved by coupling of the biased excitable network to inhibitory mechanisms that act on the larger scale of the entire cell, for example, via a global inhibitor or a local excitation global inhibition (LEGI) network (Devreotes et al., 2017). In particular, LEGI networks can adapt to sense small changes in chemoattractant over a wide range of their concentrations (Levchenko and Iglesias, 2002). A coupled LEGI-BEN could thereby sense shallow chemoattractant gradients over a wide concentration range (Devreotes et al., 2017). However, mechanisms that couple extracellular chemoattractant concentrations to the excitability threshold are currently unknown.

Interestingly, the excitability of cell protrusion signals needs to be tightly controlled to enable effective chemotaxis. For example, if the positive feedback of protrusion excitability is too high, cells either oscillate globally between protrusion and retraction, or they form a stably polarized, keratocyte-like cell shape that is not responsive to chemoattractant cues (Miao et al., 2017). Only at an intermediate level of positive feedback, cells can generate exploratory protrusions that can be biased and promoted by chemoattractants (Miao et al., 2017).

Coupling a LEGI-BEN with cellular short-term memory that acts on longer temporal scales could explain the stable segregation of front and back regions in polarized cells (Iglesias and Devreotes, 2012). For example, in the persistent, more directional migration mode that

is induced by starvation, pseudopods in Dictyostelium cells only rarely form *de novo*, but are instead generated by splitting of existing pseudopods, with an alternating left-right sequence (Bosgraaf and Van Haastert, 2009b). It was suggested that this strong bias in the generation of pseudopods is based on a local signal that is dependent on the *PLA2* gene, which could thereby act as a form of cellular short-term memory (Bosgraaf and Van Haastert, 2009a,b).

Similar mechanisms could also operate on the excitability of cell contraction during mechanosensitive processes (Graessl et al., 2017). For example, if cells encounter a gradient of extracellular matrix stiffness during durotaxis, a local gradient of elasticity could lower the threshold of Rho excitability to generate more frequent myosin pulses at the cell front that could pull the cell center forward. Coupled mechanical symmetry-breaking mechanisms (Goehring and Grill, 2013) that could also act as a cellular short-term memory could generate more persistent front-back polarization to drive directed cell migration.

In addition to controlling cellular morphogenesis on relatively short time scales, exploratory processes could also modulate long-term cell fate decisions such as cell proliferation and differentiation. This coupling would require a form of cellular long-term memory that is likely established by slowly acting transcriptional programs. For example, pulsatile sub-cellular contraction dynamics that are modulated by extracellular matrix stiffness (Graessl et al., 2017) could modulate mechanosensitive proliferation and cell differentiation programs. This idea of a role for dynamic, exploratory cell contraction dynamics in such processes is supported by the observation that cyclic stretch is a stronger stimulator of mechanosensitive cell growth compared to equivalent constant strain (Cui et al., 2015). The underlying mechanotransduction mechanism could primarily involve the mechanosensitivity of myosin itself, which was shown to accumulate at sites of increased strain (Luo et al., 2012). Subsequently, nuclear translocation of the contraction associated transcription factors MRTF-A and YAP was suggested to drive cyclic stretch-induced changes in cell growth (Cui et al., 2015).

In conclusion, multiple, coupled positive and negative feedback mechanisms that can generate excitable system dynamics were identified that generate local pulses or waves of signal network activity. Such signal networks enable exploratory cell dynamics that act as an active sense of touch during cellular navigation and fate decisions.

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References

Abercrombie, M., Heaysman, J.E., and Pegrum, S.M. (1970). The locomotion of fibroblasts in culture. I. Movements of the leading edge. Exp. Cell Res. *59*, 393–398.

Andrew, N. and Insall, R.H. (2007). Chemotaxis in shallow gradients is mediated independently of PtdIns 3-kinase by biased choices between random protrusions. Nat. Cell Biol. *9*, 193–200.

Aoki, K., Nakamura, T., Fujikawa, K., and Matsuda, M. (2005). Local phosphatidylinositol 3,4,5-trisphosphate accumulation recruits Vav2 and Vav3 to activate Rac1/Cdc42 and initiate neurite outgrowth in nerve growth factor-stimulated PC12 cells. Mol. Biol. Cell *16*, 2207–2217.

Arai, Y., Shibata, T., Matsuoka, S., Sato, M.J., Yanagida, T., and Ueda, M. (2010). Self-organization of the phosphatidylinositol lipids signaling system for random cell migration. Proc. Natl. Acad. Sci. USA *107*, 12399–12404.

Arrieumerlou, C. and Meyer, T. (2005). A local coupling model and compass parameter for eukaryotic chemotaxis. Dev. Cell *8*, 215–227.

Arthur, W.T., Quilliam, L.A., and Cooper, J.A. (2004). Rap1 promotes cell spreading by localizing Rac guanine nucleotide exchange factors. J. Cell Biol. *167*, 111–122.

Asano, Y., Nagasaki, A., and Uyeda, T.Q. (2008). Correlated waves of actin filaments and PIP3 in *Dictyostelium* cells. Cell Motil. Cytoskeleton *65*, 923–934.

Baird, M.A., Billington, N., Wang, A., Adelstein, R.S., Sellers, J.R., Fischer, R.S., and Waterman, C.M. (2017). Local pulsatile contractions are an intrinsic property of the myosin 2A motor in the cortical cytoskeleton of adherent cells. Mol. Biol. Cell *28*, 240–251.

Bear, J.E., Svitkina, T.M., Krause, M., Schafer, D.A., Loureiro, J.J., Strasser, G.A., Maly, I.V., Chaga, O.Y., Cooper, J.A., Borisy, G.G., et al. (2002). Antagonism between Ena/VASP proteins and actin filament capping regulates fibroblast motility. Cell *109*, 509–521.

Bement, W.M., Leda, M., Moe, A.M., Kita, A.M., Larson, M.E., Golding, A.E., Pfeuti, C., Su, K.C., Miller, A.L., Goryachev, A.B., et al. (2015). Activator-inhibitor coupling between Rho signalling and actin assembly makes the cell cortex an excitable medium. Nat. Cell Biol. *17*, 1471–1483.

Benard, V., Bohl, B.P., and Bokoch, G.M. (1999). Characterization of rac and cdc42 activation in chemoattractant-stimulated human neutrophils using a novel assay for active GTPases. J. Biol. Chem. *274*, 13198–13204.

Berg, H.C. and Brown, D.A. (1972). Chemotaxis in *Escherichia coli* analysed by three-dimensional tracking. Nature *239*, 500–504.

Blanchard, G.B., Murugesu, S., Adams, R.J., Martinez-Arias, A., and Gorfinkiel, N. (2010). Cytoskeletal dynamics and supracellular

organisation of cell shape fluctuations during dorsal closure. Development *137*, 2743–2752.

Bosgraaf, L. and Van Haastert, P.J. (2009a). Navigation of chemotactic cells by parallel signaling to pseudopod persistence and orientation. PLoS One *4*, e6842.

Bosgraaf, L. and Van Haastert, P.J. (2009b). The ordered extension of pseudopodia by amoeboid cells in the absence of external cues. PLoS One *4*, e5253.

Bretschneider, T., Diez, S., Anderson, K., Heuser, J., Clarke, M., Muller-Taubenberger, A., Kohler, J., and Gerisch, G. (2004). Dynamic actin patterns and Arp2/3 assembly at the substrate-attached surface of motile cells. Curr. Biol. *14*, 1–10.

Cain, R.J. and Ridley, A.J. (2009). Phosphoinositide 3-kinases in cell migration. Biol. Cell *101*, 13–29.

Case, L.B. and Waterman, C.M. (2011). Adhesive F-actin waves: a novel integrin-mediated adhesion complex coupled to ventral actin polymerization. PLoS One *6*, e26631.

Collinet, C., Rauzi, M., Lenne, P.F., and Lecuit, T. (2015). Local and tissue-scale forces drive oriented junction growth during tissue extension. Nat. Cell Biol. *17*, 1247–1258.

Cooper, M.S. and Schliwa, M. (1986). Motility of cultured fish epidermal cells in the presence and absence of direct current electric fields. J. Cell Biol. *102*, 1384–1399.

Coravos, J.S., Mason, F.M., and Martin, A.C. (2017). Actomyosin pulsing in tissue integrity maintenance during morphogenesis. Trends Cell Biol. *27*, 276–283.

Cui, Y., Hameed, F.M., Yang, B., Lee, K., Pan, C.Q., Park, S., and Sheetz, M. (2015). Cyclic stretching of soft substrates induces spreading and growth. Nat. Commun. *6*, 6333.

David, D.J., Tishkina, A., and Harris, T.J. (2010). The PAR complex regulates pulsed actomyosin contractions during amnioserosa apical constriction in *Drosophila*. Development *137*, 1645–1655.

Dent, E.W., Gupton, S.L., and Gertler, F.B. (2011). The growth cone cytoskeleton in axon outgrowth and guidance. Cold Spring Harb. Perspect Biol. *3*, a001800.

Devreotes, P.N., Bhattacharya, S., Edwards, M., Iglesias, P.A., Lampert, T., and Miao, Y. (2017). Excitable signal transduction networks in directed cell migration. Annu. Rev. Cell Dev. Biol. *33*, 103–125.

Ehrlich, J.S., Hansen, M.D., and Nelson, W.J. (2002). Spatio-temporal regulation of Rac1 localization and lamellipodia dynamics during epithelial cell-cell adhesion. Dev. Cell *3*, 259–270.

Fivaz, M., Bandara, S., Inoue, T., and Meyer, T. (2008). Robust neuronal symmetry breaking by Ras-triggered local positive feedback. Curr. Biol. *18*, 44–50.

Fleming, I.N., Gray, A., and Downes, C.P. (2000). Regulation of the Rac1-specific exchange factor Tiam1 involves both phosphoinositide 3-kinase-dependent and -independent components. Biochem. J. *351*, 173–182.

Frantz, C., Karydis, A., Nalbant, P., Hahn, K.M., and Barber, D.L. (2007). Positive feedback between Cdc42 activity and H^+ efflux by the Na-H exchanger NHE1 for polarity of migrating cells. J. Cell Biol. *179*, 403–410.

Gerisch, G., Malchow, D., and Hess, B. (1974). Cell communication and cyclic-AMP regulation during aggregation of the slime mold *Dictyostelium discoideum*. In: Biochemistry of Sensory Functions, L. Jaenicke, ed. (Heidelberg, Germany: Springer), pp. 279–298.

Giannone, G., Dubin-Thaler, B.J., Dobereiner, H.G., Kieffer, N., Bresnick, A.R., and Sheetz, M.P. (2004). Periodic lamellipodial contractions correlate with rearward actin waves. Cell *116*, 431–443.

Goehring, N.W. and Grill, S.W. (2013). Cell polarity: mechanochemical patterning. Trends Cell Biol. *23*, 72–80.

Goldberg, D.J. and Burmeister, D.W. (1986). Stages in axon formation: observations of growth of *Aplysia* axons in culture using video-enhanced contrast-differential interference contrast microscopy. J. Cell Biol. *103*, 1921–1931.

Graessl, M., Koch, J., Calderon, A., Kamps, D., Banerjee, S., Mazel, T., Schulze, N., Jungkurth, J.K., Patwardhan, R., Solouk, D., et al. (2017). An excitable Rho GTPase signaling network generates dynamic subcellular contraction patterns. J. Cell Biol. *216*, 4271–4285.

Guilluy, C., Garcia-Mata, R., and Burridge, K. (2011). Rho protein crosstalk: another social network? Trends Cell Biol. *21*, 718–726.

Han, J., Luby-Phelps, K., Das, B., Shu, X., Xia, Y., Mosteller, R.D., Krishna, U.M., Falck, J.R., White, M.A., and Broek, D. (1998). Role of substrates and products of PI 3-kinase in regulating activation of Rac-related guanosine triphosphatases by Vav. Science *279*, 558–560.

Hodge, R.G. and Ridley, A.J. (2016). Regulating Rho GTPases and their regulators. Nat. Rev. Mol. Cell Biol. *17*, 496–510.

Huang, C.H., Tang, M., Shi, C., Iglesias, P.A., and Devreotes, P.N. (2013). An excitable signal integrator couples to an idling cytoskeletal oscillator to drive cell migration. Nat. Cell Biol. *15*, 1307–1316.

Iglesias, P.A. and Devreotes, P.N. (2012). Biased excitable networks: how cells direct motion in response to gradients. Curr. Opin. Cell Biol. *24*, 245–253.

Kasza, K.E. and Zallen, J.A. (2011). Dynamics and regulation of contractile actin-myosin networks in morphogenesis. Curr. Opin. Cell Biol. *23*, 30–38.

Krause, M. and Gautreau, A. (2014). Steering cell migration: lamellipodium dynamics and the regulation of directional persistence. Nat. Rev. Mol. Cell Biol. *15*, 577–590.

Lauffenburger, D.A. and Horwitz, A.F. (1996). Cell migration: a physically integrated molecular process. Cell *84*, 359–369.

Lee, C.S., Choi, C.K., Shin, E.Y., Schwartz, M.A., and Kim, E.G. (2010). Myosin II directly binds and inhibits Dbl family guanine nucleotide exchange factors: a possible link to Rho family GTPases. J. Cell Biol. *190*, 663–674.

Levchenko, A. and Iglesias, P.A. (2002). Models of eukaryotic gradient sensing: application to chemotaxis of amoebae and neutrophils. Biophys. J. *82*, 50–63.

Lim, J.I., Sabouri-Ghomi, M., Machacek, M., Waterman, C.M., and Danuser, G. (2010). Protrusion and actin assembly are coupled to the organization of lamellar contractile structures. Exp. Cell Res. *316*, 2027–2041.

Luo, T., Mohan, K., Srivastava, V., Ren, Y., Iglesias, P.A., and Robinson, D.N. (2012). Understanding the cooperative interaction between myosin II and actin cross-linkers mediated by actin filaments during mechanosensation. Biophys. J. *102*, 238–247.

Macnab, R.M. and Koshland, D.E., Jr. (1972). The gradient-sensing mechanism in bacterial chemotaxis. Proc. Natl. Acad. Sci. USA *69*, 2509–2512.

Martin, A.C., Kaschube, M., and Wieschaus, E.F. (2009). Pulsed contractions of an actin-myosin network drive apical constriction. Nature *457*, 495–499.

Martin, A.C., Gelbart, M., Fernandez-Gonzalez, R., Kaschube, M., and Wieschaus, E.F. (2010). Integration of contractile forces during tissue invagination. J. Cell Biol. *188*, 735–749.

Mason, F.M., Xie, S., Vasquez, C.G., Tworoger, M., and Martin, A.C. (2016). RhoA GTPase inhibition organizes contraction during epithelial morphogenesis. J. Cell Biol. *214*, 603–617.

Mato, J.M., Losada, A., Nanjundiah, V., and Konijn, T.M. (1975). Signal input for a chemotactic response in the cellular slime mold *Dictyostelium discoideum*. Proc. Natl. Acad. Sci. USA *72*, 4991–4993.

McNeill, H., Ryan, T.A., Smith, S.J., and Nelson, W.J. (1993). Spatial and temporal dissection of immediate and early events following cadherin-mediated epithelial cell adhesion. J. Cell Biol. *120*, 1217–1226.

Medina, F., Carter, A.M., Dada, O., Gutowski, S., Hadas, J., Chen, Z., and Sternweis, P.C. (2013). Activated RhoA is a positive feedback regulator of the Lbc family of Rho guanine nucleotide exchange factor proteins. J. Biol. Chem. *288*, 11325–11333.

Miao, Y., Bhattacharya, S., Edwards, M., Cai, H., Inoue, T., Iglesias, P.A., and Devreotes, P.N. (2017). Altering the threshold of an excitable signal transduction network changes cell migratory modes. Nat. Cell Biol. *19*, 329–340.

Ming, W., Li, S., Billadeau, D.D., Quilliam, L.A., and Dinauer, M.C. (2007). The Rac effector p67phox regulates phagocyte NADPH oxidase by stimulating Vav1 guanine nucleotide exchange activity. Mol. Cell Biol. *27*, 312–323.

Munjal, A., Philippe, J.M., Munro, E., and Lecuit, T. (2015). A self-organized biomechanical network drives shape changes during tissue morphogenesis. Nature *524*, 351–355.

Murray, J.D. (2002). Mathematical Biology: I: An Introduction, Third Edition (New York, USA: Springer).

Murray, J.D. (2003). Mathematical Biology: II: Spatial Models and Biomedical Applications, Third Edition (New York, USA: Springer).

Nishikawa, M., Naganathan, S.R., Julicher, F., and Grill, S.W. (2017). Controlling contractile instabilities in the actomyosin cortex. eLife *6*, e19595.

Ohta, Y., Hartwig, J.H., and Stossel, T.P. (2006). FilGAP, a Rho- and ROCK-regulated GAP for Rac binds filamin A to control actin remodelling. Nat. Cell Biol. *8*, 803–814.

Petrie, R.J. and Yamada, K.M. (2016). Multiple mechanisms of 3D migration: the origins of plasticity. Curr. Opin. Cell Biol. *42*, 7–12.

Plotnikov, S.V. and Waterman, C.M. (2013). Guiding cell migration by tugging. Curr. Opin. Cell Biol. *25*, 619–626.

Plotnikov, S.V., Pasapera, A.M., Sabass, B., and Waterman, C.M. (2012). Force fluctuations within focal adhesions mediate ECM-rigidity sensing to guide directed cell migration. Cell *151*, 1513–1527.

Prentice-Mott, H.V., Meroz, Y., Carlson, A., Levine, M.A., Davidson, M.W., Irimia, D., Charras, G.T., Mahadevan, L., and Shah, J.V. (2016). Directional memory arises from long-lived cytoskeletal asymmetries in polarized chemotactic cells. Proc. Natl. Acad. Sci. USA *113*, 1267–1272.

Radde, N. (2008). The effect of time scale differences and time delays on the structural stability of oscillations in a two-gene network. Adv. Complex Syst. *11*, 471–483.

Ridley, A.J. (2015). Rho GTPase signalling in cell migration. Curr. Opin. Cell Biol. *36*, 103–112.

Rodriguez-Viciana, P., Warne, P.H., Dhand, R., Vanhaesebroeck, B., Gout, I., Fry, M.J., Waterfield, M.D., and Downward, J. (1994). Phosphatidylinositol-3-OH kinase as a direct target of Ras. Nature *370*, 527–532.

Rosenfeldt, H., Castellone, M.D., Randazzo, P.A., and Gutkind, J.S. (2006). Rac inhibits thrombin-induced Rho activation: evidence of a Pak-dependent GTPase crosstalk. J. Mol. Signal *1*, 8.

Sander, E.E., ten Klooster, J.P., van Delft, S., van der Kammen, R.A., and Collard, J.G. (1999). Rac downregulates Rho activity: reciprocal balance between both GTPases determines cellular morphology and migratory behavior. J. Cell Biol. *147*, 1009–1022.

Sanz-Moreno, V., Gadea, G., Ahn, J., Paterson, H., Marra, P., Pinner, S., Sahai, E., and Marshall, C.J. (2008). Rac activation and inactivation control plasticity of tumor cell movement. Cell *135*, 510–523.

Sasaki, A.T., Janetopoulos, C., Lee, S., Charest, P.G., Takeda, K., Sundheimer, L.W., Meili, R., Devreotes, P.N., and Firtel, R.A. (2007). G protein-independent Ras/PI3K/F-actin circuit regulates basic cell motility. J. Cell Biol. *178*, 185–191.

Shin, I., Kim, S., Song, H., Kim, H.R., and Moon, A. (2005). H-Ras-specific activation of Rac-MKK3/6-p38 pathway: its critical role in invasion and migration of breast epithelial cells. J. Biol. Chem. *280*, 14675–14683.

Skoge, M., Yue, H., Erickstad, M., Bae, A., Levine, H., Groisman, A., Loomis, W.F., and Rappel, W.J. (2014). Cellular memory in eukaryotic chemotaxis. Proc. Natl. Acad. Sci. USA *111*, 14448–14453.

Te Boekhorst, V., Preziosi, L., and Friedl, P. (2016). Plasticity of cell migration *in vivo* and *in silico*. Annu. Rev. Cell Dev. Biol. *32*, 491–526.

Tyson, J.J., Chen, K.C., and Novak, B. (2003). Sniffers, buzzers, toggles and blinkers: dynamics of regulatory and signaling pathways in the cell. Curr. Opin. Cell Biol. *15*, 221–231.

van den Boom, F., Dussmann, H., Uhlenbrock, K., Abouhamed, M., and Bahler, M. (2007). The myosin IXb motor activity targets the myosin IXb RhoGAP domain as cargo to sites of actin polymerization. Mol. Biol. Cell *18*, 1507–1518.

van Haastert, P.J., Keizer-Gunnink, I., and Kortholt, A. (2017). Coupled excitable Ras and F-actin activation mediates spontaneous pseudopod formation and directed cell movement. Mol. Biol. Cell *28*, 922–934.

Vicker, M.G. (2002). F-actin assembly in Dictyostelium cell locomotion and shape oscillations propagates as a self-organized reaction-diffusion wave. FEBS Lett. *510*, 5–9.

Weiner, O.D., Marganski, W.A., Wu, L.F., Altschuler, S.J., and Kirschner, M.W. (2007). An actin-based wave generator organizes cell motility. PLoS Biol. *5*, e221.

Wittmann, T., Bokoch, G.M., and Waterman-Storer, C.M. (2003). Regulation of leading edge microtubule and actin dynamics downstream of Rac1. J. Cell Biol. *161*, 845–851.

Wozniak, M.A., Kwong, L., Chodniewicz, D., Klemke, R.L., and Keely, P.J. (2005). R-Ras controls membrane protrusion and cell migration through the spatial regulation of Rac and Rho. Mol. Biol. Cell *16*, 84–96.

Wu, M., Wu, X., and De Camilli, P. (2013). Calcium oscillations-coupled conversion of actin travelling waves to standing oscillations. Proc. Natl. Acad. Sci. USA *110*, 1339–1344.

Xiao, S., Tong, C., Yang, Y., and Wu, M. (2017). Mitotic cortical waves predict future division sites by encoding positional and size information. Dev. Cell *43*, 493–506 e493.

Xiong, Y., Huang, C.H., Iglesias, P.A., and Devreotes, P.N. (2010). Cells navigate with a local-excitation, global-inhibition-biased excitable network. Proc. Natl. Acad. Sci. USA *107*, 17079–17086.

Xiong, D., Xiao, S., Guo, S., Lin, Q., Nakatsu, F., and Wu, M. (2016). Frequency and amplitude control of cortical oscillations by phosphoinositide waves. Nat. Chem. Biol. *12*, 159–166.

Yang, H.W., Collins, S.R., and Meyer, T. (2016). Locally excitable Cdc42 signals steer cells during chemotaxis. Nat. Cell Biol. *18*, 191–201.

Yang, Y., Xiong, D., Pipathsouk, A., Weiner, O.D., and Wu, M. (2017). Clathrin assembly defines the onset and geometry of cortical patterning. Dev. Cell *43*, 507–521 e504.

Yao, M., Qiu, W., Liu, R., Efremov, A.K., Cong, P., Seddiki, R., Payre, M., Lim, C.T., Ladoux, B., Mege, R.M., et al. (2014). Force-dependent conformational switch of a-catenin controls vinculin binding. Nat. Commun. *5*, 4525.

Yip, S.C., El-Sibai, M., Coniglio, S.J., Mouneimne, G., Eddy, R.J., Drees, B.E., Neilsen, P.O., Goswami, S., Symons, M., Condeelis, J.S., et al. (2007). The distinct roles of Ras and Rac in PI 3-kinase-dependent protrusion during EGF-stimulated cell migration. J. Cell Sci. *120*, 3138–3146.