

The excitable signal transduction networks: movers and shapers of eukaryotic cell migration

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ABSTRACT In response to a variety of external cues, eukaryotic cells display varied migratory modes to perform their physiological functions during development and in the adult. Aberrations in cell migration result in embryonic defects and cancer metastasis. The molecular components involved in cell migration are remarkably conserved between the social amoeba *Dictyostelium* and mammalian cells. This makes the amoeba an excellent model system for studies of eukaryotic cell migration. These migration-associated components can be grouped into three networks: input, signal transduction and cytoskeletal. In migrating cells, signal transduction events such as Ras or PI3K activity occur at the protrusion tips, referred to as 'front', whereas events such as dissociation of PTEN from these regions are referred to as 'back'. Asymmetric distribution of such front and back events is crucial for establishing polarity and guiding cell migration. The triggering of these signaling events displays properties of biochemical excitability including all-or-nothing responsiveness to suprathreshold stimuli, refractoriness, and wave propagation. These signal transduction waves originate from a point and propagate towards the edge of the cell, thereby driving cytoskeletal activity and cellular protrusions. Any change in the threshold for network activation alters the range of the propagating waves and the size of cellular protrusions which gives rise to various migratory modes in cells. Thus, this review highlights excitable signal transduction networks as key players for coordinating cytoskeletal activities to drive cell migration in all eukaryotes.


KEY WORDS: *signaling wave, polarity, PIP3, macrophage, phagocytosis, cytokinesis, cancer*

***Dictyostelium* paves the way for understanding cell migration in human health and disease**

In eukaryotic cells, cell migration is crucial for a multitude of physiological processes. During embryogenesis, migration of individual or groups of cells, in response to external cues, leads to formation of various glands and organs, and wiring of the nervous system (Montell, 2008). Examples include the coordinated movement of epithelial cell sheets at the onset of gastrulation and neurulation (Keller, 2005, Leptin, 2005, Theveneau and Mayor, 2012, Yang *et al.*, 2002), movement of primordial germ cells across the embryo towards the developing somatic gonads (Blaser *et al.*, 2006, Richardson and Lehmann, 2010) or glial and neural precursor cell migration in the central and peripheral nervous systems (Klambt, 2009). In adults, directed migration is observed during host inflam-

matory responses when immune cells move through tissues and vessels towards invading pathogens (Nourshargh and Alon, 2014, Weninger *et al.*, 2014), or various cellular regenerative processes such as wound healing performed by concerted movement of fibroblasts and keratinocytes (Shaw and Martin, 2009). Cells are able to sense and integrate a variety of external cues from the environment and each other, including chemicals (Bagorda and Parent, 2008, Tessier-Lavigne, 1994), electric fields (Cortese *et*

Abbreviations used in this paper: PIP3, phosphatidylinositol (3,4,5)-trisphosphate; PI(3,4)P2, phosphatidylinositol 3,4-bisphosphate; PI(4,5)P2, phosphatidylinositol 4,5-bisphosphate; PTEN, phosphatase and tensin homolog; PI3K, phosphoinositide 3-kinase; GPCR, G-protein-coupled receptor; cAMP, 3',5'-cyclic adenosine monophosphate; cARs and FARs, cAMP and folic acid receptors; STEN, signal transduction excitable networks; CEN, cytoskeletal excitable networks.

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#Note: The indicated authors contributed equally to this article.

Submitted: 14 June, 2019; Accepted: 14 August, 2019.

al., 2014, Gao et al., 2011, Zhao et al., 2006), light (Armitage and Hellingwerf, 2003), temperature (Ramot et al., 2008, Whitaker and Poff, 1980) and mechanical forces (Harland et al., 2011, Lo et al., 2000). Irregularities or defects in cell migration are responsible for pathogenesis of several inflammatory diseases such as acute respiratory distress syndrome, several allergies (asthma, allergic rhinitis and atopic dermatitis), arthritis, atherosclerosis, periodontal disease, sarcoidosis and Wiskott-Aldrich syndrome (Lakshman and Finn, 2001, Moulding et al., 2013). Cell migration is also a crucial phenomenon during cancer metastasis when tumor cells detach and spread from their primary site of origin to colonize other tissues and organs of the body (Kedrin et al., 2007).

Eukaryotic cells perform their crucial physiological functions by displaying a variety of migratory behaviors. Migration in these cells is achieved by coordinated extension of actin-rich protrusions at the leading edge of the cell, and actomyosin filaments-based contraction at the trailing edge (Fig. 1A). Variations of this cytoskeletal organization in the cell give rise to a vast repertoire of migratory behaviors. Leukocytes, hematopoietic stem cells and several metastatic cancer cells translocate by amoeboid motility, a rhythmic extension and retraction of actin-filled pseudopodia

leading to cell movement in random directions. Primordial germ cells use an unusual type of amoeboid motion, termed as blebbing, which involves extension of rounded cytoplasmic bulges caused by detachment of plasma membrane from actomyosin cortex due to myosin-based contraction (Blaser et al., 2006, Fackler and Grosse, 2008, Yoshida and Soldati, 2006). Keratocyte-like migration, seen in mesenchymal-derived corneal stromal cells, is characterized by large, actin-driven, fan-like lamellipodia at the front and sides of the cell causing them to move in a rolling motion (Barnhart et al., 2010). This mechanism leads to the fastest motion and cells can maintain constant direction and speed over several cell lengths (Anderson and Cross, 2000). "Mesenchymal" migration, seen in fibroblasts, is much slower than amoeboid or keratocyte-like motility and is mediated by lamellipodia at the leading edge of the cell (Hou et al., 2012). This type of migration depends on focal adhesions between cell and extracellular matrix whereas amoeboid or keratocyte migration involves more transient attachment of cells to the substratum (Mogilner and Keren, 2009, Parsons et al., 2010). In this review, the focus primarily would be on understanding the signal transduction events in amoeboid migration in cells, although it is explained how cells can abruptly switch between migratory modes.

Most of the present-day knowledge regarding amoeboid-type migration in eukaryotic cells was first revealed from seminal studies in the social amoeba, *Dictyostelium*. For example, identification of actin binding proteins such as coronin (de Hostos et al., 1991), functional redundancy of cytoskeletal components involved in cell migration (Andre et al., 1989, Jung et al., 1996, Witke et al., 1992), role of myosin II in cytokinesis and cell migration (De Lozanne and Spudich, 1987, Wessels et al., 1988), identification of chemoattractant receptors as GPCRs (Klein et al., 1988), confinement of phosphoinositide lipids and G protein signaling events to the leading edge of migrating cells (Iijima and Devreotes, 2002, Parent et al., 1998), involvement of Ras GTPases in cell migration (Insall et al., 1996, Kae et al., 2004, Sasaki et al., 2007), and discovery of actin waves (Vicker, 2002). Due to evolutionary conservation between

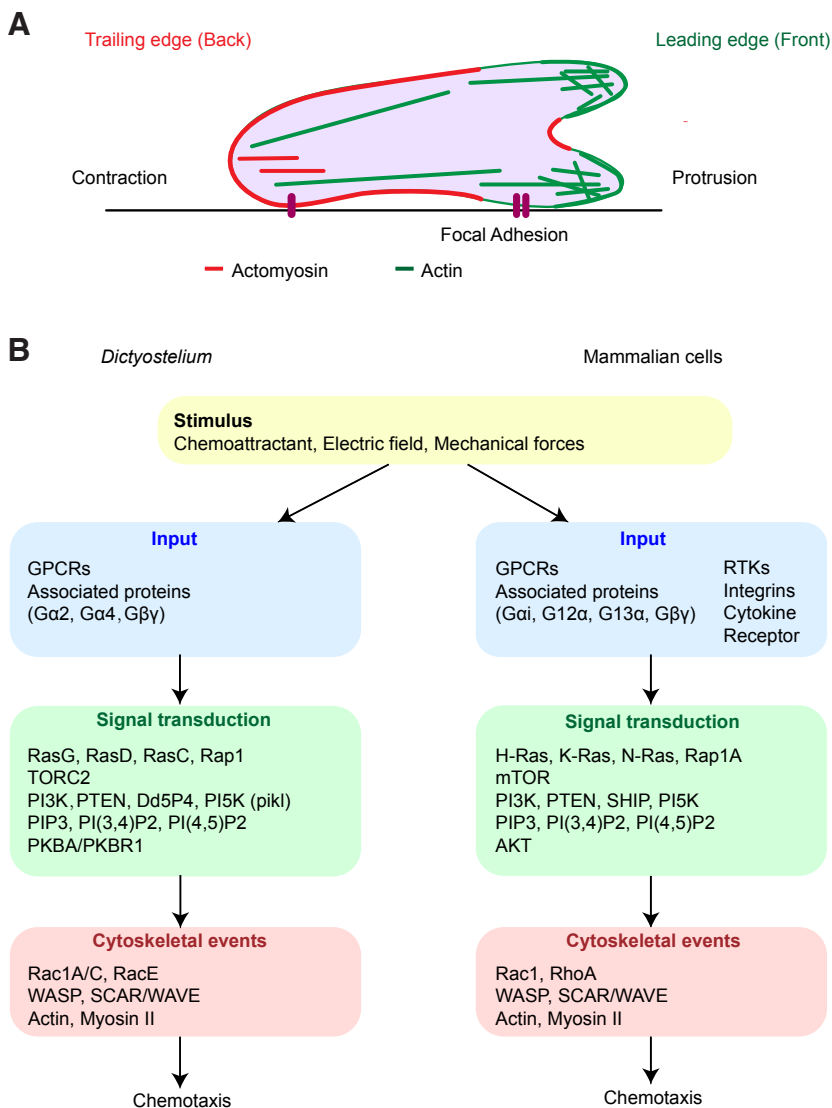


Fig. 1. Molecular components involved in eukaryotic migration. (A) Eukaryotic migration is achieved by extending actin-rich protrusions at the leading edge of the cell (as shown in dark green), coordinated with actomyosin-based contraction at the trailing edge (denoted in dark red). These cells develop active sites for actin polymerization, called focal adhesions, underneath the leading edge for integrin-dependent adhesion and migration. (B) Independent genetic and biochemical experimentation have identified various components involved in directed cell migration which can be grouped into 3 networks- input, signal transduction, and cytoskeletal events. The important components for each of these networks have been highlighted in the cartoon. A variety of external stimuli, such as chemoattractant (chemicals), electric fields and mechanical forces, locally activate the signal transduction networks through input networks, leading to cytoskeletal events such as F-actin polymerization at the front and actomyosin-based contraction at the back of the cell. The coordinated activation of these networks results in chemotaxis (directed cell migration) and is functionally conserved in *Dictyostelium* (left) and mammalian cells (right).

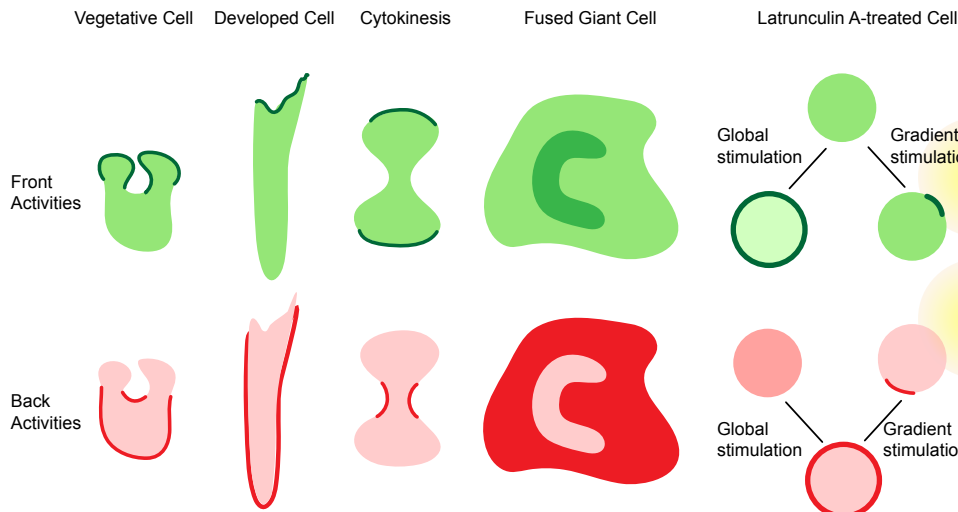


Fig. 2. Complementary distribution pattern of front and back activities in *Dictyostelium* cells undergoing various morphological changes.

Front activities, such as Ras or PI3K activation, occur at the macropinocytic cups or protrusions of migrating vegetative or developed cells, respectively (denoted in dark green, top row). These front activities are complemented with back activities at the trailing edge of the macropinocytic cups or cell (denoted in dark red, bottom row). During cytokinesis, these front molecules are found at the poles of the dividing cells (denoted in dark green, top row) and the back molecules accumulate at the cleavage furrow (shown in dark red, bottom row). In motile 'giant' *Dictyostelium* cells, generated by

electric-pulse-induced fusion, majority of the basal surface is marked by biosensors for back molecules (shown in dark red, bottom row), except in the region of F-actin polymerization where the front molecules are present (shown in dark green, top row). Upon global or gradient chemoattractant stimulation, latrunculin A-treated cells show opposite distribution of front (shown in dark green, top row) and back (shown in dark red, bottom row) activities.

Dictyostelium and higher eukaryotes, these scientific discoveries paved the way for understanding the role of migration-associated signal transduction and cytoskeletal networks in human physiology and pathology (Bagorda *et al.*, 2006, Parent, 2004).

The amenability of *Dictyostelium* to experimentation enabled these discoveries. It is easily cultivable in the laboratory, and is well suited for live cell imaging. It has a haploid genome which is completely sequenced and annotated, thereby facilitating genetic manipulations (Muller-Taubenberger *et al.*, 2013). Moreover, *Dictyostelium* are naturally migratory cells which respond to chemoattractants, such as cAMP, as part of their development program, and folic acid to seek nutrients (Chisholm and Firtel, 2004, Mahadeo and Parent, 2006). These processes can be mimicked in the laboratory with relative ease which makes the amoeba the premier model system for investigating cell migration.

Spatiotemporal control of signal transduction networks regulates cell migration

The components involved in cell migration can be grouped into input, signal transduction, and cytoskeletal networks, which show functional conservation between *Dictyostelium* and mammalian cells (Fig. 1B). Chemical, electrical or mechanical stimuli locally activate the signal transduction networks through input networks which consists of different receptors or sensors (Allen *et al.*, 2013, Meng *et al.*, 2011, Miao *et al.*, 2017, Zhao *et al.*, 2002, Zhao *et al.*, 2006). In *Dictyostelium*, the input network is comprised of cAMP and folic acid GPCRs (cARs and FARs respectively) along with associated G proteins ($G\alpha_2$, $G\alpha_4$, $G\alpha_9$ and $G\beta\gamma$) (Kimmel and Parent, 2003, Klein *et al.*, 1988, Pan *et al.*, 2016, Parent and Devreotes, 1996, Wu and Devreotes, 1991). The input network of mammalian cells consists of GPCRs and associated G proteins ($G\alpha_i$, $G_{12\alpha}$, $G_{13\alpha}$ and $G\beta\gamma$), RTKs, integrins and chemokine receptors (Fig. 1B) (Baggiolini, 2001, Manes *et al.*, 2005, Senoo *et al.*, 2016, Stephens *et al.*, 2008). Chemokine receptors have been found to be functionally similar to cARs and FARs in *Dictyostelium*. These sensors detect gradients of chemoattractants (cAMP and

folic acid for *Dictyostelium* or chemokines, cytokines and growth factors for mammalian cells) in the environment, thereby triggering downstream signal transduction components and enabling the cell to migrate along the gradient (Jin *et al.*, 2008).

The signal transduction network is comprised of components which are arranged in several interconnected or parallel pathways. In *Dictyostelium*, it consists of various molecules including Ras proteins (RasG, RasD and RasC), phosphoinositide lipids [phosphatidylinositol (3,4,5)-trisphosphate or PIP3, phosphatidylinositol 3,4-bisphosphate or PI(3,4)P2 and phosphatidylinositol 4,5-bisphosphate or PI(4,5)P2], and several lipid kinases/phosphatases (PI3K, PTEN and Dd5P4) and protein kinases/phosphatases (PKBA/PKBR1). The signaling network in mammalian cells is analogous including H-Ras, K-Ras, PIP3, PI(3,4)P2, PI(4,5)P2, PI3K, PTEN, SHIP and AKT (Fig. 1B) (Devreotes and Horwitz, 2015, Devreotes *et al.*, 2017, Simanshu *et al.*, 2017, Tang *et al.*, 2011, Wilkins and Insall, 2001). Studies suggest that the role of the Ras-PIP3 network in mammalian cell migration is remarkably consistent with that in *Dictyostelium* (Artemenko *et al.*, 2014). Other reports have shown that interactions between Ras/Rap1 family proteins and TORC2 are conserved and thereby coordinate migration in *Dictyostelium* and mammalian cells (Charest *et al.*, 2010, Khanna *et al.*, 2016). Importantly, these network events are spontaneously activated leading to cell migration even in absence of an external cue (Arai *et al.*, 2010, Bosgraaf and Van Haastert, 2009, Sasaki *et al.*, 2007, Xiong *et al.*, 2010).

The signal transduction network connects to the cytoskeletal network, and controls organization of the acto-myosin cytoskeleton which drives cell migration. In *Dictyostelium* and mammalian cells, the cytoskeletal network is made up several components which include Rho family G proteins, myosin II, SCAR/WAVE complex, Arp 2/3 complex and coronin (Fig. 1B) (Alvarez-Gonzalez *et al.*, 2014, Wilkins and Insall, 2001). Of these components, the Rho family GTPases are a crucial convergence point of migration-associated signaling. In mammals, cell migration-related research has been focused on Rac1, RhoA and Cdc42 proteins of the Rho family whereas 15 Rho family proteins are present in *Dictyostelium*.

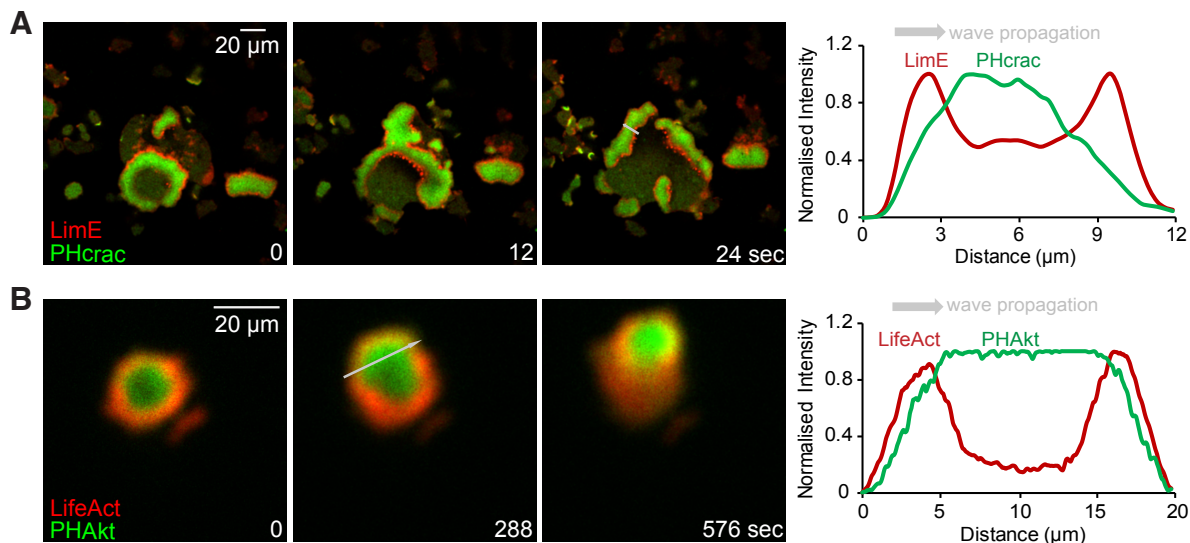


Fig. 3. Conservation of PIP3 and F-actin waves in *Dictyostelium* and mammalian cells. (A) Time lapse merged confocal images showing distribution of F-actin biosensor, LimEΔcoil or “LimE” (red) and PIP3 biosensor, PHcrac (green) in waves at the basal surface of a migrating fused *Dictyostelium* cell (left). Intensity plot across the white arrow in image “24 sec” (right). Prior to imaging, fused cells were generated by electroporation of single cells at 1,000 V, 3 μF for once, and 1,000 V, 1 μF for three times, with 1–2 secs between pulses. (B) Time lapse merged confocal images of F-actin biosensor, LifeAct (red) and PIP3 biosensor, PHAkt (green) in waves at the basal surface of a RAW 264.7 macrophage cell undergoing frustrated phagocytic spreading on an anti-BSA IgG coated glass surface (left). Intensity plot across the white arrow in image “288 sec” (right).

These proteins act on a number of effectors, thereby regulating protrusion, adhesion and polarization during migration (Lawson and Ridley, 2018, Lim *et al.*, 2002, Ridley, 2015). Studies have shown that there is functional conservation between mammalian Rac1 and RhoA and *Dictyostelium* Rac1 A/C and RacE, respectively (Filic *et al.*, 2012, Wang *et al.*, 2013a, Wang *et al.*, 2013b). Further evidence for this was put forward in a recent study which shed light on a novel interaction between phosphorylated GDP-bound RacE or RhoA and mTORC2 kinase, resulting in downstream AKT phosphorylation in *Dictyostelium* and mammalian cells, respectively (Senoo *et al.*, 2019).

In migrating cells, many components of the signal transduction network selectively translocate to, or are activated on, protrusions tips while others, initially present on the cortex dissociate from the protrusions. These sets of components are referred to as “front” or “back”, respectively (Fig. 2). For example, “front” biosensors for Ras or PI3K activation and PIP3 accumulation reside in the cytosol and are recruited to the tips of pseudopodia as protrusions form. On the other hand, “back” proteins such as PTEN and myosin are present uniformly in the cortex and dissociate from the regions where protrusions are formed (Filic *et al.*, 2012, Kriebel *et al.*, 2008, Lam *et al.*, 2012, Matsuoka and Ueda, 2018, Parent, 2004, Tsai *et al.*, 2019, Xu *et al.*, 2017). Such complimentary distribution of front and back components is observed in an extensive set of other morphological changes. For example, the pattern is preserved in macropinosomes and pseudopods and during cytokinesis where front molecules accumulate at the poles of the dividing cell and back molecules localize to the cleavage furrow (Fig. 2) (Janetopoulos *et al.*, 2005). A complementary pattern of front and back events is also observed in motile polykaryotic *Dictyostelium* cells, which are formed in response to electric pulsing of single cells, and have been recently used for imaging large-scale pattern dynamics independently of boundary effects (Gerisch *et al.*, 2013). A major

portion of the basal surface of these fused ‘giant’ cells is marked by biosensors for back components, except in the region of F-actin polymerization where the front molecules are present (Fig. 2). Furthermore, in presence of a global chemoattractant stimulation, all front components of an actin polymerization inhibitor (latrunculin A)-treated cell translocate from the cytosol to distribute uniformly over the cortex or membrane whereas the back molecules fall off from the cell periphery into the cytosol (Fig. 2). When latrunculin A-treated cells are subjected to a chemoattractant gradient, membrane patches for the front components are enhanced at the high side of the gradient, and suppressed at the low side. The back components follow an exactly complementary localization pattern on the membrane in response to a spatial gradient (Fig. 2) (Iijima and Devreotes, 2002, Iijima *et al.*, 2004, Janetopoulos *et al.*, 2004, Parent *et al.*, 1998, Sasaki *et al.*, 2004). A comprehensive list of signaling components that translocate to front or back of cells is provided previously (Swaney *et al.*, 2010).

Asymmetric accumulation of front and back components is crucial for promoting actin polymerization and producing cellular protrusions for cell migration. In cells expressing constitutively active RasC (Q62L), F-actin polymerization appears at ectopic sites around the cell periphery resulting in increased protrusions and reduced directionality to the chemoattractant source (Cai *et al.*, 2010). Excess accumulation of PIP3 along the entire cell periphery in neutrophils lacking SHIP1 or *Dictyostelium* lacking PTEN, gives rise to ectopic lateral pseudopodia outside the leading edge. If these cells are treated with LY294002 (PI3K inhibitor), this phenotype is reverted to a single anterior pseudopod in the cells (Chen *et al.*, 2003, Funamoto *et al.*, 2002, Iijima and Devreotes, 2002, Nishio *et al.*, 2007). Various studies involving uniform activation or pharmacological inhibition of PI3K have shown that altering levels of PIP3 alone is sufficient to affect directed and random migration in neutrophils, fibroblasts, germ cells, cancer cells, and *Dictyostelium*

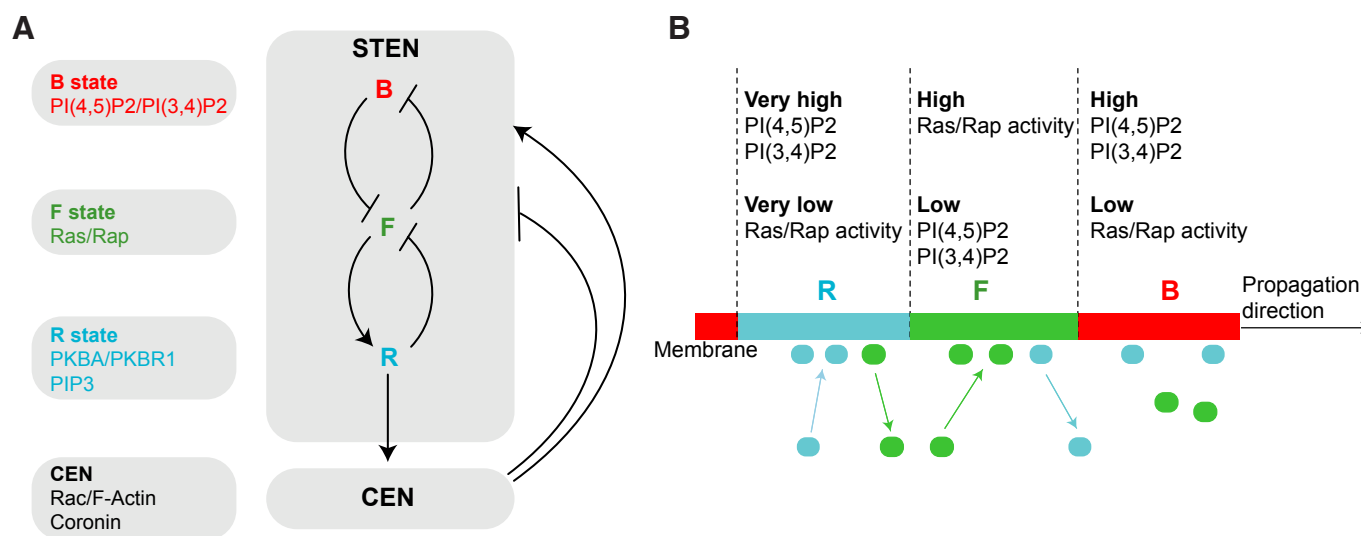


Fig. 4. Feedback loops and state of network components of signal transduction excitable networks (STEN). (A) The STEN-CEN model architecture showing B (inactive, red), F (active, green) and R (refractory, blue) states of STEN and the feedback connections between STEN and CEN. The positive feedback is indicated through the arrowhead, whereas the bar at the end of the arrow refers to negative feedback. Positive feedback is due to mutual inhibition between F (activation of Ras/Rap) and B (PIP2) states at the cell cortex, and a delayed negative feedback from R, due to delayed PKB activation by PIP3, to F state. The PKBs (R state) feeds into CEN and promotes F-actin polymerization which, in turn, provides a fast positive and slow negative feedbacks to STEN. (B) A representative portion of the cortical membrane depicting regions where the STEN is in B (red), F (green) and R (blue) states. Due to the various positive and negative feedbacks in STEN-CEN, the F state of the cell cortex is characterized by high Ras/Rap activity and low PI(4,5)P2/PI(3,4)P2 level whereas the B state is denoted by high PI(4,5)P2/PI(3,4)P2 level and low Ras/Rap activity. The R state, which follows the F state, is characterized by very high PI(4,5)P2/PI(3,4)P2 level and very low Ras/Rap activity. These positive and negative feedbacks along with diffusion of network components, advances the wave unidirectionally (black arrow). Abbreviations: STEN, signal transduction excitable networks; CEN, cytoskeletal excitable networks.

(Chen *et al.*, 2003, Devreotes and Horwitz, 2015, Dumstrei *et al.*, 2004, Ferguson *et al.*, 2007, Hoeller and Kay, 2007, Inoue and Meyer, 2008). In cells lacking the 5-phosphatase, Dd5P4, depletion of PI(3,4)P2 leads to increased Ras activity, cell spreading and aberrant migratory behavior (Li *et al.*, 2018). Therefore, role of signal transduction networks in cell migration across various species is well-established.

Role of excitability in *Dictyostelium* and mammalian cells

Although the networks shown in Fig. 1B depict a linear interaction between inputs, signal transduction and cytoskeletal events, such a linear interaction is not sufficient to explain increasing instances of actin wave propagation. Seminal experiments of M.G. Vicker provided the first evidence of spiral localization patterns of actin filament assembly in vegetative-stage *Dictyostelium* cells (Vicker, 2002). Actin wave generation and propagation in *Dictyostelium* cells was further characterized and confirmed by Gerisch *et al.*, (Bretschneider *et al.*, 2004, Gerisch, 2010). Coordinated waves of Ras activity, PIP3, PTEN and actin were observed in *Dictyostelium* which provided a basis for modeling the nonlinear interactions that produce spatio-temporal patterns in the actin system of cells (Arai *et al.*, 2010, Fukushima *et al.*, 2019, Gerisch *et al.*, 2012, Xiong *et al.*, 2016, Xiong *et al.*, 2010). Signal transduction or cytoskeletal waves were then reported by a growing number of studies in various types of mammalian cells such as neutrophils (Hepper *et al.*, 2012, Weiner *et al.*, 2007), macrophages (Masters *et al.*, 2016), fibroblasts (Case and Waterman, 2011), *Xenopus* eggs (Bement *et al.*, 2015), mast cells (Colin-York *et al.*, 2019, Wu *et al.*, 2013, Xiong

et al., 2016), keratocytes (Barnhart *et al.*, 2011), cultured neurons (Winans *et al.*, 2016), and cancer cells (Marchesin *et al.*, 2015). Waves of signaling (PIP3) and cytoskeletal components (actin filaments) show a distinct dynamic pattern with respect to each other (Gerhardt *et al.*, 2014, Gerisch *et al.*, 2011). In fused *Dictyostelium* cells, F-actin (denoted by its biosensor, LimE) appears as narrow “leading” and “trailing” bands separated with intermediate intensity while PIP3 (denoted by its biosensor, PHcrac) initiates with the leading edge and trails off through the entire region. PIP3/F-actin waves in fused *Dictyostelium* show similar patterns in mammalian cells such as macrophages. An example is shown in Fig. 3.

To explain wave propagation, and other features of the signal transduction networks (shown in figures 1B and 4A) including spontaneous activations, and refractory periods, we have proposed that this network is excitable. Within the signal transduction excitable network (STEN), an activator triggers a fast, autocatalytic loop that generates positive feedback, and a slower inhibitor forms a delayed negative feedback loop. As the activator and inhibitor diffuse, the activities of these processes travel throughout the medium in the form of a propagating wave (Iglesias and Devreotes, 2012). A number of mathematical models consisting of such reaction–diffusion equations for actin waves have also been proposed to capture wave formation and propagation (Bement *et al.*, 2015, Hecht *et al.*, 2010, Meinhardt and de Boer, 2001, Miao *et al.*, 2019, Xiong *et al.*, 2010). Further refinement of this model proposes that local regions of the cell cortex transition between inactive, active, and refractory states, designated as back (B), front (F), and refractory (R) states, respectively. The back and front markers such as PTEN and PI3K, usually associate with the B and F states of the cell

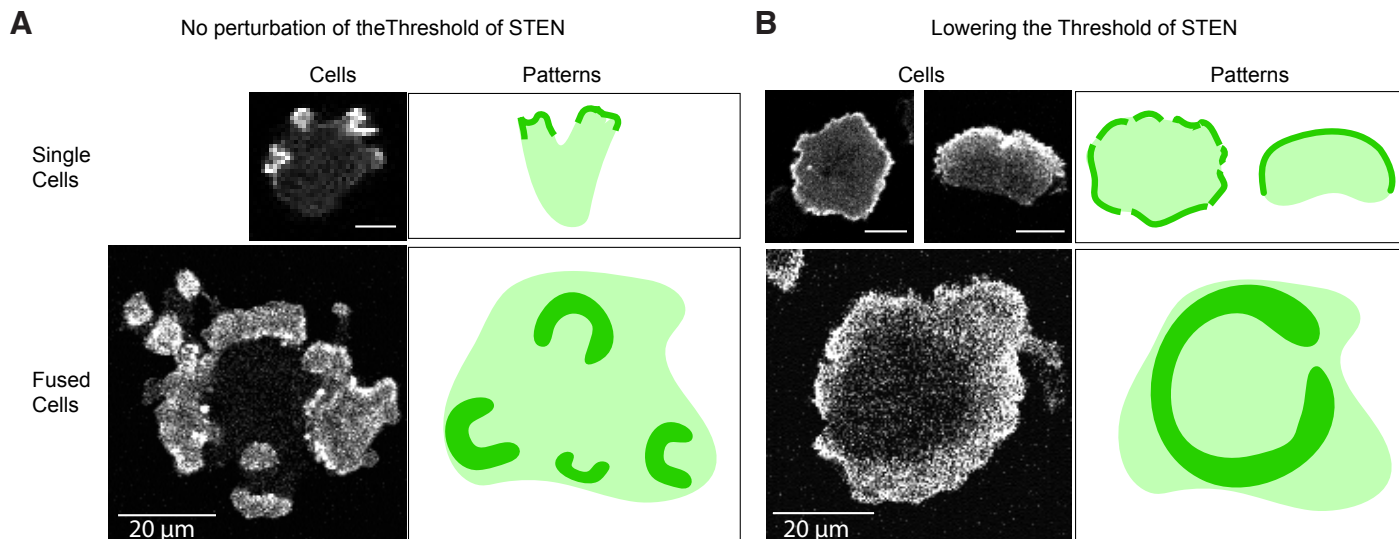


Fig. 5. Perturbation of signal transduction excitable networks (STEN) threshold alters wave behavior and ultimately changes protrusion pattern necessary for cell migration. Confocal images or cartoons showing *LimE* (F-actin biosensor) wave patterns on the basal surface of single (top) or fused (bottom) *Dictyostelium* cells, in absence (A) or presence (B) of synthetic perturbation to the threshold for STEN activation. In these experiments, the threshold for STEN activation was lowered by reducing PIP2 levels or increasing Ras/Rap activities at the cell cortex using the chemically inducible dimerization (CID) system. In single cells, such a perturbation causes the size of cellular protrusions to expand from small macropinosomes or pseudopodia (A) (top row) to wide, sheet-like protrusions resembling lamellipodia (B) (top row). This ultimately changes the migratory mode of the cells from amoeboid to oscillatory or fan-shaped. For the amoeboid cells, the waves start at a distinct point and extinguish after a few micrometers of travelling (A) (top row). In case of the ‘oscillators’, the wave initiates from a much bigger region, expands and covers the basal membrane of the cell momentarily, before extinguishing from the centre outward resulting in the collapse of the cell (B) (top row). In the fan-shaped cell, the expansion of the wave is similar, but asymmetric and therefore pushes out a wide band on the front of the cell (B) (top row). After acute lowering of PIP2 at the cortex of the fused cells, the waves propagate symmetrically without stopping in all directions (B) (bottom row), in comparison to waves in the unperturbed cells, which have asymmetric expansion patterns, smaller range and break up easily (A) (bottom row). These waves also propagate with a significantly faster speed in comparison to those seen in the unperturbed cells.

cortex. The B and F regions are mutually inhibitory, and therefore, create the positive feedback loop. The F and R states are related through a delayed negative feedback loop (Fig. 4A). In resting cells, most of the cortex is in the B state. Once initiated, waves propagate outwardly because diffusion of F-state components triggers activation in adjoining B but not R regions. The trailing R region ensures unidirectionality of the waves and their annihilation upon crossing each other (Fig. 4B) (Miao et al., 2017).

Studies in multiple labs are beginning to delineate the architecture of the signal transduction and cytoskeletal networks. In STEN, evidence suggests that a mutually exclusive interaction between Ras/Rap and PI(4,5)P2/PI(3,4)P2 can be assigned to F and B, respectively, whereas there is negative feedback through PKBA/PKBR1, which is assigned to R. Interestingly, PIP3 as an activator of PKBA, plays a negative role in STEN, while it is also an important positive regulator to actin polymerization (Figs. 1B and 4). The cytoskeletal network is also excitable and is referred to as the cytoskeletal excitable network (CEN) (Figs. 1B and 4A) (Li et al., 2018, Miao et al., 2019). The activities of CEN are more localized and rapid than those of STEN. Studies in *Dictyostelium* have shown that in the absence of signal transduction, these cytoskeletal events generate short, narrow protrusions, whereas coupling of STEN and CEN produces waves at the edge of cells followed by forward expansion of the membrane in the form of pseudopodia or broad lamellipodia-like protrusions (Huang et al., 2013, Miao et al., 2019, Shi et al., 2013, Tang et al., 2014, Vicker, 2002). Such correlations between signal transduction and actin

waves, and leading edge protrusions are also found in mammalian cells such as neutrophils (Weiner et al., 2007), fibroblasts (Jalal et al., 2019, Zhang et al., 2018), keratocytes (Barnhart et al., 2011) and cancer cells (Yang et al., 2018).

The non-linear feedback models suggest that alteration in individual components can have a significant impact on the overall excitability of the networks (Iglesias and Devreotes, 2012). In recent studies, the threshold for STEN activation was synthetically reduced, by either decreasing PIP2 level or increasing Ras/Rap activities at the cell cortex, which resulted in significantly increased speed and range of F-actin waves in *Dictyostelium* cells (Miao et al., 2017). Such a change in wave propagation resulted in expansion of small, cup-like protrusions to very wide, lamellipodia-like protrusions, and ultimately, caused changes in cellular migratory modes from amoeboid to keratocyte-like and oscillatory (Fig. 5) (Miao et al., 2019, Miao et al., 2017). This work further supports the idea that STEN-CEN coupling generates sustained protrusions of migrating cells. Moreover, such abrupt and reversible changes in migratory modes between amoeboid and gliding indicate that migratory behaviors demonstrated by various eukaryotic cells could arise from a basic mechanism of STEN-CEN coupling.

***Dictyostelium* makes predictions about eukaryotic migration**

We propose that, as in *Dictyostelium*, wave patterns of signal transduction components control protrusive activities in mammalian

cells. It has been previously reported that local production of PIP3 or Rac1 activation, by optogenetics, is sufficient to initiate actin growth cone-like “waves” in developing neurons or reorient polarity and guide migration in neutrophils (Graziano *et al.*, 2017, Kakumoto and Nakata, 2013). Moreover, the ability of manipulations of key signaling molecules or enzymes to create migratory and hyperactive phenotypes in mammalian cells, can be interpreted as changes of wave propagation. Examples include SHIP1 or PTEN deletions in neutrophils show increased motility and recruitment to inflamed sites (Sarraj *et al.*, 2009), overexpression of cancer-associated, membrane translocation-incompetent PTEN mutations showed increased cell proliferation and migration in breast epithelial cells (Nguyen *et al.*, 2015), and mutations of K-Ras promote migration and invasion in various cancers (Chu *et al.*, 2018, Kang *et al.*, 2018, Mann *et al.*, 2016, Millien *et al.*, 2018, Stuelten *et al.*, 2018). These studies point towards the possibility that STEN is the integration site of extrinsic cues which control migration. There is an increasing appreciation of the ability of cells to integrate environmental signals, such as chemoattractant stimuli, electric fields and mechanical forces, and to move accordingly. Integration of these environmental signals with STEN and CEN, and the various regulatory feedback loops involved between them may be sufficient to explain the vast array of protrusions and migratory modes observed in diverse eukaryotic cells under physiological and pathological conditions.

Acknowledgements

We thank all members of the P.N.D. laboratory for helpful suggestions for this work. We thank the Johns Hopkins University (JHU) Microscope Facility for confocal microscopy. This work was supported by NIH grant R35 GM118177 (to P.N.D.), AFOSR MURI FA95501610052, DARPA HR0011-16-C-0139, as well as NIH Grant S10 OD016374 (to S. Kuo of the JHU Microscope Facility).

Author contributions

D.S.P., T.B. and Y.M. contributed experimental data; D.S.P., X.L. and P.N.D. wrote the manuscript; T.B. conceptualized figure 2, X.L. prepared figures 1, 2, 4 and 5, and D.S.P. made figure 3; D.S.P., X.L., T.B., Y.M. and P.N.D. contributed to manuscript revision and approved the submitted version of the manuscript.

Competing financial interests

The authors declare no competing financial interests.

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