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Excitable Signal Transduction Networks in Directed Cell Migration

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Abstract

Although directed migration of eukaryotic cells may have evolved to escape nutrient depletion, it has been adopted for an extensive range of physiological events during development and in the adult organism. The subversion of these movements results in disease, such as cancer. Mechanisms of propulsion and sensing are extremely diverse, but most eukaryotic cells move by extending actin-filled protrusions termed macropinosomes, pseudopodia, or lamellipodia or by extension of blebs. In addition to motility, directed migration involves polarity and directional sensing. The hundreds of gene products involved in these processes are organized into networks of parallel and interconnected pathways. Many of these components are activated or inhibited coordinately with stimulation and on each spontaneously extended protrusion. Moreover, these networks display hallmarks of excitability, including all-or-nothing responsiveness and wave propagation. Cellular protrusions result from signal transduction waves that propagate outwardly from an origin and drive cytoskeletal activity. The range of the propagating waves and hence the size of the protrusions can be altered by lowering or raising the threshold for network activation, with larger and wider protrusions favoring

gliding or oscillatory behavior over amoeboid migration. Here, we evaluate the variety of models of excitable networks controlling directed migration and outline critical tests. We also discuss the utility of this emerging view in producing cell migration and in integrating the various extrinsic cues that direct migration.

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DIVERSITY AND IMPORTANCE OF DIRECTED CELL MIGRATION

Nearly all cells in living organisms move spontaneously and with direction from extrinsic cues, although the mechanisms of propulsion and sensing are extremely diverse. It is likely that cell migration initially evolved to escape nutrient depletion, and it has been adopted for an enormous variety of fascinating processes. Bacteria and many free-living eukaryotic cells such as protozoa move with flagella, cilia, or related internal or external appendages. Other free-living cells, such as amoebae, and most metazoan cells move with morphological extensions such as pseudopodia, lamellipodia, or blebs, although many have cilia and some, such as sperm, rely on flagella for propulsion (**Figure 1**). A multiplicity of extrinsic cues direct migration, including light (Armitage & Hellingwerf 2003), chemicals (Bagorda & Parent 2008, Tessier-Lavigne 1994), mechanical forces (Harland et al. 2011, Lo et al. 2000), electric fields (Cortese et al. 2014, Gao et al. 2011, Zhao et al. 2006), and temperature (Ramot et al. 2008, Whitaker & Poff 1980). Some of these cues are illustrated in **Supplemental Figure 1**. Cells are capable of integrating these cues, which come from the environment as well as from other cells (Haeger et al. 2015, Rørth 2011) (see **Supplemental Table 1**).

Cell migration is critical for an extensive range of physiological events. During metazoan development, the concerted movements of cell sheets bring about gastrulation (Keller 2005, Leptin 2005, Yang et al. 2002) and neurulation (Theveneau & Mayor 2012), and groups of cells migrate coordinately during the formation of organs and glands (Montell 2008). Single neural crest cells travel to distal sites, participating in a wide array of different tissues, and germ cells traverse through the embryo to find the gonad (Blaser et al. 2006, Richardson & Lehmann 2010). Neural precursors and glial cells (Klämbt 2009) migrate directionally, whereas neuronal growth cones, tethered to growing axons, seek specific targets (Wen & Zheng 2006). In the adult, immune

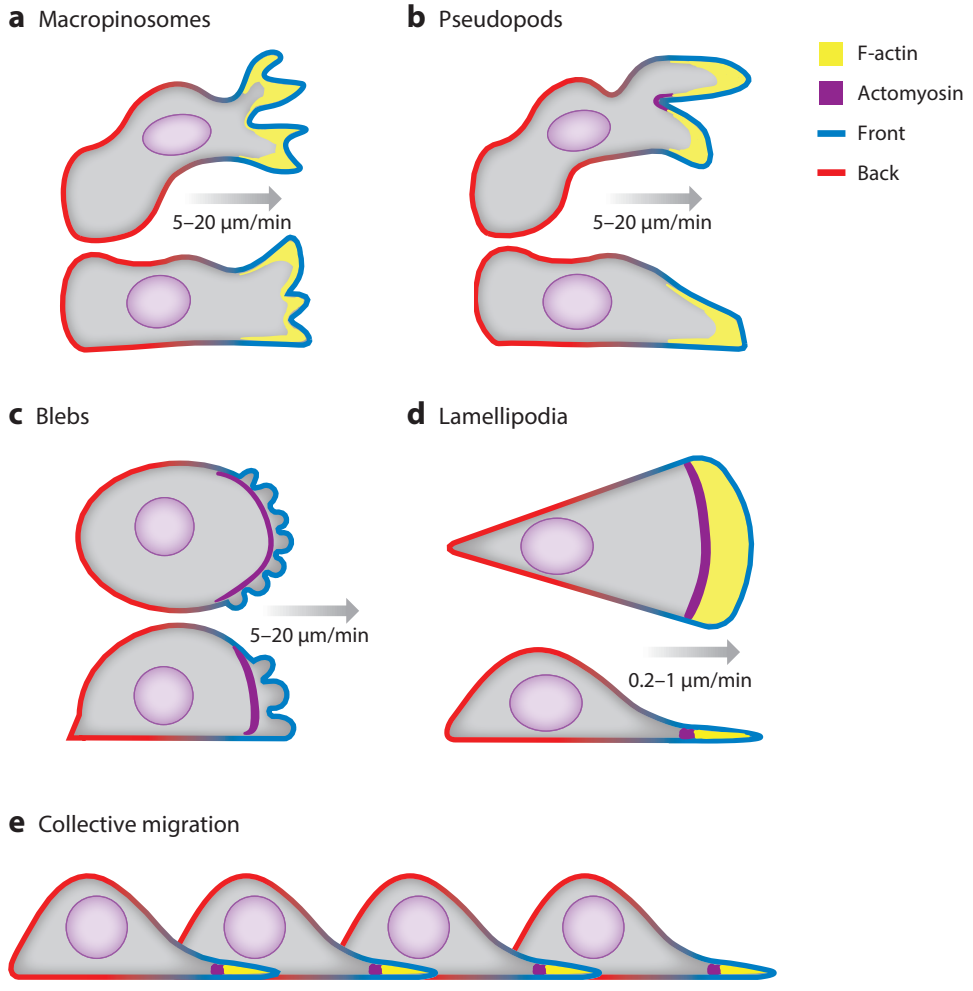

Figure 1

The diverse array of projections that move migratory cells forward are diagrammed in coronal and sagittal slices. The blue membrane represents the front of the cell, and the red membrane represents the back of the cell. In these representations, only the polymerizing F-actin and contractile actomyosin at the leading edges of cells are highlighted. (*a*) Macropinosomes are wide, cuplike-shaped structures at the top and sides of the cell. (*b*) Pseudopods are narrower protrusions usually found closer to the substrate. (*c*) Blebs are a result of the plasma membrane detaching from the actomyosin cortex due to contractile pressure. (*d*) Lamellipodia are sheetlike structures containing distinct actin and actomyosin zones. (*e*) Collective migration of cells connected and partially driven by cryptic lamellipodia.

cells traverse the vessels and lymphatics and seek invading substances (Nourshargh & Alon 2014, Weninger et al. 2014), fibroblasts and keratinocytes close wounds (Shaw & Martin 2009), regenerative stem cells mobilize from niches (Baumann 2014, Wright et al. 2001), and neurons remodel connections.

When these orchestrated movements occur improperly or are subverted, disease results. For example, defects in leukocyte migration cause sarcoidosis, infections, Wiskott-Aldrich syndrome, and other leukopenias (Lakshman & Finn 2001, Moulding et al. 2013). Excessive inflammatory

responses, in which cellular accumulations fail to resolve, underlie many disorders, including atherosclerosis, asthma, arthritis, and periodontal disease, as well as much of injury-associated pain (Lakshman & Finn 2001). An ostensible disease process involving cell migration is cancer metastasis, in which cells move away from primary tumors or enter the circulation and later extravasate to colonize new sites (Condeelis et al. 2005, Reymond et al. 2013).

Supplemental Material

There are salient similarities and differences in the migration behaviors of different cells (**Figure 1**; **Supplemental Table 1**). Most eukaryotic cells move by extending actin-filled protrusions at the cell front, coupled with actomyosin-based contraction, usually at the rear of the cell or the base of the projections. Variations on this general scheme can give rise to a diversity of migration modes. Amoeboid cells, such as leukocytes and some metastatic cancer cells, rhythmically extend and retract discrete actin-filled pseudopodia, producing intermittent advances in the cell front. Under some conditions, amoeboid motion can be characterized by the extension of cytoplasmic extensions termed blebs in which the actin polymerization is weakened relative to the actomyosin contraction (Blaser et al. 2006, Yoshida & Soldati 2006). Primordial germ cells (Blaser et al. 2006, Richardson & Lehmann 2010) and some metastatic cancer cells (Fackler & Grosse 2008, Wolf et al. 2003) exclusively deploy blebs. Keratinocytes or keratocytes move with a rocking, gliding motion, led by a wide, flat, actin-filled protrusion that often covers nearly three-quarters of the cell perimeter (Barnhart et al. 2010, Keren & Theriot 2008). Amoeboid and keratocyte-like motility is usually rapid, with cells able to move at 15 to 30 μm/min (Anderson & Cross 2000). Fibroblastic or mesenchymal migration involves gliding lamellipodia and is typically much slower, with cells advancing at 0.2 to 1 μm/min (Hou et al. 2012). These disparate speeds are reflected in the transient attachments made by amoeboid and keratocyte-like cells versus the developed focal adhesions that fibroblastic cells make to the extracellular matrix (Mogilner & Keren 2009, Parsons et al. 2010, Satulovsky et al. 2008). Apparently similar motions are also observed in collected groups and sheets of epithelial cells that form structures during development and regeneration. These movements are highly coordinated, likely through mechanical forces exerted directly through cell-cell attachments or adhesions to the extracellular matrix (Gupton & Waterman-Storer 2006). This review focuses primarily on the signal transduction events involved in amoeboid migration; however, many of the molecular events and concepts described below can be extended to account for other cell behaviors, including those seen during collective migration and movement through 3D environments.

With this vast assortment of migratory modes and mechanisms and the large number of independent investigators studying them, there is a need to define some of the major terms. Directed migration can be described conceptually as involving motility, polarity, and directional sensing (**Figure 2**). Motility refers to the ability of a cell to extend protrusions, coordinated with appropriate contractions and attachments, and to thereby translocate. Polarity indicates the relatively stable axis with a definite front and rear displayed by many cells; polarity provides persistence of movement and is distinct from the momentary asymmetry displayed by a motile cell extending a protrusion. Directional sensing denotes the capacity of a cell to sense a spatially heterogeneous cue and to respond biochemically independently of motility or polarity. In directed cell migration, these three processes occur concurrently and coordinately. Additional terms that are useful and prevalent in describing directed migration are included in a glossary (**Supplemental Table 2**).

COMPLEXITY OF SIGNAL TRANSDUCTION EVENTS INVOLVED IN DIRECTED CELL MIGRATION

The list of signal transduction events associated with directed cell migration is continuously growing. Much of this information is derived from genetic, biochemical, and biosensory analysis

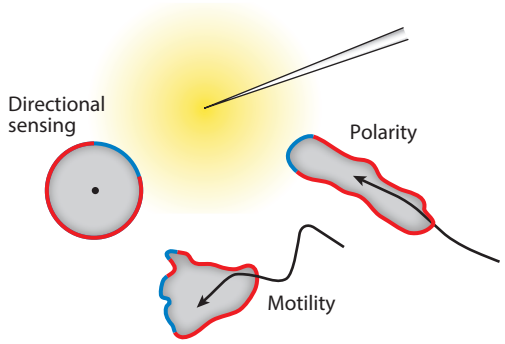

Figure 2

The three distinct processes—motility, polarity, and directional sensing—that coordinate to bring about directed migration toward the chemoattractant gradient (*yellow*). The region of the cortex facing the needle forms the front (*blue*); the quiescent back is demarcated by red.

Supplemental Material

of *Dictyostelium* cells responding to chemoattractant (**Supplemental Table 3**). Many external signals feed into a network of pathways (**Figure 3**). cARs and FARs (cAMP and folic acid GPCRs) and associated G proteins are essential for migration toward the respective chemical gradients and trigger many signal transduction events, which, as far as has been tested, are also locally activated under the guidance of electric fields (Allen et al. 2013; Meng et al. 2011; Miao et al. 2017; Zhao et al. 2002, 2006) or shear force (Artemenko et al. 2016, Décave et al. 2003). Importantly, network events are activated and cells move even in the apparent absence of external cues (Arai et al. 2010, Bosgraaf & Van Haastert 2009, Sasaki et al. 2007).

The network has a number of notable features. First, it contains multiple parallel or compensating pathways, as evidenced by the fact that individual disruptions at many nodes are relatively inconsequential whereas activating perturbations at the same ones produce striking phenotypes. For example, cells can move in the absence of PI3K signaling (Chen et al. 2003, Hoeller & Kay 2007, Takeda et al. 2007, Weiger & Parent 2012), but ectopic production of PIP3 is sufficient to initiate protrusions (Inoue & Meyer 2008, Kakumoto & Nakata 2013, Weiner et al. 2002), and excess accumulation of PIP3 leads to multiple persistent extensions (Iijima & Devreotes 2002, Sarraj et al. 2009). Inhibition of three or four parallel pathways is required for a significant loss of protrusion formation (Artemenko et al. 2016, Chen et al. 2007, Veltman et al. 2008) (**Supplemental Video 1**). Second, increments in chemoattractant trigger transient changes at many points in the network, with characteristic time courses (Caterina & Devreotes 1991) (**Figure 3**; **Supplemental Video 2**). For example, after a delay of a few seconds, PIP3 rises to a peak at 20 s, rapidly declines, and increases again in a series of secondary peaks during the next few minutes (Chen et al. 2003, Huang et al. 2013). Although the initial peak of F-actin occurs before that of PIP3, it is also followed by secondary peaks (Chen et al. 2003). The initial transient burst of F-actin is global, causing cells to freeze and then contract uniformly, whereas the secondary peaks are localized, producing spreading protrusions (Condeelis et al. 1990, Futrelle et al. 1982, Postma et al. 2003, Xiong et al. 2010). Other responses, such as PKB activation, are delayed with respect to PIP3, but most display the characteristic initial and secondary peaks (Kamimura et al. 2008). Whereas most of these events start at a basal level and increase, others begin at an elevated level and decrease (**Figure 4**). For example, PTEN transiently leaves the membrane and then returns (Funamoto et al. 2002, Iijima & Devreotes 2002). Remarkably, the same signal transduction events with the similar time courses are triggered by a brief application of shear force (Artemenko et al. 2016). Third, most of these actions take place at the membrane or cortex. In migrating and

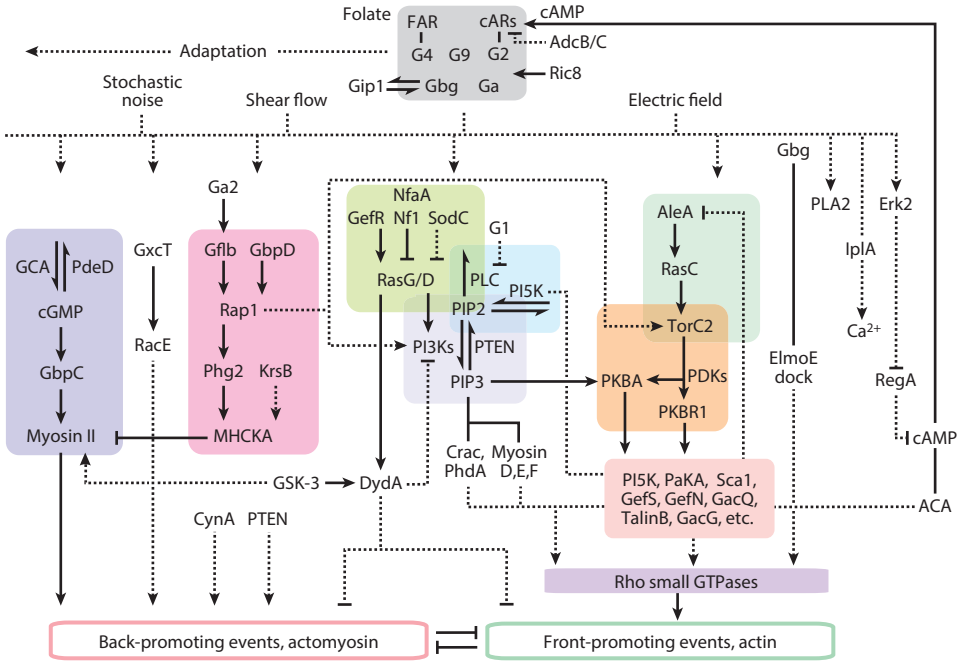

Figure 3

The network of signal transduction pathways involved in directed cell migration in *Dictyostelium*. Architecture is based on biochemical and genetic analyses and reflects an extensive series of experiments by independent investigators. In most cases, connections represent interpretations of phenotypes or stimulus-induced biochemical and biosensory behavior, contrasted with wild type, in cells carrying single or multiple gene deletions. Half-arrows are substrate-product relationships; solid connectors represent direct interactions between components, and dashed connectors denote inferred or indirect links. The references supporting these interactions are included in **Supplemental Table 3**.

Supplemental Material

chemotaxing cells, events such as Ras and PI3K activity occur at the tips of protrusions and are loosely referred to as *front*, whereas other events such as PTEN dissociation from pseudopods are designated as *back* (**Figure 4**; **Supplemental Video 3**). A list of front and back events is shown in **Supplemental Table 4**.

Reporting on migration-associated signaling events in mammalian cells is somewhat limited in depth, although there are extensive observations. Many GPCRs, RTKs, and other receptors influence migration. A large set of chemokines and chemokine receptors are functionally parallel to cARs and FAR in *Dictyostelium* (Jin et al. 2008). Elegant studies in macrophages have shown that local activation of RGS proteins (regulators of G protein signaling) is sufficient to direct migration (O'Neill et al. 2016) (**Supplemental Video 4**), which is consistent with earlier indirect studies in *Dictyostelium* and neutrophils (Neptune et al. 1999, Wu et al. 1995). Furthermore, studies of the role of Ras and PIP3 activation in mammalian cell migration are largely consistent with the *Dictyostelium* model (Artemenko et al. 2014). Research in mammalian cells has focused significantly on the role of the small GTPases Cdc42, Rac, and Rho as activators of SCAR (suppressor of cAR) proteins and formins and, consequently, as key regulators of cellular protrusions. Unfortunately, direct parallels are complicated by the presence of 15 Rho family proteins in *Dictyostelium* that do not readily fall into the three classes on the basis of sequence (Lim et al. 2002). Recent evidence suggests that *Dictyostelium* Rac1A/C and RacE may function similarly to mammalian Rac1 and

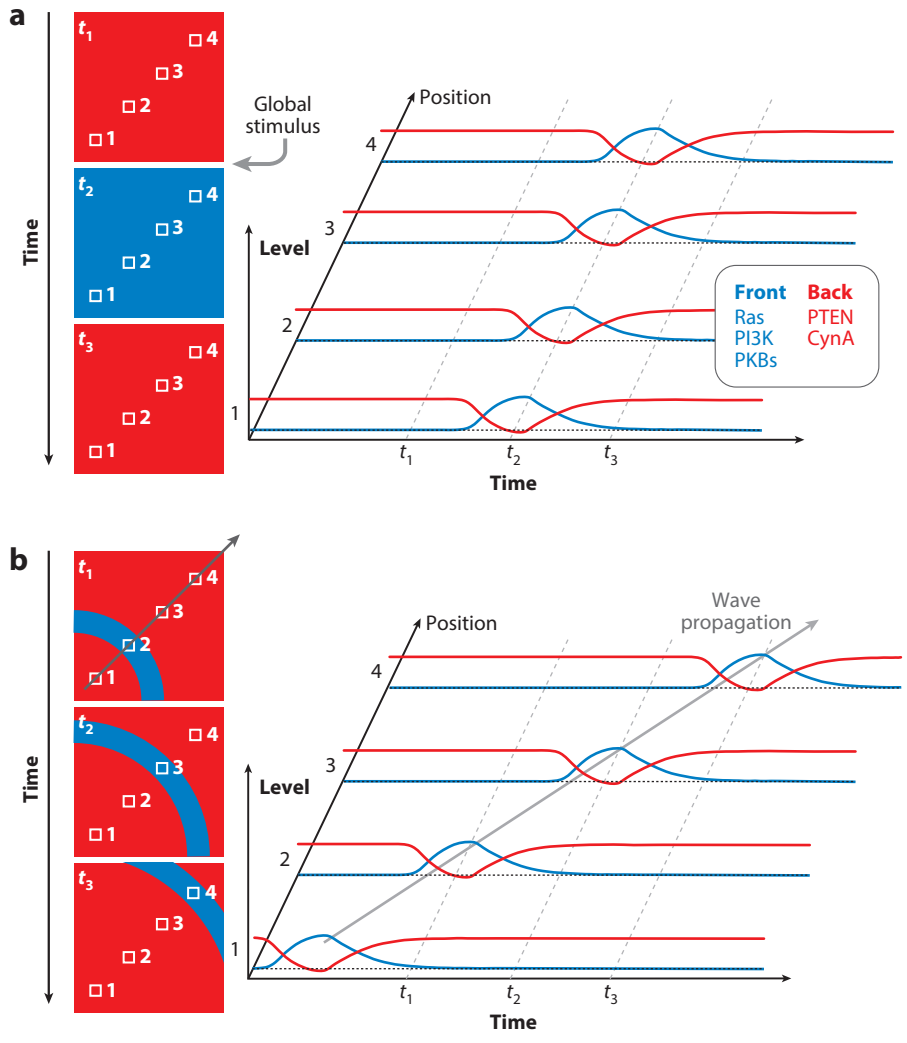

Figure 4

Correlation between spatial and temporal activities of front (*blue*) and back (*red*) proteins. (*a*) The colored boxes at the left represent sequential time instants. The small numbered white squares in each box denote spatial references. The plots at the right show how the global stimulus manifests as a time course at each spatial position and how the front and back proteins or biosensors (representative activities shown in the *adjoining box*) associate and dissociate, respectively. (*b*) Same format as in panel *a*, but showing the position of a propagating wave at three sequential times. The presentation of the stimulus as an advancing wave causes the temporal protein activities at each position to occur sequentially, in contrast to the synchronized events triggered by the global stimulus shown in panel *a*.

RhoA, respectively (Filić et al. 2012, Wang et al. 2013). With more parallel observations, such as the involvement of Ras and Rap family proteins (Khanna et al. 2016), TorC2, and PKBs upstream of the Rho family G proteins, understanding of the networks in the different systems appears to be converging (Filić et al. 2012). However, there are persistent differences, for instance, with respect to the regulation of myosin; in this case, cGMP plays a major role in *Dictyostelium*,

whereas ROCK and MLC kinase are key in many mammalian cells (Bosgraaf & van Haastert 2006, Vicente-Manzanares et al. 2009).

THE SIGNAL TRANSDUCTION EXCITABLE NETWORK (STEN)

The triggering of these events in the signal transduction network displays properties of biochemical excitability, including all-or-nothing responses to suprathreshold stimuli and a refractory period found in *Dictyostelium*. First, with saturating concentrations of chemoattractant, the initial peak of Ras or PI3K activity is the same whether the stimulus duration is 2 or 60 s (Huang et al. 2013). Second, whereas the response to increasing doses is graded, micrometer-sized patches behave as all-or-nothing elements (Nishikawa et al. 2014). Different sensitivities among patches as well as individual cells feed into the dose-response behavior. More sensitive methods have detected smaller, perhaps subthreshold, patches of Ras activity that do not expand (van Haastert et al. 2017). Third, refractory periods for chemoattractant and shear force–elicited PIP3 production were demonstrated by applying short, paired stimuli and by monitoring the response to the second stimulus (Artemenko et al. 2016, Huang et al. 2013, Nishikawa et al. 2014). All-or-nothing responses and refractory period are also displayed by immobilized neutrophils, although the kinetics are slower (Wang et al. 2014). The term signal transduction excitable network (STEN) was coined to encompass this behavior (Huang et al. 2013).

Excitability is also apparent in propagating waves of signal transduction and cytoskeletal components. Actin waves were first reported by Vicker in *Dictyostelium* (Vicker 2002) and were extensively characterized by Gerisch and colleagues (Bretschneider et al. 2004, 2009; Gerisch 2010; Gerisch et al. 2009, 2011; Schroth-Diez et al. 2009). Waves of the biosensors for Ras and PI3K activation were also observed in cells during migration (Gerisch et al. 2001, Xiong et al. 2010). Studies of (*a*) the recruitment of SCAR subunits, F-actin binding proteins, and biosensors for Ras, PI3K, and Rac activation and (*b*) coordinated dissociation of PTEN and myosin from the cell cortex suggest that the other components in the signal transduction and cytoskeletal networks would also show the same relative changes that have been established for responses to chemotactic stimulation (**Supplemental Video 5**). The correspondence between the temporal responses to global stimuli and the spatial distribution of the same components and activities in propagating waves is striking (**Figure 4**). That is, front components and activities are associated with the leading edge of the propagating wave, whereas back events, present on the rest of the cortex, are transiently absent from the active zone.

Similar propagating waves and evidence for excitability of signal transduction and cytoskeletal events have been observed in neutrophils (Weiner et al. 2007), mast cells (Wu et al. 2013), fibroblasts (Case & Waterman 2011, Ryan et al. 2012), and other cells (Winans et al. 2016). In some cases, similar phase relationships among various components have been documented. In mast cells, for example, F-actin zones correspond to troughs in PIP2 waves (Xiong et al. 2016). It remains to be determined whether these dynamic events are displayed by a few cell types under specific conditions or whether they are an unrecognized general feature of migrating cells and perhaps play further roles in cell physiology.

Many investigators have appreciated that the propagating waves reflect the properties of an excitable medium, whereby the activity in a restricted region is relayed forward and a trailing refractory zone assures unidirectionality and annihilation of crossing waves. As a means of formalizing these concepts, Miao et al. (2017) proposed that local regions of the cell cortex transition between inactive, active, and refractory states, designated as B, F, and R states, respectively (**Figure 5**). The B and F states are mutually inhibitory, creating a positive feedback loop. The F and R states are related through a delayed negative feedback loop. In resting cells, most of the cortex is in the

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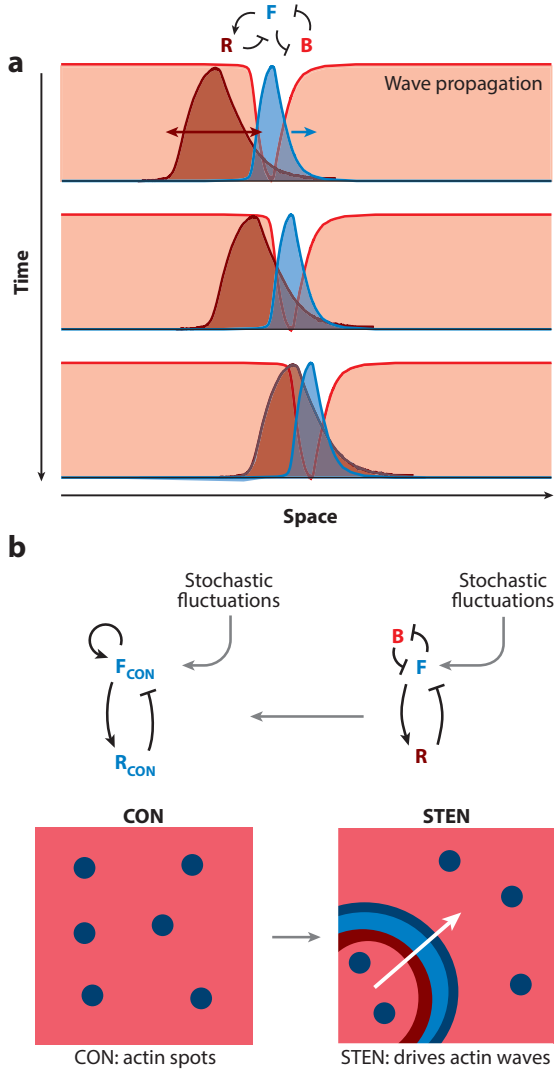

Figure 5

(*a*) A representation of how an excitable wave propagates on the membrane. The reciprocal front (F) and back (B) zones (*blue* and *red*, respectively) propagate, and the refractory (R) zone (*deep red*) trails, ensuring unidirectional front propagation. (*b*) Coupling between [CON (cytoskeletal oscillatory network)] and signaling [STEN (signal transduction excitable network)] activities generates propagating waves of cytoskeletal activity. In the absence of STEN activity, there are flashes of actin polymerization, as shown by the dark blue spots in the box at the left (a random 2D area on the cortex). Triggering of STEN activity causes the CON spots to expand in space, driven by the traveling STEN wave front (*blue* trailed by *deep red*; *arrow* shows direction); this development creates actin waves.

B state. Once initiated, waves propagate outwardly because diffusion of F-state components triggers activation in adjoining B but not R regions. This model also captures many observed features of signal transduction networks, including all-or-nothing and refractory behavior.

The spontaneous signal transduction and cytoskeletal activities are independent but can couple (**Figure 5**). On one hand, a prescient study demonstrated that caffeine induced reciprocal waves

of PIP3 and PTEN to propagate around the perimeter of immobilized *Dictyostelium* cells (Arai et al. 2010). Several other studies showed that Ras, PIP3, and PTEN activity waves occur spontaneously on the basal surfaces of immobilized cells, albeit with diminished frequency (Taniguchi et al. 2013, Xiong et al. 2010). One study showed that spontaneous, dynamic PI3K activation occurs in neutrophils that were immobilized by a cocktail of cytoskeletal inhibitors (Wang et al. 2014). The theme of independent coupled networks was advanced by studies demonstrating a spatiotemporal association of larger waves of cytoskeletal activities with STEN reporters (Weiger et al. 2010). Furthermore, in cells migrating over perforations, F-actin is polymerized at points of high curvature, but only in regions of accumulation of PIP3 (Jasnin et al. 2016). On the other hand, when multiple signal transduction pathways were inhibited, the cytoskeletal reporters reverted to rapid flashes or oscillations, and Huang et al. (2013) proposed the designation cytoskeletal oscillatory network (CON). The flashes and oscillations do not expand as waves, arguing against molecular models of propagation that rely solely on interactions among cytoskeletal components. Taken together, these studies suggest that the cytoskeletal waves are essentially a readout of the propagating signal transduction waves, which the spontaneous cytoskeletal events help initiate. However, under certain conditions the cytoskeletal oscillations could be synchronized and cells could be steered, apparently independently of STEN (Hoeller et al. 2016, Yang et al. 2016).

There are many unanswered questions surrounding the STEN wave theory of cell migration. First, the highly parallel nature of the network noted above, in which activation at single nodes lowers the threshold but no single perturbation eliminates the signal transduction waves or blocks migration, has hampered efforts to identify the core feedback loops underlying excitability and wave propagation. In the analogous case of the nerve action potential, the unifying concept of membrane potential provides a strong framework for comprehending the roles of the many participating ion channels. The realization of a similar principle would greatly facilitate the understanding of the many contributing components and responses in the STEN. Second, the molecular mechanism of coupling of STEN waves to cytoskeletal activity is not known, although Rho family GTPases are leading candidates. In *Dictyostelium*, the Rac binding domain from mammalian Pak1 localizes to the leading edges of migrating cells and travels coordinately with the STEN waves that drive large protrusions, but this domain does not appear to associate with the unaccompanied rapid flashes and oscillations of cytoskeletal activity (Huang et al. 2013). In neutrophils, production of PIP3, without activation of the chemoattractant receptor, leads to activation of Rac (Inoue & Meyer 2008, Toettcher et al. 2011, Weiner et al. 2002). Given the extensive observations of the roles of Cdc42 and Rac in promoting and regulating cytoskeletal events, these small GTPases should represent a natural point of coupling between STEN and cytoskeletal activities. Third, the topological connection of laterally expanding waves on the cell cortex to the various types of protrusions that cells display (see **Figure 1**) is a current active area of research.

PROPAGATING STEN WAVES AND THE PROTRUSIONS THAT UNDERLIE MOTILITY

The coupling of STEN waves to cytoskeletal activity appears to explain the topology of certain protrusions involved in migration of amoeboid cells, and given their flexibility, similar excitable systems may shape the great variety of protrusions made by cells (**Figure 6**). Careful examination of the waves propagating on the basal surfaces of cells shows that SCAR subunits and actin binding proteins outline the wide advancing waves of Ras and PI3K activity in the F-state region (Gerisch 2011, Huang et al. 2013, Schroth-Diez et al. 2009, Weiner et al. 2007). The expanding cuplike protrusions seen in lattice light sheet microscopy (Chen et al. 2014) of randomly migrating vegetative *Dictyostelium* cells could be explained by propagating STEN waves driving

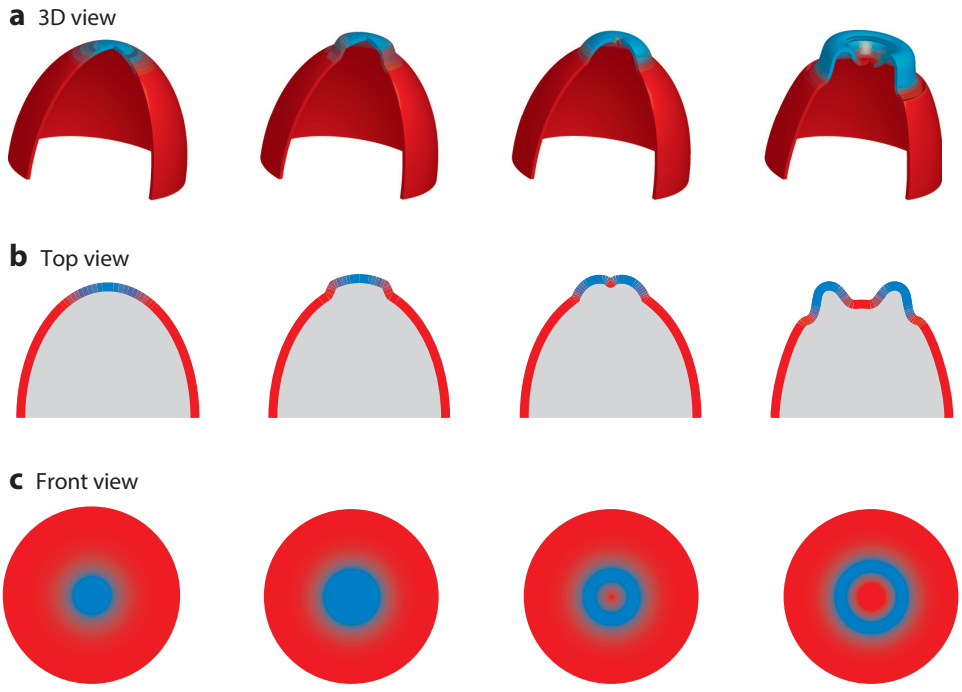

Figure 6

Representation of the coupling between wave propagation and the topology of cellular protrusions. (*a*) 3D representation of the formation of cuplike structures on the cortex as a wave propagates [*blue* and *red* denote front (F) and back (B) activity, respectively]. (*b*,*c*) Top and front views of these structures show how these protrusions are born out of wave propagation as the front activity spreading outward creates a hole in 2D, which translates to a cuplike protrusion in 3D.

cytoskeletal activity. Consistently, actin binding proteins rim the cuplike structure, which is filled with a broader zone of Ras and PI3K activity (Pollitt et al. 2006, Veltman et al. 2016). These structures, referred to as macropinosomes, are abundant in axenic cells that have been selected for fluid uptake (Maniak 2001). The mutation that confers axenic growth capacity is a homolog of the human Ras GAP NF1, consistent with the elevated Ras activity in these cuplike structures (Bloomfield et al. 2015). However, when near the substrate, many macropinosomes clearly function in moving the cells, questioning the functional distinction between these structures and pseudopods. Some distinctions between macropinosomes and pseudopods are found below and in **Supplemental Table 2**. Further evidence that these structures underlie motility is the fact that changing their properties dramatically alters migration: Perturbations at key nodes of the STEN lower the threshold for network activation, increase wave speed and range, expand the cuplike structures, and switch the mode of migration from amoeboid to keratocyte like and oscillatory (Miao et al. 2017) (**Supplemental Videos 6** and **7**).

Supplemental Material

The pseudopodia of migrating, differentiated *Dictyostelium* cells and other amoeboid cells likely have a similar basis in a spontaneous local activation of STEN and wave propagation, although there are salient differences in the structures formed. Pseudopodia are narrower and protrude further than macropinosomes. Thus, the propagating STEN wave expands differently to accommodate these different structures. These morphologically distinct protrusions can exist simultaneously in different locations on the same cell. Furthermore, under certain conditions,

chemoattractants can elicit pseudopodia without abolishing the waves underlying macropinosomes (Ecke & Gerisch 2017). We know from theoretical considerations that small changes in the set point of the excitable network can lead to structures with quite different dimensions and durations (Shi et al. 2013). The fact that small perturbations in the network can rapidly switch cuplike structures to gliding lamellipodia-like protrusions is consistent with the idea that the various types of protrusions are on a continuum arising from the same basic mechanism (Miao et al. 2017).

We speculate that further modification of the basic STEN wave scheme may account for the blebs mediating the migration of germ cells, some cancer cells, and constrained amoebae and may therefore provide a clue as to the mechanism of guidance of blebbing cells. The idea is as follows: As the STEN wave expands, the response at the origin subsides, and the region becomes refractory, recruiting back proteins such as myosin and causing contraction. If actin polymerization is curtailed during this process and protrusion at the rim is selectively impaired, the local contraction might cause separation of the cortex and membrane, producing the bleb. Consistent observations include the appearance of myosin at the bases of blebs in germ cells (Blaser et al. 2006) and, in *Dictyostelium*, the prevalence of blebs following the chemoattractant-elicited actin burst when myosin is highest or in cells with impaired actin polymerization (Langridge & Kay 2006). If blebbing sites are driven by STEN activity, a mechanism for steering blebbing cells would be provided.

THE BIASED EXCITABLE NETWORK (BEN) CONCEPT: BIASING OF STEN ACTIVITY BY EXTERNAL CUES AND DIRECTED MIGRATION

The concept of cell migration described above, captured computationally with independent coupled excitable modules for STEN and CON, would be sufficient to allow the unstimulated cells to generate protrusions leading to random motility, but directional movement requires that the protrusions be more likely in the direction of the external gradient (**Figure 7**). Extrinsic cues, such as chemoattractants or mechanical signals, could mediate directional sensing by biasing the threshold for activation. For example, if the threshold level drops as receptor occupancy increases, then the part of the cell facing the chemoattractant source will show a greater rate of pseudopod formation, and likely larger protrusions, than the rear. Indeed, such guidance mechanisms, broadly referred to as biased excitable network (BEN) schemes, are plausible and likely operate in many systems.

In *Dictyostelium* cells and human neutrophils exposed to chemotactic gradients, fewer protrusions are seen at the rear of a responding cell. Similarly, in immobilized cells, STEN activity is suppressed on the side of the cell away from the gradient (Tang et al. 2014). These findings suggest that a shallow difference in receptor occupancy across the cell lowers the threshold at one end and raises it at the other end. As this suppression cannot be achieved using only local information, a means of sharing information globally is needed, and various models incorporating global inhibitors have been proposed (Levchenko & Iglesias 2002, Levine et al. 2006, Meinhardt 1999, Parent & Devreotes 1999). When linked to an excitable network, these models are referred to as biased excitable network–global inhibitor (BENGI) and local excitation–global inhibition–biased excitable network (LEGI-BEN) schemes (Tang et al. 2014) (**Figure 8**). These closely related classes of models differ in how the inhibitory signal is generated. In BENGI schemes, the signal arises as the result of a feedback loop from the activity of the excitable system (Meinhardt 1999). This feedback may be the result of signaling components or cytoskeletal activity, as in the pseudopod-centered model (Insall 2010). In LEGI-BEN schemes, the inhibitory signal is the result of a feedforward loop from receptor occupancy itself, which generates a response regulator. Mathematical models show that both mechanisms can spatially bias the activity of STEN, cytoskeletal activity, and protrusions (Hecht et al. 2011, Meinhardt 1999, Neilson et al. 2011, Xiong et al. 2010), resulting in directed motility. Experiments on *Dictyostelium* mutants lacking

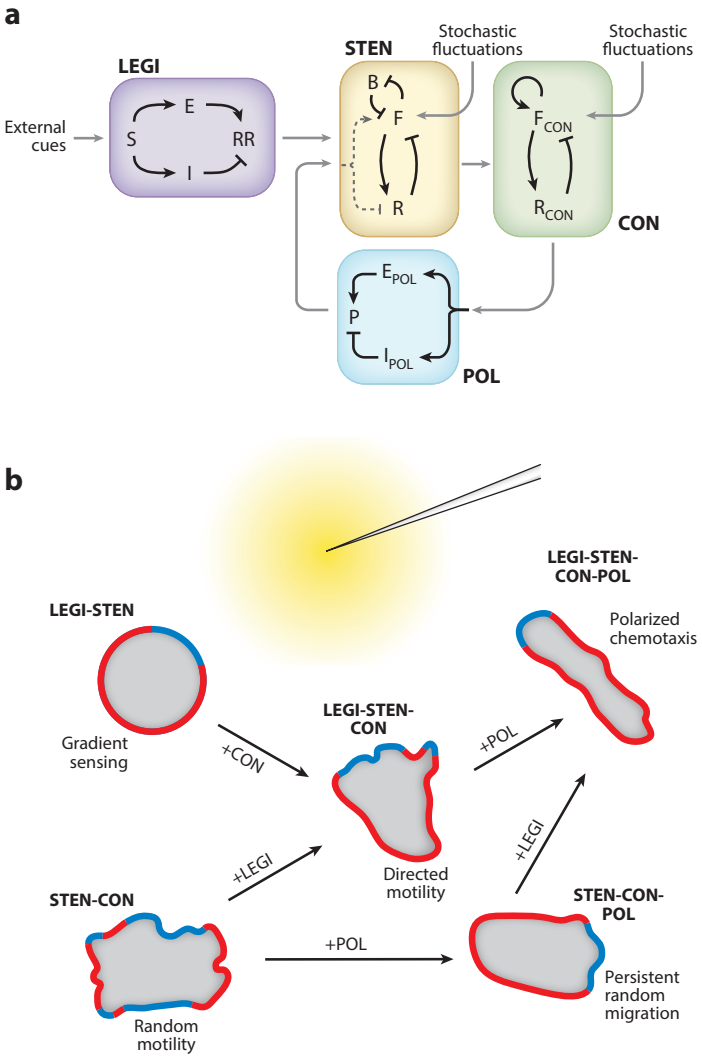

Figure 7

A representation of the various modules and networks involved in directed cell migration. (*a*) A schematic showing the coupling between the modules and networks. (*b*) How these modules and networks coordinate to orchestrate different kinds of cell motility in the presence of a chemoattractant gradient (*yellow*), with blue and red depicting the front (F) and back (B) activities, respectively. Abbreviations: CON, cytoskeletal oscillatory network; E, local excitation; E_{POL} and I_{POL}, feedforward exciter and inhibitor mediating polarity; I, global inhibition; LEGI, local excitation–global inhibition module; P, polarity; POL, polarity module; R and R_{CON}, negative feedback regulators of STEN and CON; RR, response regulator; S, external input; STEN, signal transduction excitable network.

G protein subunits and wild-type cells exposed to multiple temporal stimuli favor a LEGI circuit biasing mechanism (Tang et al. 2014) (**Figure 8**). Conveniently, the global inhibitor in the feedforward LEGI module can also account for the ability of chemotactic cells to ignore the ambient level of stimulus and to respond only to the steepness of the gradient.

These models link directional sensing to motility, but they do not account for the stable polarization displayed by differentiated *Dictyostelium* cells, human neutrophils, and many other

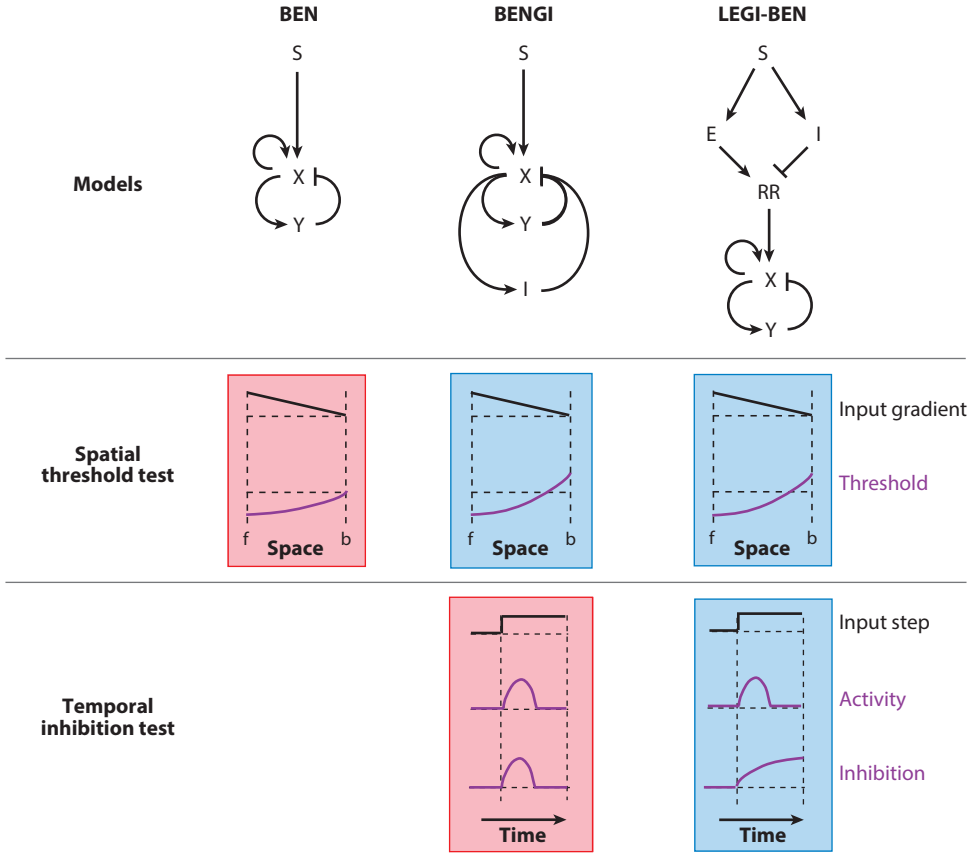

Figure 8

A representation of how LEGI-BEN (local excitation–global inhibition–biased excitable network) emerged as a realistic model of directional sensing. (*Top*) Three such proposed models are put through two specific tests. Structures are shown at the top: E, local excitation; I, global inhibition; RR, response regulator; S, external input; X and Y, activator and inhibitor of the excitable system. (*Middle*) The outputs shown schematically represent the responses observed. The blue shading and red shading represent passed and failed tests, respectively. For the spatial threshold test, when an input gradient is applied (f, front of cell; b, back of cell), all three models display a lowered threshold at the front. In the BENGI (biased excitable network–global inhibitor) and LEGI-BEN models, which have global inhibitors, the threshold is raised at the back. This results in better directed movement. (*Bottom*) To distinguish between these two models, the cells were put through a temporal inhibition test, whereby the global inhibitor level is quantified for a homogeneous input of chemoattractant. For the BENGI model, as the inhibition follows the activator level, it dies down with time. However, in the LEGI-BEN scheme, the inhibitor is derived from the input itself, causing the level of inhibition to rise with time as the step is sustained, matching real cell observations.

cell types even in the absence of a gradient. Polarity can be accomplished by competing local excitatory and global inhibitory signals formed as the result of positive and negative feedback loops (**Figure 7**). The positive feedback loop essentially lowers the threshold at locations where a firing has taken place, enabling unstimulated cells to move persistently (Cooper et al. 2012, Shi et al. 2013) (**Figure 7**). This or a similar mechanism may account for the low number of de novo pseudopods seen in migrating amoeboid cells. In *Dictyostelium* and neutrophils, this intrinsic polarity mechanism is used both for memory and to sharpen the response of cells to external

cues (Janetopoulos et al. 2004, Wang et al. 2014). Most of the polarity is actin dependent, but an actin-independent component has been observed (Skoge et al. 2014).

FROM A CACOPHONY OF INHIBITORS COMES ORCHESTRATED MOVEMENT

The inhibitors of the BENGI and LEGI-BEN models are distinct from the negative feedback loops that cause shutoff and refractoriness in the excitable networks. Depending on how these global inhibitors are deployed, they can explain the capacity of cells to cease responding to a uniform stimulus while remaining responsive to subsequent increases, referred to as adaptation (Tang et al. 2014). In *Dictyostelium* cells, the refractory periods of the STEN are shorter than the time courses of adaptation and must involve faster inhibitors. This hierarchy of inhibitors with different kinetics is needed to account for the complex kinetics of many of the biochemical responses in STEN and CON, such as cGMP production, Ras and PI3K activation, and actin polymerization: Uniform stimuli trigger an initial peak response that shuts off within 45 s and is followed by a series of secondary events that subside after a few minutes. Additional responses can be triggered by incrementing the chemoattractant or by removing it, allowing for a period of recovery, and then reapplying the same level. The concatenation of these two kinetic behaviors was apparent in the earliest observations of oscillations of light scattering displayed by shaken *Dictyostelium* cells (Gerisch & Hess 1974) but has led to considerable confusion in terminology, which **Supplemental Table 2** attempts to sort out by including these terms in a glossary.

Supplemental Material

The proposed schemes and descriptions of directed migration place numerous constraints on the molecular events that mediate inhibitory activities. There are global inhibitors, delayed feedback inhibitors, and polarity inhibitors. **Supplemental Table 5** lists the proposed inhibitors. Despite the compelling phenomenology, molecular events have been tentatively assigned to only a few inhibitors. First, evidence shows that PKBs play an important role in negative feedback regulation of Ras activity because cells lacking PKBA and PKBR1 display persistently high RasC activity in pull-down assays (Charest et al. 2010) and because, in immobilized cells lacking PKBs, RBD patches are more frequent but rapidly quenched by recruitment of PKBA (Miao et al. 2017). Second, in neutrophils, membrane tension is an important negative regulator (Diz-Muñoz et al. 2013, 2016). Typically, 10–15-μm cells induced to lengthen to 50 μm still display distinctive front and rear characteristics (Houk et al. 2012). Upon severing, thereby releasing membrane tension, the rear portion instantly forms a new SCAR-decorated front. Similarly, highly polarized *Dictyostelium* cells lacking KrsB sometimes break during migration, whereupon the rear portion instantly forms a new front (Artemenko et al. 2012). Moreover, cells lacking dynacortin, which contributes to cortical viscoelasticity, do not become as polarized as wild-type cells (Kabacoff et al. 2007). Third, in *Dictyostelium*, disruption of ostensible negative regulators such as SodC (Veeranki et al. 2008), the Ras GAPs NF1 and Nfa (Zhang et al. 2008), and PTEN (Iijima & Devreotes 2002) appears to lengthen the duration of chemoattractant-triggered responses and promotes protrusive behavior. Other genes, including those encoding KrsB, a set of novel regulator of adhesion and motility (RAM) proteins, and myosin, appear to play a similar role (Artemenko et al. 2012, Lampert et al. 2017). Of course, it is difficult to distinguish negative regulators from feedforward and negative feedback inhibitors, and further studies are needed to define the precise roles that each of these genes plays.

EMERGENCE OF STEN AS A CONTROLLER OF MIGRATION

The concept of STEN as a regulator of cell migration has advantages for directional sensing. First, if the external cue differentially regulates the threshold across the cell, as proposed in the BEN

mechanisms incorporating global inhibitors, slight shifts in the threshold can lead to enhanced activity at the front or to completely suppressed activity at the rear. Although direct measurement of local thresholds on a cell exposed to a gradient is technically challenging, a difference in sensitivity at the ends of a stably polarized cell has been repeatedly demonstrated (Jin et al. 2000, Skoge et al. 2014, Swanson & Taylor 1982, Wang et al. 2014). Second, the excitable character of the STEN produces stochastic projections characteristic of amoeboid motion, which is effective in sensing extrinsic cues. The repeated extension of pseudopodia from a defined region at the anterior of the polarized cell coupled to contractility at the rear provides rapid motility. However, the cell's track is readily correctable by favoring pseudopodia on one side of the front over the other during exposure to a directional cue. Less is known about the role of the STEN in leukocytes, although signal transduction events similar to those observed in *Dictyostelium* occur at the leading edges of migrating neutrophils. The complex ruffling at the leading edges of leukocytes, in contrast to the rhythmic pseudopodia of *Dictyostelium*, suggests that the STEN in these cells would be tuned slightly differently. At first glance, the amoeboid behavior of *Dictyostelium* and leukocytes seems removed from the slow gliding behavior of fibroblastic, mesenchymal cells, yet some of the same signal transduction events do occur at the leading edges (Haugh et al. 1999, Kaur et al. 2006, Weiger et al. 2010). Furthermore, the fact that *Dictyostelium* cells can be abruptly and reversibly shifted from amoeboid to gliding migration, with protrusions resembling classic lamellipodia, suggests that the migration profiles displayed by diverse cells are slight variations of a basic mechanism (Miao et al. 2017). The behavior of mesenchymal cells may differ primarily in the much stronger adhesive properties of these cells. Interestingly, whereas the fast-moving keratocyte-like *Dictyostelium* cells ignore guidance cues, fibroblastic cells can be guided, perhaps because the slow movement allows for long-term integration of the gradient (Miao et al. 2017).

An optimal level of protrusive activity is required for efficient motility in a given environment (**Figure 9**). This can be understood in light of the STEN concept. On one hand, if the set point of the network precludes stochastic noise from crossing the threshold, there will be few large protrusions, and cells will barely move. These characteristics are observed when multiple signal transduction pathways are inhibited in *Dictyostelium*, leaving only brief, uncoordinated cytoskeletal

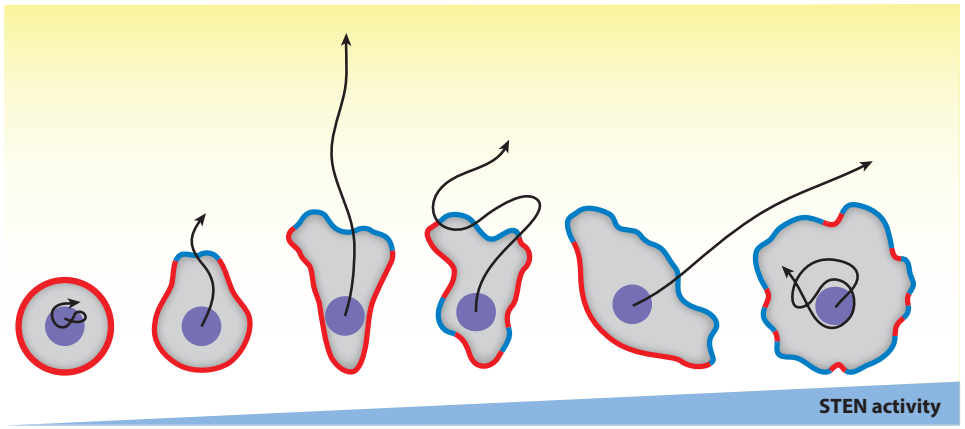

Figure 9

An optimal level of STEN (signal transduction excitable network) activities is required for effective directed cell migration. Cells with increasing STEN activities are illustrated from left to right, with blue representing front events and red representing back events. Black arrows represent centroid tracks of each cell moving toward a chemoattractant gradient (*yellow*) in the same amount of time.

events (Artemenko et al. 2016, Chen et al. 2007, Huang et al. 2013, Veltman et al. 2008). Some cells move more in uniform chemoattractant, a process referred to as chemokinesis (Ferguson et al. 2007, Petrie et al. 2009 Vicker 1994). According to most BEN schemes, chemokinesis is expected if uniform stimuli lower thresholds globally. On the other hand, the overall level of excitability can become too high, causing simultaneous projections in multiple directions and effectively blocking migration. In one example of this phenomenon, *Dictyostelium* cells lacking the PTEN display numerous spiky protrusions in all quadrants. To a lesser extent, similar phenotypes are displayed by vegetative *Dictyostelium* cells, 18 RAM mutants, and neutrophils lacking PTEN or SHIP1 (Lampert et al. 2017, Liu et al. 1999, Sarraj et al. 2009, Sasaki et al. 2007). Where tested, damping down signal transduction events or partially decoupling them from cytoskeletal activity improves migration. Thus, increased STEN activity leads to greater motility, but at too high a level, such increased activity becomes counterproductive. In the future, it will be informative to ask why migration is impaired or enhanced in various contexts by focusing on the number, localization, and characteristics of protrusions.

The STEN is potentially the site of integration of extrinsic cues controlling migration. There is an increasing appreciation of the ability of cells to integrate environmental signals and to move accordingly, but the mechanisms are poorly understood (Haeger et al. 2015, Huang et al. 2013, Theveneau et al. 2010). It seems evident that multiple chemoattractants can be integrated by activating the same set of signal transduction events, for instance, by activating a common G protein $\beta\gamma$ subunit, but less obvious is how mechanical, electrical, and optical signals are brought in. That cells migrating under shear force and in electric fields activate the same signal transduction events as do chemoattractants suggests that the various inputs may be integrated at this level. Further evidence of this idea is that in *Dictyostelium* a brief exposure to shear force activates the STEN, precisely as do chemoattractants. If multiple extrinsic cues controlling migration fed into a common STEN, altering its set point, the STEN would serve as an integrator of these signals.

There may be a profound evolutionary basis for the concurrence between the pathways involved in cell migration and cell growth. It is striking that Ras GTPases and PI3K pathways play a central role in both processes, and there are parallels in the effects of mutations. For example, oncogenic mutations in Ras GTPases and PI3Ks, which promote growth in mammalian cells, lead to more active *Dictyostelium* cells with additional protrusions (Miao et al. 2017). Loss of the tumor suppressors NF1 and PTEN has a similar equivalence with their mammalian counterparts (Bloomfield et al. 2015, Iijima & Devreotes 2002, Sarraj et al. 2009). These parallels extend further, for example, to *Dictyostelium* Rap1 and KrsB, which are homologs of the mammalian oncogene Rap1 and tumor suppressors Hippo/Mst1–2, respectively (Artemenko et al. 2012). Additional examples are found throughout the pathways. The effects on migration stand out in *Dictyostelium* because these perturbations have minimal effects on growth rates, whereas research in mammalian cells has focused primarily on growth. We propose a possible explanation for this apparent connection between the regulation of growth and that of motility: Growth pathways may have evolved first in nonmotile cells, and as expanding colonies depleted nutrients, cells able to repurpose those same pathways for migration were able to seek further nutrients and were selected. As explained above, macropinosomes, which engulf nutrients, mediate motility when they contact the substrate.

CONCLUSIONS

Directed cell migration, although critical in health and disease, is a fundamental physiological process displayed by nearly all eukaryotic cells. Research by numerous investigators is beginning to describe the mechanisms of migration and to understand how the hundreds of involved proteins act coordinately. Broadly, all cells displaying directed migration must link together directional

sensing, motility, and polarity. We suggest that motility results from spontaneous activity within a STEN that directs cytoskeletal events. Inputs to STEN from directional sensing and polarity modules increase persistence and bring about directed migration. The coupled network or modular view of cell migration emerging from recent studies has many advantages for experimental and computation analyses. Subtle variations in the molecular composition, dynamic properties, and linkage of these modules may be sufficient to explain the vast array of migration modes and sensing abilities displayed by diverse cells throughout phylogeny.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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