Developmental Cell
Perspective

Whence Directionality: Guidance Mechanisms in Solitary and Collective Cell Migration

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As individual cells or groups of cells move through the complex environment of the body, their migration is affected by multiple external cues. Some cues are diffusible signaling molecules, and some are solid biophysical features. How do cells respond appropriately? This perspective discusses the relationship between guidance input and the cellular output, considering effects from classical chemotaxis to contact-dependent guidance. The influences of membrane trafficking and of imposed constraints on directional movement are also considered. New insights regarding guidance and dynamic cell polarity have emerged from examining new cell migration models and from re-examining well known ones with new approaches and new tools.

The Basic Concerns of Migrating Cells

Directional cell movement has interested biologists for a long time. It is a fascinating cell behavior that can be observed even with simple microscopes. Of medical relevance, it is critical for the functionality of our immune system, both for finding and eliminating intruders and for allowing the cell-cell interactions that shape immune responses. Unfortunately, cell migration also adds to the difficulties of effectively battling cancer by contributing to spreading and metastasis of tumor cells. New experimental approaches for studying cell migration include sophisticated probes for use in light microscopy as well as the ability to image whole tissues or animals, both of which have increased our understanding. But the basic features of eukaryotic cell migration have been clear for some time (Lauffenburger and Horwitz, 1996; Ridley et al., 2003): when a cell migrates, it makes protrusions, plasma-membrane bound cellular processes. In many cells, these are F-actin rich, such as large flat lamellipodia or small filopodia, but they can also be ''blebs'' of locally extruded cytoplasm and membrane (Insall and Machesky, 2009). For actual movement, the cell and the cell processes must adhere to, and gain traction on, the substrate. The nature of the substrate, whether strings (1D), surfaces (2D), or complex environments (3D) of extracellular matrix (ECM) or other cells, helps determine which adhesion molecules can perform this task and which cellular protrusions are made. The cell must also exert force to pull itself forward, with the amount of force needed influenced by the resistance in the environment. All of these processes are dependent on the actin cytoskeleton, on dynamic actin polymerization, and on force generation on F-actin cables via myosin motors. Microtubules play a role as well, but generally as regulators and this is more cell-type specific. Finally, for cell movement to occur, the processes described above must be polarized such that there is a functional difference between the front and the back of a cell (Figure 1). If the cell is not polarized, it just spreads or contracts. Front-back polarization is spontaneous in many cell types but it may also be stimulated or directed by external factors, such as guidance cues.

There are different types of migratory cells. One way of categorizing them is as ''professional'' migrators versus cells with a migratory phase. Professional migrators include cells of the immune system and the amoebae *Dictyostelium discoideum*, both popular models of eukaryotic chemotaxis (Servant et al., 2000; Van Haastert and Devreotes, 2004). These cells migrate relatively fast, around 10 microns per minute, and can change direction quickly. Immune cells, such as neutrophils, can respond to cytokines released by local cells and to substances released from target cells, such as the peptide fMLP derived from bacteria or damaged cells. The aim of professional migrators is to get to the target fast, be it intruders or food, so their movement is likely to be optimized for speed as well as directionality. Many other animal cells can migrate directionally, but do so as part of a developmental or regenerative program. These cells generally move more slowly, 1 micron per minute or less, similar to the guided movement of axon growth cones. Their movement is likely to be optimized for fidelity, allowing multiple input and corrections, and not for speed, as most developmental morphogenesis occurs relatively slowly.

In physiological settings, many cells do not migrate alone, but collectively, as part of a group. A number of recent reviews have discussed occurrence of collective migration in development and disease, as well as the features of such migration (Friedl and Gilmour, 2009; Rorth, 2009; Weijer, 2009). As for many solitary migrating cells, collectively migrating cells can display inherent cell motility that is steered by, but not induced by, external guidance cues (Haas and Gilmour, 2006; Poukkula et al., 2011). In addition to these basic migratory features, collectively migrating cells also interact with each other. This can provide non-cell-autonomous pushing and pulling forces to affect overall movement. The collectively migrating cells also have the potential to provide each other with spatial input as they occupy discrete places in substrate space.

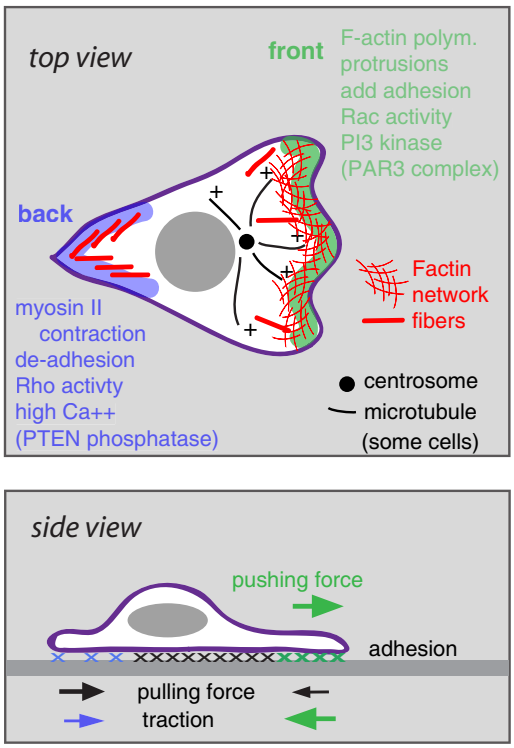

Figure 1. Basic Features of a Migrating Eukaryotic Cell
Indicated are features typical of a migrating cell, with protrusions at the front and myosin-dependent contraction at the back. Below is the same cell viewed from the side, indicating substrate adhesion (shown as stronger in the front) and relevant cellular forces. Not all features are found in all cells: robust F-actin polymerization is a common front feature, but some cells use blebs (Insall and Machesky, 2009). Also, the indicated polarity may be stable or labile. Positive interactions and feedback reinforce the front (and back) domains, and mutual inhibition may keep them separate.

This perspective will discuss guidance mechanisms for cell migration, in particular the relationship between input and output. Input in this context means the guidance information originating from outside the migrating cell and interpreted by this cell. Chemotaxis triggered by soluble input will be considered first to allow a discussion of some principles of signal interpretation. The focus will subsequently be on guidance by touch, the use of local and physical interactions to direct movement. This is quite widely used but has particular relevance for collective migration. When considering output, the intracellular signaling output created by migrating cells in response to the input is clearly important. But the actual cellular output can be defined as the feature(s) directly changed by guidance input such that the cell moves in the right direction: preferential addition of cell cortex/protrusions, preferential adhesion, or preferential direction of pulling. The simplicity, both technically and conceptually, of looking at outgrowth of protrusions makes it tempting to focus exclusively on this cellular feature. But while important, it is unlikely to be the whole story. As various examples will show, the relationship between guidance input and output is not fixed. They can be essentially separate features of cell migration, as in classical bacterial chemotaxis, or they may be more intimately connected. The latter is evident when considering guidance by touch as well as the effects of trafficking and the role of constraints in directional movement.

Graded Guidance Input and Cellular Switches

Guidance of cell migration by soluble cues, a straightforward form of chemotaxis, has been a key focus of analyses in both prokaryotes, which swim, and eukaryotes, which crawl. Although bacteria move by a different mechanism, their strategy of chemotaxis is well understood and of interest for this discussion. Integrating and computing the incoming guidance signals is interesting and complex (Baker et al., 2006; Falke et al., 1997), but the outcome is simple: a switch-like control of the orientation of flagellar rotation (Berg, 2003), which in turn determines one of only two possible outcomes, forward movement of the cell (run) or tumbling to give a new, random direction. Signal comparison is temporal, with an increasing concentration of attractant over time favoring the run state. The resulting biased random walk provides effective net directionality, showing that well-guided cell movement can occur from control of binary cellular decisions.

Contrary to bacterial cells, eukaryotic cells, including fast neutrophils and amoebae, can sense chemotactic gradients purely spatially (Herzmark et al., 2007; Van Haastert and Devreotes, 2004; Zigmond, 1974). In addition, there is ample opportunity for spatial control of local effects. The initial stimulus may be the stochastic chance of ligand binding to a uniformly distributed receptor (Ueda et al., 2001), giving an intracellular signal that directly converts the extracellular ligand concentration into a graded average frequency of receptor activation. The relevant cellular outputs, such as F-actin polymerization or adhesion strength, can be local and clearly they are not inherently binary. Nevertheless, switches are conspicuous in the control circuitry for eukaryotic cell migration. On cellular level, there is a front/back switch or front/back polarity (Figure 1), making a section of the cell either front-like or back-like at any one point in time, rather than a bit of both. The existence of such intrinsic polarity explains why many eukaryotic cells can move straight and effectively within short timeframes, even without clear guidance input. That front/back is a major polarity axis can also be seen from recent proteome analyses comparing front protrusions and cell body of chemotactic cells (Pertz et al., 2008; Wang et al., 2007). Front/back polarity shares some components with the stable cell polarity found in epithelial cells (apical/basal) and other polarized cells (Pegtel et al., 2007). But for guided, migrating cells, the polarity must be designed to either turn over or be steerable.

The control circuitry for eukaryotic cell migration is also characterized by feedback regulation at many levels, including from migration output to input. When cells migrate, localized signals can be detected in the front and in the back (Figure 1). Local accumulation of the plasma membrane lipid PIP3, product of phosphoinositide 3-kinase (PI3K) activity, has attracted much attention because—in some cells—its accumulation is stimulated by guidance signaling and it an excellent front marker along with protrusion and actin polymerization (Van Haastert and Devreotes, 2004). Characteristic of the back in many cells is higher activity of myosin and of the small GTPase RhoA (Wong et al., 2006). The front and the back signaling outputs are each subject to positive feedback regulation as well as mutual

negative cross-regulation (Weiner et al., 2002; Wong et al., 2006; Xu et al., 2003). The front is particularly linked to high activity of the GTPase Rac, and the recent generation of a photo-activatable form of Rac (Wu et al., 2009) has elegantly shown that elevated Rac activity can be sufficient for front directional activity in both single cells and cell groups (Wang et al., 2010; Wu et al., 2009; Yoo et al., 2010). These experiments also confirmed the positive feedback between Rac and PI3K activity and the general negative relationship between Rac and Rho GTPases. Another front/back mutually inhibitory loop, via effects on plasma membrane recruitment, involves PI3K and the phosphatase PTEN that catalyzes the opposite reaction (Funamoto et al., 2002; Iijima and Devreotes, 2002). Although elegant in design, this mechanism seems not to be general. For example in neutrophils, PTEN appears to have a different but very fascinating role in chemotaxis: helping immune cells prioritize their guidance responses such that they ignore general cytokine attractants when they are close to an actual target: a bacterium (Heit et al., 2008). Overall, there is some understanding of front/back polarity, but much remains to be understood and may differ between various migratory cells. One key issue is whether there is one central polarity controlling all migratory features and receiving all guidance input or whether it is all parallel processing with extensive crosstalk.

How does graded guidance input make cells move in the right direction and what is the relationship to front and back cell features? There are several views on this, which in part reflects the different cell types that have been analyzed. One view is that guidance signals directly set up the front/back polarity of the cell, for example by front-biased PI3K or Rac activation, and the original small differences between levels of signaling in the front and back are amplified until it becomes all or none, front or back (Parent and Devreotes, 1999) (Figure 2A). The amplification could follow a local excitation/global inhibition model, which is theoretically satisfying, but, as yet, not experimentally validated. Alternatively, the mutual inhibition of front and back features discussed above could be responsible. Another view is that the polarity of the migrating cell is inherent and not directly affected by the graded guidance signals. The guidance signal, in turn, is not part of a global comparison network, but its local concentration controls the frequency of small, stochastic front signaling events (Figure 2B). When tested computationally, this model also provides effective directional movement in gradients (Arrieumerlou and Meyer, 2005). Finally, observations of guided cells indicate that guidance controls the persistence of large cellular protrusions. For Dictyostelium cells (Andrew and Insall, 2007) and neurons migrating in the mammalian brain (Martini et al., 2009), front protrusions often split in two: the one pointing most effectively up the attractive gradient is maintained and the other is retracted (Figure 2C). In migrating cell clusters, we found that the difference between protrusions from front cells (pointing toward the attractant) and those from back cells (pointing away) is their lifetime, as well as the effectiveness of their grip on the substrate (Poukkula et al., 2011). Together, these studies indicate that protrusions have an inherent retraction tendency, and guidance input may counteract this tendency. In summary, the final cellular output of guidance signaling can take a number of conceptually different forms: reorientation of the front/back polarity, incremental additions of front features, and less-well-understood features of overall protrusion stability.

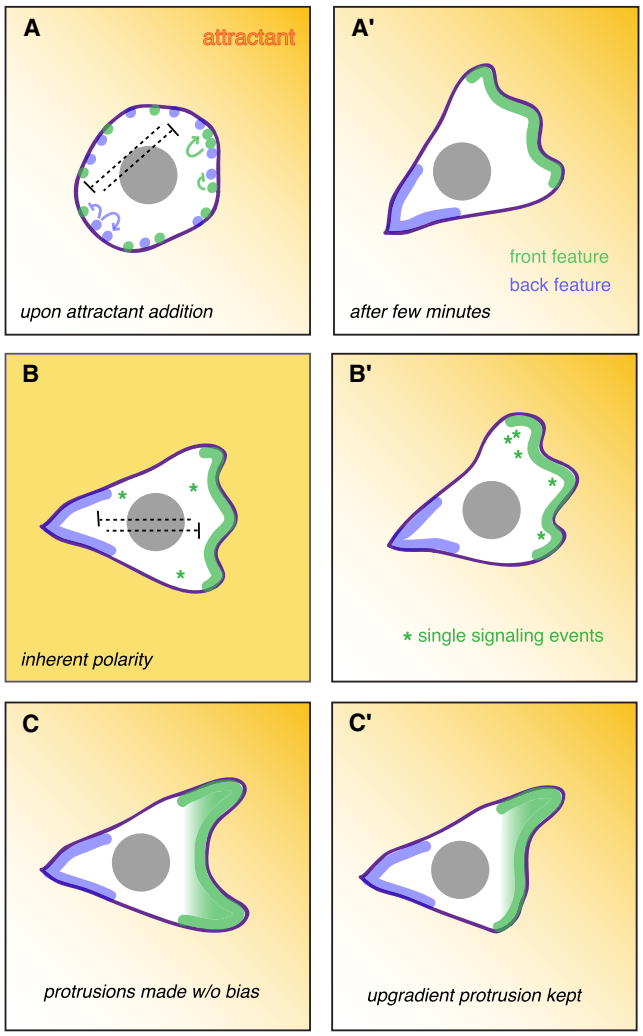

Figure 2. Relationship between Guidance Input and Cell Polarity in Chemotaxis

(A)–(C) shows the initial situation for three possible scenarios, and (A′)–(C′) shows the situation after reaction to the attractant gradient.

(A) Without the attractant, the cell is unpolarized. Slightly asymmetric attractant stimulates front features relative more on the up-gradient side and/or back features on the down-gradient side. Positive self-reinforcement and mutual inhibition (possibly via a diffusible inhibitor) causes sorting to two domains, which may happen stochastically as the attractant is uniform. This strategy is used by some professional migrators (Van Haastert and Devreotes, 2004).

(B) Even without or in uniform attractant, the cell is inherently polarized. The attractant causes local signaling event stochastically, but the frequency depends on the local concentration: only the front domain will respond with local protrusion, allowing the cell to turn (slightly). A strategy seen in fibroblast and dendritic cells (Arrieumerlou and Meyer, 2005).

(C) New protrusions are formed by splitting of existing main front protrusion (pseudopod) or triggered by other stochastic events. The protrusion going up-gradient or with the highest level of signal is stabilized, the other(s) retract. This is seen for single cells and collectives. Retraction is not well understood, but may be linked to nonproductive adhesion.

Spatial visualization of signaling activities is very informative in the analysis of chemotaxis. But increased sophistication of the methodology also changes what is observed and distributions

that appeared simple are suddenly not. Such updating has happened in several areas. One observation was that of transient local bursts of PIP3 accumulation in addition to the general front enrichment (Arrieumerlou and Meyer, 2005). This contributed to the second view of guidance input discussed above but maintains PIP3 accumulation as a front characteristic. Another front activator with complex spatio/temporal distribution is the Scar/WAVE complex, a Rac-regulated activator of the Arp2/3 complex and, hence, of actin polymerization. TIRF microscopy allowed visualization of membrane recruitment of the Scar complex, which occurred in apparent traveling waves (Millius et al., 2009; Weiner et al., 2007). Interestingly, Scar recruitment was modulated by feedback from F-actin. Also, wave characteristics reflected both internal cell polarity and chemotactic gradients, supporting their relevance for directionality of migration. With respect to back characteristics, it has long been appreciated that cytoplasmic Ca^{2+} is elevated in the back (Brundage et al., 1991; Hahn et al., 1992). More recently, ''flickers'' or local bursts of Ca^{2+} have been observed in the front of migrating cells and responding spatially to graded guidance cue (Wei et al., 2009). It is not yet clear how direct the link between Ca^{2+} flickers and guidance signaling is, but it may involve local IP3 production. This explains how some Ca^{2+}-regulated processes can occur in the front with others in the back, but it obviously blurs the most simplistic front/back distinction. Similarly complicated is the case of the small GTPase Rho, which, at least in fibroblasts, is not only active at the back but also at the front of the cell (Pertz et al., 2006). A recent detailed spatial/temporal analysis indicated that active Rho was more closely linked to the protrusive front of the cell than active Rac (Machacek et al., 2009). So while the front/back dichotomy remains relevant, the real distinction for these signaling components may be in network characteristics and dynamics rather than in absolute distributions.

Clearly PI3K, as well as Rac and Rho GTPases, are important for cell migration, and their activity states are nicely detectable and, hence, experimentally attractive. But as the discussion of front/back polarity illustrates, what they report can be far removed from the actual guidance signal. So what does the initial intracellular guidance signal look like in migrating cells? This important question remains largely unanswered, in part because it is technically challenging to report unamplified signaling events. As discussed below, single-molecule tracking can be used to follow receptors, but the activity state is usually unclear. For Dictyostelium cells, G protein signaling in response to cAMP gradients has been monitored directly and showed a shallow intracellular gradient (Xu et al., 2005) reflecting a shallow ligand-binding gradient (Ueda et al., 2001). Detection of active guidance receptor was recently achieved for a receptor tyrosine kinase guiding cell migration in vivo, but in this case, receptor activity appeared to be modified by cell-cell interactions in addition to the ligand input (Janssens et al., 2010). Going forward, direct visualization of guidance signaling in multiple systems, including with optimized live activity probes, should help further clarify the logic of signal perception and mechanism of transmission into downstream signals.

Shaping Guidance Input and Output by Trafficking

Guidance receptor signaling events at the cell surface may be faithful transmissions of the extracellular ligand concentration transformed only by the probability of ligand engagement and dependent on the Kd for the ligand-receptor interaction. However, receptor concentration is not always uniform and may be modified by trafficking or other relocalization events in a signaling-dependent manner. If an attractant receptor is targeted to the front of a cell, guidance decisions could become consolidated, ''locking in'' the direction or at least not allowing minor ligand fluctuations to alter directionality. Guidance signaling has been thoroughly studied in axonal growth cones, providing possible insights for guided cell migration. The same guidance cues can steer growth cones and migrating cells, and there is significant overlap in the cellular processes involved. One difference is that growth cones have a fixed and inherent back: the connection to the axon shaft (Figure 3). In growth cones of spinal cord neurons, activation of the GABA receptor is chemoattractive. Over time, the receptor becomes enriched toward the gradient source (Figure 3A′). Single molecule imaging by quantum dots showed that this enrichment occurred by a biased movement of receptors in this direction, dependent on receptor activity and microtubules (Bouzigues et al., 2007). Subsequent modeling found that such positive enrichment makes the directional response resistant to short-lived fluctuations in ligand concentration (Bouzigues et al., 2010). This could ensure that turning responses are more robust in noisy, complex environments. Also, as the biased transport seems to reflect development of an underlying microtubule bias, this effect could allow cross-regulation of guidance receptors, as well as of other directional cues.

Guidance receptors may also be redistributed in the cell via endocytosis and recycling. It was previously shown that endocytosis of a receptor tyrosine kinase could shape guidance signaling and cell migration in vivo (Jekely et al., 2005). Recent visualization of active receptors demonstrated directly that both receptor density and degree of receptor activation was spatially controlled in this manner (Janssens et al., 2010). A requirement for recycling controlled by Rab11 was also indicated. In agreement with the notion of Rab11-dependent recycling enhancing directional signals, Rab11 inhibition in epithelial cells diminished directional movement, although random movement was stimulated (Prigozhina and Waterman-Storer, 2006). Finally, trafficking may affect the signaling properties of guidance receptors: downstream pathways may preferentially be activated from the plasma membrane or from an internal compartment and, thus, require receptor endocytosis. Such spatial selectivity has been shown for numerous receptors in different contexts.

Obviously, membrane endocytosis, exocytosis, and recycling can have multiple roles in directed migration. In addition to shaping signaling by transporting guidance receptors, movement of specific adhesion molecules may be regulated, as may movement of membrane material as such. For example, in fast-moving Dictyostelium, the plasma membrane turns over fast in order to make dynamic protrusions and motility is blocked by inhibition of endocytosis or exocytosis (Traynor and Kay, 2007; Zanchi et al., 2010). Also, experiments looking at guidance of growth cones have shown that graded presence of attractive cues can promote local exocytosis (Figure 3B), whereas repulsive cues can promote locally increased endocytosis (Figure 3C), both effects that would help shape the membrane such that the

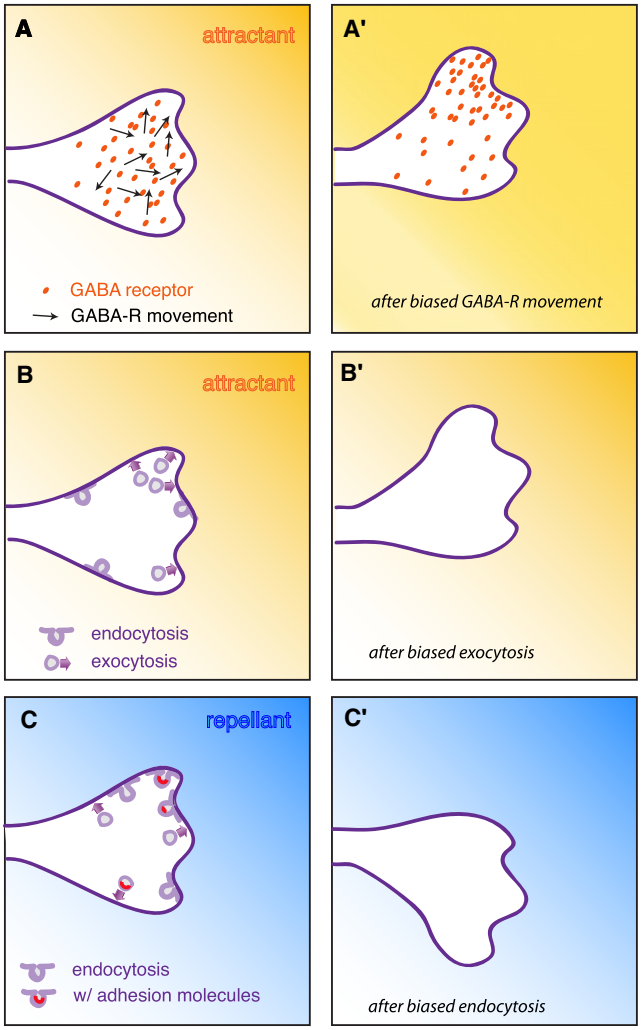

Figure 3. Effect of Receptor Redistribution and Other Trafficking on Guided Movement

All examples are shown as guided movement of a growth cone, but could be moving cells. (A)–(C) shows the initial situations and (A′)–(C′) after reaction to the gradient.

(A) The attractant receptor (here GABA-R) becomes enriched at the higher concentration of ligand, for example by signal-dependent biased movement along an underlying, signaling-biased cytoskeleton. If the attractant gradient temporarily collapses, directionality is kept due to the nonuniform receptor distribution.

(B) Endocytosis happens everywhere, but exocytosis is stimulated at regions with higher local attractant stimulus (here, the upper part of the growth cone). This leads to increased surface area on the up-gradient side, decreased surface area on the down-gradient side, and thus, turning toward the attractant source as shown in (B′).

(C) Exocytosis happens everywhere, but endocytosis is stimulated at regions with higher local repellant stimulus. The net effect is loss of surface area on the up-gradient side, leading to turning away from the repellant source as shown in (C′). For both (B) and (C), vesicle trafficking may include guidance receptors (as in A) and/or specific adhesion molecules. Selective trafficking of productive adhesion molecules can give biased adhesion toward (B) or away (C) from the attractant or repellant source, respectively.

growth cone turns in the right direction (Kolpak et al., 2009; Tojima et al., 2007, 2010) (Figure 3B′ and 3C′). Localized trafficking effects on integrin adhesion molecules have been observed in similar experiments, at least in response to chemorepulsion (Hines et al., 2010). For invasive migration of tumor cells, a Rab11 family member called Rab25 appears to be important, possibly due to its ability to promote recycling of specific integrins to the front of the cell (Caswell et al., 2007). These findings confirm that spatially controlled endocytosis and exocytosis can be important cellular outputs in directional migration.

Given that both guidance receptors and the molecules representing cellular output in directional migration are controlled by membrane trafficking, this raises the possibility that the effects could be closely connected. Perhaps one set of molecules is "hitchhiking" with the other. However, studies have so far focused exclusively on one aspect or the other, so it is difficult to gauge the overall significance of this.

Guidance by Touch and Clasp

The principle of locally acting and nonsecreted guidance cues has long been appreciated in the study of growth cone guidance (Tessier-Lavigne and Goodman, 1996). For example, signaling of Ephrins and Eph receptors, which is cell-contact-dependent signaling, controls guidance as well as cell movement in vivo (Poliakov et al., 2004). More recently, similar effects were observed for nerve regeneration as well (Parrinello et al., 2010). Restricted presence or graded distribution of permissive versus nonpermissive substrates for migration can also result in guidance: a type of guidance is called haptotaxis. The substrate may be other cells or it may be ECM. The ECM may, in turn, be modulated biochemically or mechanically (Provenzano et al., 2008) by other cells. The physical properties of the substrate may be sensed and used to select direction, a process called durotaxis. Finally, transient mechanical prodding can polarize cells (Verkhovsky et al., 1999). physical obstacles can actively reorient cell directionality and affect the intracellular signaling when doing so (Weiner et al., 2007). What all these effects have in common is that they represent contact-dependent communication. Communication by touch has two salient features: it is inherently localized and it can link directly to the physical properties of the interacting parties. These features are both particularly relevant for directional cell migration. Localized signaling is useful for a process that is spatially controlled, like guided migration. Also, cell migration is a process that requires exertion of physical force to move cells forward or even invade tissues. When force is exerted, tension may be sensed. The combination of being local and being linked to cellular force allows potentially much closer linking of the input and output in contact-dependent steering of cell migration.

Increased interest in the role of contact-dependent communication for directional migration has come in part from the study of collective migration. Collectively migrating cells physically interact with one another in addition to their interactions with the substrate. These interactions can be in the form of cell-cell adhesion and mechanical coupling, static or dynamic, or may primarily be signaling interactions. In parallel, studies of mechanical aspects of cell signaling have lead to the identification of molecules, in addition to the well-established membrane channels, that respond to tension and other mechanical input (Giannone and Sheetz, 2006; Ingber, 2006; Orr et al., 2006). Importantly, this is now being coupled with the development of tools that will allow better reporting of tension and forces. Until now, forces have generally been reported indirectly, via

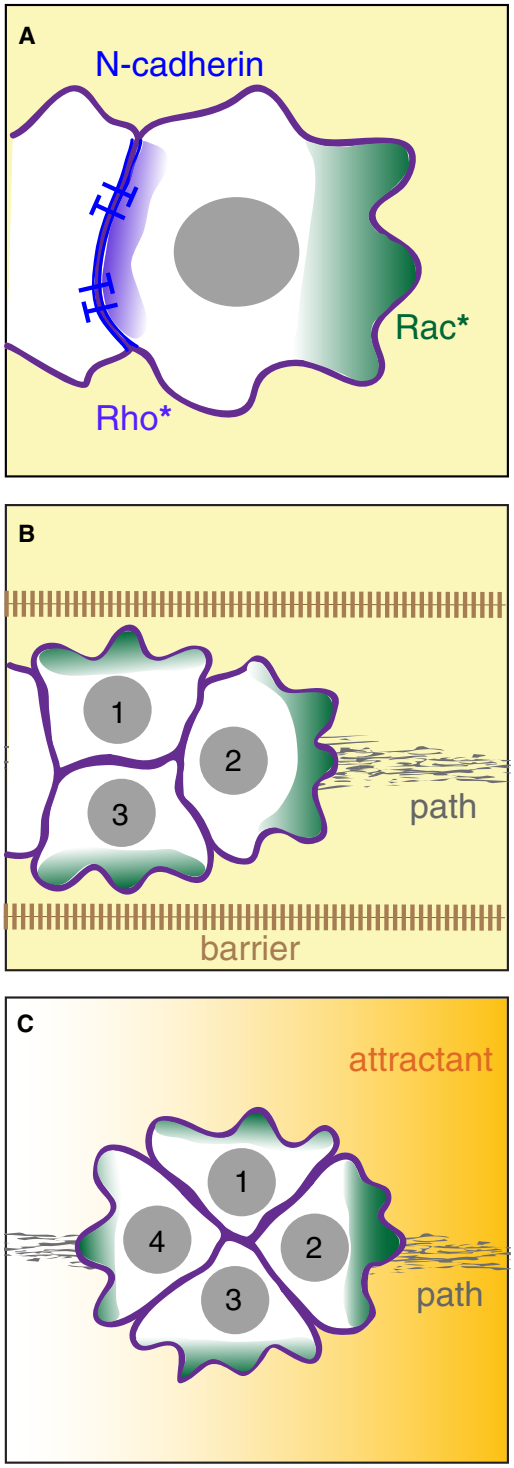

Figure 4. Contact-Dependent Guidance Effects in Collective Migration

These effects are in addition to those illustrated in previous figures. Some contact effects are also relevant for solitary cell migration: the positive, permissive effect of a path as well as constraining effects or barriers.

(A) Illustration of signaling seen in neural crest cells, where N-cadherin-dependent cell-cell contact induces local inhibition of front features (protrusion) via local Rho activation. This also involves non-canonical Wnt signaling. This will polarize/orient a cell with free edges.

(B) For a polarized cell cluster (cell no. 2 is front), contact-dependent effects as described in (A) (here shown as outwards-elevated Rac activity), together with either a permissive "path" or constraining "barriers," can be sufficient for guidance: the cluster will move to the right. Gradients can also contribute.

(C) For a nonpolarized cell cluster, additional external information is needed for directionality, such as the shown attractant gradient. Contact-dependent effects may polarize each cell outwards, here shown to synergize with the gradient effect to give highest Rac activity in the front of the front cell. The gradient may act by local effects as in solitary migration or cell-based, making cell no. 2 different from cell no. 4 by virtue of higher attractant signal levels (collective guidance). A cellular difference, such as higher motility or pulling force in cell no. 2, can give directional movement if the cluster is mechanically coupled.

deformation of specialized substrates (Dembo et al., 1996; du Roure et al., 2005; Galbraith and Sheetz, 1997), which has significant limitations. Recently, a very promising FRET-based live intracellular tension sensor probe has been developed, based on vinculin and its properties as a tension-sensitive, focal adhesion component (Grashoff et al., 2010). At cell-cell interactions, a key component of cadherin-based adherence junctions, called alpha-catenin, also appears to undergo tension-dependent conformational change (Yonemura et al., 2010). Perhaps these changes can also be reported live. With the right tools, mechano-sensing together with the other aspects of communication by touch should become a very fruitful area for the study of directed cell migration.

The role of cell-cell contact in specific collective migrations has been addressed by a number of recent studies. Neural crest cells migrate directionally in "streams" in vivo, with frequent dynamic interactions between the cells (Kulesa and Fraser, 2000; LaBonne and Bronner-Fraser, 1999). For *Xenopus* neural crest, explants and in vivo analyses indicated that these cells affect one another with what may be called local contact-inhibition-of-locomotion at the site of interaction (Carmona-Fontaine et al., 2008) (Figure 4A). The induced signal depends on noncanonical Wnt signaling and locally induces Rho activation. Considering the front/back polarity discussed above, this can be interpreted as locally inducing a back feature, which could direct cell migration at least in part by forcing front activity to be elsewhere. A subsequent study confirmed that Rac activity was inhibited at the sites of cell-cell contact, forcing front activities to be only at the free, noncontacted edges of cells (Theveneau et al., 2010) (Figure 4A). Similar "free edge" logic is likely to apply to directed movement of sheets of cells, which are not guided in the classical sense (see Rorth, 2009). If the migrating cell stream is inherently polarized, such cell-contact constraints can provide instructive information for directionality (Figure 4B), in particular in combination with external permissive cues or constraints.

The cell-cell adhesion molecule N-cadherin was required for the touch-based communication in neural crest cells. And surprisingly, cell-cell contact was essential for guided migration, even in the presence of a graded source of the appropriate chemo-attractant SDF. A specific cadherin was also shown to be required for directionality of cerebellar neurons when performing collective chain migration (Rieger et al., 2009). Interestingly, for mesendoderm cells, cadherin-based contact was required for collective migration, but not required when the same cells were allowed to migrate as individual cells in the embryo (Arboleda-Estudillo et al., 2010). These studies highlight

common features of contact-dependent effects in collective migration as well as differences in how essential the collective aspect is for directionality. Apart from the fact that both the cell types and the mode of analysis differed in these studies, it seems likely that the relative strength of diverse directional input will vary in different situations. One can view this as continuity of collective and individual migration phenomena; effects from both can be integrated to provide guidance in vivo.

Guidance by touch can be positive as well as negative. One useful system using large neurons from *Aplysia* was set up to mimic the positive interaction of a guided growth cone with target cells. A large bead was covered with the adhesion molecule ApCAM, related to mammalian NCAM, and placed on top of a growing, splayed out growth cone to interact via homophilic adhesion (Figure 5). Unrestrained, the bead was carried backward to the axon shaft by the so-called retrograde actin flow. But if the bead was physically restrained, mimicking growth cone attachment to another fixed object, it triggered a complex cellular response ending in turning of the growth cone toward the bead (Suter et al., 1998). The response had a time lag of a few minutes and required Src kinase activity, with localized signaling dependent on adhesion and traction force (Suter and Forscher, 2001). Further analyses have revealed the cytoskeletal changes occurring in response to this local, positive touch-based engagement (Schaefer et al., 2008). Overall, these studies suggested that the key cellular output in such cases is engagement of a "clutch" that couples cell-cell adhesion to the underlying actin cytoskeleton in order to generate traction and thereby directed movement. F-actin polymerization and treadmilling are needed, but not directly regulated. Exactly how the clutch works is unclear, but a biophysical motor-clutch model can describe it (Chan and Odde, 2008). The requirement for tension during the signaling phase of the "restrained bead" response with ApCAM suggests that some engagement with the cytoskeleton is present from the start, but that this engagement changes in type or strength as the cell responds.

How general are such force-dependent effects in directional cell movement? Retrograde actin flow has been observed in other cell types, and potential clutch engagement via adhesion complexes has been investigated in some detail for epithelial cells migrating in vitro (Hu et al., 2007; Ji et al., 2008). A similar mechanism was also proposed for migration of germ cells in vivo, with cadherin as the tension-bearing cell-cell adhesion molecule (Kardash et al., 2010). These effects can be interpreted as basic force-generating mechanisms for movement but also as potential sources of guidance input, or contact-based instructive signals, for example, by triggering local Src activation. As such, it is an example of how signaling input for directional movement can be closely linked to the cellular output, spatially and biophysically.

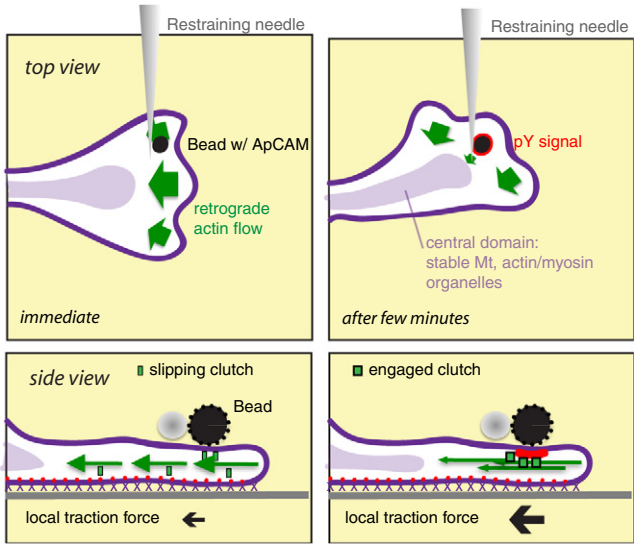

Figure 5. Contact- and Force-Dependent Steering of Movement
The example of a giant *Aplysia* growth cone with a "restrained bead" is shown, but a similar scenario may be seen in other cases of positive, adhesion-dependent local interactions. The lamellar retrograde actin flow is loosely coupled to adhesion at the cell surface (above and below) and will move a bead covered with an abundant cell-cell adhesion molecule away from the front. If the bead is physically held in place, or restrained, a tension dependent signal develops that includes local activation of Src tyrosine kinase (pY signal, red). This signal induces strong coupling to the underlying F-actin network, attenuating retrograde flow and increases local traction, in the clutch hypothesis described as an engaged clutch. A biophysical motor-clutch model can describe such phenomena (Chan and Odde, 2008). These cytoskeletal effects orient the stable central domain and thereby overall growth of the structure.

Directionality by Constrained Motility

When contact-dependent communication is considered, the distinction between permissive and instructive cues for directional migration can become fuzzy. As for neural crest cells discussed above, guidance of the lateral line primordium in fish requires the secreted molecule SDF (Ghysen and Dambly-Chaudiere, 2007), which is a known chemo-attractant. However, for both of the collective migrations, the directionality of movement may arise primarily from cell-cell interactions spatially constraining the production of free edges, with SDF possibly playing permissive role (as in Figure 4B). Motility, on the other hand, is generally considered permissive for cell migration. But if a group of cells are mechanically coupled and interact such that there are free edges only around the periphery, simply making one cell more motile or more force-bearing than the rest can provide directionality (Rorth, 2007) (see Figure 4C). Thus, it can be sufficient to spatially augment or constrain motility to get directional migration in collective cell migration. Tissue-constraints may provide further information. For example, some collectively migrating cancer cells invade tissues or ECM matrix by following a leading cell, reusing its path or tunnel (Friedl and Gilmour, 2009; Gaggioli et al., 2007). An interesting example of how the combination of motility control and tissue constraints can create directional movement was recently reported for axis elongation in vertebrates (Benazeraf et al., 2010). In this study, it was found that what at a first glance looked like guided, directional migration of mesoderm cells in response to graded FGF was something quite different. The individually migrating cells showed FGF-controlled motility, that is, FGF stimulated random dispersion with no directional bias. As a result of the cellular motility gradient and the constraints by neighboring tissues, the whole axial tissue, even the ECM, stretched in one direction. So at both cellular and tissue levels, constraints can control directionality of movement.

Concluding Remarks

Analogies can be useful when describing the relationship between guidance and motility for directional cell migration: motility is the motor, and guidance cues control the steering wheel. Or, motility is the legwork, but the nose (guidance) smells where the hotdog stand is. These analogies are apt for singular movement in an otherwise unrestricted environment. But what if there is only one road to travel on, or what if one is caught up in a crowd exiting the Metro during rush hour? Then the source of directionality becomes intertwined with substrate-interactions and the mechanism of movement. In other words, input and output are intimately connected and constraints are as informative as attractants. The latter analogies help describe important aspects of the directional movement performed by animal cells navigating their natural environment, the body. There is a lot of information to consider and the migrating cells need to integrate this in a productive way. The dynamic front/back polarity that is essential for motility, whether it functions as a central processor or as multiple parallel processes with extensive feedback, is important in this context. Diverse input, whether soluble attractants or spatial constraints, can be both integrated and translated to cellular output via these extensive feedback loops. It also helps ensure that conflicting information will not paralyze the cell. Overall, this allows directional movement to be productive with anything from very subtle to very complex guidance input. So whence directionality? Everywhere!

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