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Diverse and dynamic sources and sinks in gradient formation and directed migration

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Abstract

The traditional view of directional cell migration within a tissue is that it requires a gradient of a soluble attractive chemical that is stable in space and time due to the presence of a source and a sink. However, advances in live imaging technology and the ability to study cell migration *in vivo* have revealed that endogenous sources and sinks are typically far more varied and complex. Both sources and sinks can be made up of multiple tissues. During long-range migrations, cells tend to divide up their trajectories and follow different source signals in each segment. When a single source signal is used repeatedly in each segment, its expression is dynamically controlled. Source signals can also originate locally from neighboring migrating cells. Sinks are important in some cases but not all, to sculpt a permissive migratory path or allow cells to move from one intermediate target to another. Migrating cells themselves can function as sinks to create a gradient out of an initially uniform chemoattractant. These diverse ways of building sources and sinks allow different cell types to navigate distinct trajectories through the same embryo even as the whole embryo undergoes the dramatic changes in cell number, position, arrangement and fate that are the essence of development.

Introduction

Chemotaxis refers to the directional migration of cells up a gradient of a soluble chemical attractant [1]. Classic examples include migration of *Dictyostelium discoideum* amoebae toward cAMP [1], fibroblast attraction to growth factors [2,3], chemokine-mediated leukocyte movements [4], and axonal growth cone navigation toward Netrin [5,6]. Establishment of a chemoattractant gradient that is stable in time and space in principle would require a stable source of the molecule and a sink (Figure 1), as proposed for morphogen gradients [7].

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Using controlled conditions *in vitro*, such simple gradients can be created, and cells behave as expected, migrating toward the source [2,8]. As live imaging techniques mature, it has become possible to observe directional cell migrations *in vivo* and to begin to characterize endogenous chemoattractant sources and sinks. These studies reveal that *in vivo* migrating cells can move through extremely complex and changing environments, traverse through several different types of tissues, carve complex paths, and cover long distances. Cells can travel through three-dimensional interstitial matrices, along individual extracellular matrix fibers, on flat basement membranes, or they can squeeze in between other cells to cross a blood vessel or an epithelium. Due to this complexity, it turns out that sources and sinks are far more diverse and dynamic *in vivo* than the simple artificial gradients created *in vitro*. In this review, we use both old and new examples to describe the diverse mechanisms that nature has devised to steer cells along complex trajectories.

Morphogenesis Requires Diverse and Dynamic Sources

In development, it's not uncommon for migrating cells to travel long distances and make multiple turns. A single stable source of chemoattractant would not be able to direct cells along such complex trajectories. Therefore multiple source signals are often used. This can mean changing where a single chemoattractant is produced over time, or secreting different molecules at different locations and times and altering responsiveness of the migratory cells or growth cones as they move first toward one source and then to another.

One early example can be found in the navigation of vertebrate commissural axons, which travel long distances (several centimeters or 1000 times the diameter of the cell body) to reach their targets. Growth cones, the steering tips of axons, solve this problem by breaking down the trajectory into smaller segments thus necessitating multiple guidance cues and chemoattractant "sources" [5]. The best-studied intermediate target is the central nervous system midline, which secretes chemoattractant factors Netrin-1 and Sonic Hedgehog (Shh) (Figure 2a) [5,9]. Once the axons reach that target, they turn and follow other sources such as Wnt (Figure 2a) [10]. Different sources are thus active at different places and times, breaking a long and complicated journey into smaller steps. At the midline, axons encounter Slit, a secreted protein that causes them to turn on the expression of Roundabout (Robo), which is a negative regulator of Netrin-1 signaling [11,12]. Thus, growth cones can migrate away from the midline after crossing it, even though Netrin-1 is still present.

A different solution is found in *Drosophila* tracheal system development. A patch of about 80 tracheal cells forms on the surface of each hemisegment of the fly embryo. These cells dive into the embryo and then migrate to form a complex series of interconnected tubules. In contrast to growth cones, which switch from one chemoattractant to another, tracheal cells follow a single guidance signal, the expression of which changes dynamically to allow the cells to switch direction. The *branchless* locus encodes a fibroblast growth factor (FGF) homolog that is sequentially expressed at intermediate targets along the migration path for each of the tracheal branches. Once the leading tip cell reaches the nearest Bnl source, new expression initiates at another position along the migration path (Figure 2b) [13]. It is not completely clear what serves as a sink to remove Bnl or allows the cells to leave the first Bnl source. However it's possible that expression of Bnl in each intermediate target is simply

transient so that the protein concentration dissipates from the initial source as a new source of Bnl arises.

Cell-cell Interactions Lead to Dynamic Source Signals

Source information can be even more local. In the social amoeba *Dictyostelium discoideum*, cells relay the cyclic AMP (cAMP) signal from each migrating cell to its immediate neighbor generating a collective behavior called streaming [14]. While *Dictyostelium* cells migrate individually when nutrients are abundant, upon starvation cells switch to collective behavior, causing them to aggregate and eventually form a multicellular organism. Binding of external cAMP to a receptor known as cAR1 activates adenylyl cyclase, which in turn converts ATP into cAMP. The cAMP is then secreted from the rear of the cell to attract neighboring cells, which then produce more cAMP (Figure 3a), creating a stream. This cAMP relay mechanism can effectively provide collective cell guidance and accelerate cell aggregation [14]. Moreover due to the availability of many mutants defective in specific signaling components, the ease of biochemical analysis in this organism, and high resolution imaging, it has been possible to directly observe and measure the cAMP signal relay and response in living cells [20].

Later in the process of *Dictyostelium* aggregation, when there are tens of thousands to millions of cells coming together, the cAMP concentration oscillates, producing waves of extracellular chemoattractant (Figure 3b). Instead of forming a stable spatial gradient, which would soon flatten out due to the low molecular weight and fast diffusion rate of cAMP [15], *Dictyostelium* produces a temporal gradient of cAMP to guide the migration of its neighbors [16,17]. During the oscillations, the concentration of cAMP increases for a period of time and then decreases (Figure 3b). Cells respond to the increasing concentration by migrating directionally toward the source, and when the concentration decreases they depolarize and migrate non-directionally. This combination of responses leads to a net directional migration. The temporal gradient, relayed by many cells, can mediate robust chemotaxis of many cells over several hundred millimeters [16]. Multiple extracellular and intracellular phosphodiesterases function to degrade the large amount of cAMP produced locally, allowing the system to return to baseline [18,19]. It is possible that temporal oscillation of chemoattractant production is a general phenomenon. However better tools that allow real time measurement of chemoattractant concentrations or responses with high spatial and temporal resolution, such as those used in *Dictyostelium* studies [20], will be required to detect these types of signals in other living tissues and organisms.

Signal amplification by signal relay does not happen solely in *Dictyostelium*. During inflammation, neutrophils secrete leukotriene B₄ (LTB₄) to stimulate the chemotaxis of neighboring neutrophils [21–22]. Neutrophils make up a large proportion of leukocytes in the bloodstream. Upon wound formation, neutrophils follow signals to exit blood vessels and enter the tissue surrounding the wound site. Both *in vitro* [21] and *in vivo* [22] cells close to the wound site are able to follow primary signals such as formyl peptides (fMLP) secreted by the wound, and subsequently secrete the secondary chemoattractant LTB₄ (Figure 3c). LTB₄ secretion is proportional to the fMLP concentration, so that cells closer to

the wound secrete more LTB₄. A secondary LTB₄ gradient thus forms in parallel with that of fMLP and leads to rapid congregation of neutrophils to the wound [21].

Another form of directional migration produced by cell-cell interactions is the elegant chase-and-run mechanism found in *Xenopus* embryos [23]. Epithelial cells called placodal cells serve as a source of secreted Sdf1, which attracts neural crest cells. Upon contact of the two cell types though, neural crest cells repel the placodal cells by contact inhibition of locomotion (CIL), which is mediated by N-cadherin and PCP signaling. This drives the placodal cells further ahead of the neural crest cells. The placodal cells then initiate another round of Sdf1-mediated attraction (Figure 3d) [23]. This novel mechanism of dynamic attraction and repulsion ensures effective directional migration of both cell types. An open question is how a singular overall polarity is achieved by this interaction. The observation that polarized collective migration of cells can occur when they are explanted and cultured *in vitro* indicates that the overall polarization is self-generated and does not require external cues from neighboring tissues.

Multiple and Self-generated Sinks Ensure Directional Migration

In theory both a source and a sink are required to create a stable concentration gradient of a diffusible molecule within a confined space. Without a sink, the concentration of chemoattractant (or morphogen) would rapidly rise and soon become uniform. *In vitro* studies of chemotaxis employ microfluidic devices to generate artificial sources and sinks [24,25]. *In vivo*, sinks play important roles and help to sculpt complex migration paths through the dynamic environment characteristic of a developing embryo. However natural sinks are diverse and can be made up of a single tissue, multiple tissues, or the migrating cells themselves.

When multiple tissues serve as sinks along the path of migration, they determine the landscape of attractive versus unattractive or repellant regions collectively (Figure 4). For example, during embryonic development in *Drosophila*, primordial germ cells (PGCs) migrate out of the posterior midgut pocket to reach somatic gonadal precursors (SGP). Multiple tissues (e.g. the central nervous system [CNS] and epidermis) express the lipid phosphate phosphatases *wunen* (*wun*) and *wunen2* (*wun2*). Together, they serve as sinks that actively remove phospholipids that are attractive for PGCs. During migration, PGCs avoid *wun* and *wun2*-expressing tissues, finding themselves attracted only to regions from which *wun* and *wun2* are absent (Figure 4a) [26]. Multiple *wun* and *wun2*-expressing sinks thus sculpt a path of permissive areas.

Since migrating cells actively take up chemoattractants, they themselves can also serve as chemoattractant sinks. In this case, the same chemoattractant receptor that directs the cells to move can also serve as a sink by locally internalizing the attractant and thus depleting it from the immediate environment [27]. This depletion promotes the initial dispersal of *Drosophila* primordial germ cells from a tight cluster, which is necessary for them to cross the midgut epithelium, and then this effective repulsion achieved by local depletion of the attractant, stimulates their sorting to both sides of the embryo [28]. Similarly, human cancer cells derived from various epithelial tissues including breast, lung, and pancreas can

generate their own gradient by serving as the sink. When migrating through the confined environment of an artificial maze and presented with a uniform concentration of EGF, the majority of cells strikingly take the shortest route through the maze from the entrance to the exit [27]. The reason appears to be that the cancer epithelial cells can locally take up EGF ligands, creating a sink. Molecules then diffuse from the area of highest concentration to the depleted area, forming a gradient (Figure 4b). The cells can thus create and then follow a *de novo* EGF gradient [27]. An *in vivo* example comes from chick NC cells. Upon exiting the neural tube, NC cells become subdivided into leading and trailing populations. Leader cells locally deplete VEGF, which is a chemoattractant for NC cells, forming a gradient that the trailing cells follow [29]. These examples of autonomously generated chemical gradients eliminate the need for a pre-existing long-range chemical gradient.

A second example of directional migration in response to a uniformly presented chemoattractant is the movement of the posterior lateral line primordium, which is composed of ~100 cells that migrate collectively from head to tail during zebrafish embryonic development. Along the way, little clusters of cells stop and form major mechanosensory organs along the length of the body [30]. Sdf1 is a secreted molecule present in a stripe along the migratory path. The stripe appears uniform in intensity and cells can follow it in both directions, indicating that Sdf1 is not expressed in a gradient [31]. However a non-signaling receptor, CXCR7 is expressed by cells at the rear of the primordium, potentially creating a local sink, whereas the signaling receptor CXCR4 is expressed throughout the primordium. In a recent study, novel imaging tools allowed visualization of the **functional** Sdf1 gradient and demonstrated that the concentration of functional Sdf1 is lower in the rear of the tissue than the front, owing to specific expression of the Sdf1-sequestering molecule CXCR7 in the rear (Figure 4c) [32,33]. The lateral line primordium cells can thus migrate directionally even in response to a uniform stripe of Sdf1.

Complex Migration Requires Dynamic Source and Multiple Sinks

Similar to the lateral line primordium, zebrafish primordial germ cells (PGCs) also utilize Sdf1 and CXCR7 for directional migration. However, they follow a more complicated migration path, make multiple turns, and migrate as single cells, so a single stripe of uniform Sdf1 would not be sufficient to guide them. To guide PGCs, multiple intermediate targets along the migration path sequentially express Sdf1 (Figure 4d) [34]. PGCs migrate directionally using a “run and tumble” mechanism analogous to bacterial chemotaxis [35]. When they are moving towards a higher concentration of Sdf1, they move persistently in one direction (i.e. run) for longer periods of time than when they are moving down the concentration gradient. In between the runs, the cells “tumble”, i.e. they temporarily lose polarity and do not move forward until they reassess their surroundings and repolarize [34,36].

This mechanism requires that Sdf1 is produced in limiting amounts and that excess ligand, particularly from the prior segment of the path, is removed. *CXCR7* is expressed in multiple somatic tissues along the zebrafish PGC migration path and serves as a sink to shape the gradient of Sdf1 (Figure 4d). It sequesters and internalizes Sdf1, without inducing any downstream signaling [37]. Knocking down *CXCR7* causes PGCs to lose polarity and

migrate randomly, which can be rescued by reducing *sdf1* expression concurrently. The multiple CXCR7 sinks, together with the dynamic *sdf1* source expression, ensure that zebrafish PGCs follow the right path and reach their targets at the right time.

Conclusion and Future Directions

Generation of a stable chemoattractant gradient minimally requires a source that produces the chemical and a sink that removes it. However *in vivo* the landscape is both complex and changing, and this requires that cells employ dynamic and diverse sources and sinks for navigation. Within the same embryo different cells have to find different paths, necessitating a variety of cues, receptors, and overall strategies. In some cases, production of a single chemoattractant can be dynamic enough to guide one type of cell from one intermediate target site to another. Other types of cells see different cues at different points along the way. Within a migrating population, neighboring cells can provide guidance. Sinks are critically important and can be as varied as the sources. These multiple ways of building sources and sinks allow diverse cell types to navigate distinct trajectories through the same embryo even as the whole embryo undergoes the dramatic changes associated with development.

In the future, fluorescent probes with better spatial and temporal resolution will allow visualization of more chemoattractant signals and responses, especially weak or oscillating ones, in living organisms. More advanced imaging techniques will be employed to study the distributions of chemoattractants at single molecule level. The knowledge gleaned from studying diverse sources and sinks in cell migration *in vivo* may prove useful for tissue engineering and enhanced wound healing, where it will be critically important to steer cells along the correct paths so they can reach the desired final destination as reliably and efficiently as possible.

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Figure 1.

Traditional source and sink model in cell migration. A chemoattractant (green) is secreted from a source tissue and depleted within a sink tissue, thus forming a gradient. Migrating cells polarize in the gradient and migrate to the source.

(a) Axon Guidance: distinct chemoattractants in different segments of the migration path

(b) *Drosophila* tracheal cell migration: single guidance signal expressed dynamically at intermediate targets

Figure 2.

Diverse and dynamic sources in different segments of the migration path. (a) Axons respond to different chemoattractants prior to crossing the midline (lef) and after crossing the midline (right) [38]. (b) Bnl (purple) is sequentially expressed at each intermediate target to guide tracheal tip cell migration and branch formation (red). After reaching each intermediate target, *bnl* expression disappears (dotted circle) and initiates at new site. Arrow: direction of migration.

Figure 3.

Sources formed by neighboring cells. (a) A gradient of cAMP (red) produced by a migrating *Dictyostelium discoideum* amoeba is relayed to neighbors and causes streaming. (b) Later in the aggregation process cAMP production oscillates forming temporal instead of spatial gradients. (c) Wound sites secrete a primary signal such as fMLP peptides (yellow) and induces secretion of the secondary signal LTB₄ (orange) causing rapid aggregation of neutrophils (green). (d) Placodal cells (green) function as a source by secreting Sdf1 (orange). NC cells (red) migrate toward placodal cells but upon contact undergo CIL

(magenta arrows). Cycles of chemoattraction and contact inhibition produce net directional migration [23].

Figure 4.

Diverse and dynamic sinks. (a) *Drosophila* PGC cells (green) disperse and migrate out of the posterior midgut pocket (blue) towards somatic gonadal precursors (SGP). They avoid regions where Wun and Wun2 (yellow) phosphatases deplete the chemoattractant [26]. Grey arrow indicates direction of migration. Multiple sinks expressing *wun* and *wun2* are shown in brown. A transverse section of a *Drosophila* embryo (ventral part) is shown. (b) Migration of cancer epithelial cells (green) from one chamber to another through a connected channel. EGF ligand is shown in blue. (c) Migration of zebrafish lateral line

primordium (in red) on a self-generated gradient of Sdf1 (blue). Cxcr7 receptor (brown) is a non-signaling receptor expressed by posterior cells, which serve as a sink. (d) Zebrafish primordial germ cell (green) migration. Red arrow indicates highest point of Sdf1 expression at an intermediate target (orange). Grey arrows indicate direction of migration at two different time points when the source has changed.