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Receptors, enzymes and self-attraction as autocrine generators and amplifiers of chemotaxis and cell steering

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Abstract

Cells create their own steering cues, or modify cues from their outside, for a number of reasons. These include generating optimal, legible directional information; probing their environments for information to help decide an optimal route; symmetry breaking; generating new patterns and complexity; and bringing together collectives such as neutrophil swarms. Recent advances include more mechanisms of self-steering, in particular by using cell-generated mechanical cues, and gradients of respired oxygen. An increasing number of cell types are being found to use self-steering, in particular immune cells responding to chemokines and mesodermal cells during gastrulation. Finally, receptor modification has emerged as an important limit on the range of neutrophil swarming, allowing cells to monitor other areas as well as coming together. Self-steering is thus emerging as a dominant feature of cell motility.

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Introduction

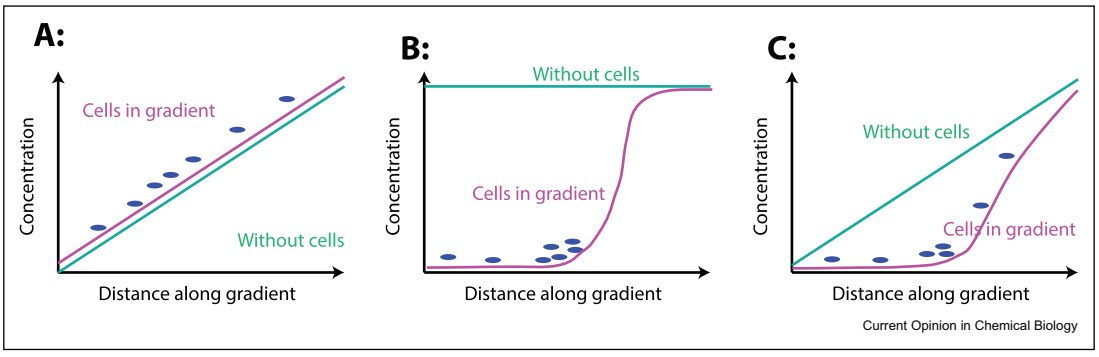
Accurate migration of cells is essential to biology. In processes such as embryogenesis and immunity, it is absolutely required that the correct cells migrate efficiently to their intended place. In embryogenesis, direct migration allows embryonic patterns to be set up and tissues precisely defined. In immune responses, leukocytes use it to attack infections or tumours but not the surrounding tissue. Similar processes are important throughout evolution.

Cells are frequently steered by chemotaxis. During this process, they read gradients of diffusible molecules and move towards the highest concentration [1]. Other, related processes are similarly important, for example durotaxis, where cells read a gradient of stiffness in the underlying matrix [2], and electrotaxis, where cells read the direction of an electric field [3]. The mechanisms that allow cells to read gradients are complex, because it is not sufficient to detect the presence of a stimulus; cells must also read its direction, comparing the strength of the stimulus at two spatially separated points. Bacteria achieve this by measuring the change in concentration as they swim in a consistent direction [4]; eukaryotic cells can read gradients without moving, by comparing receptors at different parts of their membranes [5], though it is unclear that this is the exclusive mechanism [6]. Both methods suffer from the problem of signal-to-noise ratio. If the attractant is being released from a source and removed by a sink that is any distance away, the resulting gradients are shallow when compared to the length of a cell. Similarly, if the concentration of attractant is high, receptors are similarly saturated at the front and rear of the cell; if the concentration is low, too few receptors are activated for a measurable difference.

Main text

The simple view of chemotaxis, in which cells just respond to a passive, unchanging gradient, struggles precisely because it is passive - the gradient is not altered by the locations of the cells. This means that the gradient needs to be readable throughout the cells' whole path, and information is spread thinly. If cells create their own gradients, or modify existing gradients to make them more readable (Figure 1), the information is restricted to the vicinity of the cells; gradients can be steeper and more readable. Recently we [7,8] and others [9–12] have shown that cells can generate their own steering gradients as they move. The gradient-making mechanisms are discussed in detail below, but frequently involve groups of cells locally breaking down an abundant attractant, or responding to one attractant by secreting a different one. This has many interesting advantages. It can create, amplify and complexify information, where passively reading

Figure 1



Passive chemotaxis, self-generated chemotaxis, and modification of an existing gradient. **A**: Passive chemotaxis. Cells respond to the gradient but do not alter it. The entire gradient, throughout its length must contain enough information to steer cells locally. **B**: Chemotaxis to a self-generated gradient. The chemoattractant is initially abundant and homogeneous; cells break it down locally to create a gradient in their vicinity, forming a chemotactic wave. There is little readable information outside the wave. **C**: Chemotaxis to a self-modified gradient. The chemoattractant is initially present in a gradient. Cells locally break it down to create a different, near-exponential gradient. The new gradient is oriented in the same direction as the initial one, but the level of attractant is tuned to the optimal concentration and steepness for cells to read it. A local wave may or may not form.

gradients can only lose and diminish it [13–15]. It allows cells to interact with their environments by probing the way attractants diffuse locally [12,16]. It is extremely robust to changes in cell number [9], and it addresses the problems of signal-to-noise ratio by making gradients sharp in the vicinity of the cells [17], and adjusting the attractant concentration so it is legible by the cells' receptors [18].

Self-steering is common

Despite these practical advantages to cells generating their own cues, self-steering is only described in a tiny proportion of publications that describe chemotaxis or related steering mechanisms. This might be taken to imply that it was rare. However, another interpretation - more accurate, in our opinion - is that it is usually not considered, and most ways of measuring steering are actively or passively designed not to detect it. Transwell/Boyden chamber assays, for example, which dominate the chemotaxis literature (unfortunately, as they are subject to a range of artefacts as well as concealing self-steering), give a positive result if any kind of chemotaxis has occurred; they do not differentiate passive and self-steered chemotaxis. For example, if cells respond to a chemical in the reservoir by secreting an autocrine chemoattractant, then any cell that gets through the membrane will attract all its neighbours; the chemical will incorrectly be scored as an attractant, even though the actual attractant was made by the cells. More modern devices (for example [19] or [20]) can identify self-generated gradients, but only if they are specifically sought. This is particularly important, because in most biological cases (see below) there are elements of both passive chemotaxis, because there is some underlying gradient, and self-steering, because cells must modify or reinforce gradients in order to read them.

However, the mechanisms described below are widely prevalent in steered cells. Mammalian leukocytes often secrete chemokines to which they also have receptors. Almost every known chemoattractant is attacked by locally produced enzymes, such as cell-surface peptidases [21] and proteases [22] (for peptide signals), and enzymes like phosphodiesterases [23] for chemical signals. Essentially all receptors used for chemotaxis are also endocytosed, taking their ligands with them [24]. In general, it seems likely that self-steering is possible with almost every attractant; if the passive gradient is steep enough it may dominate, but in real biological contexts this appears to be rare.

Sometimes self-steering is mistaken for other mechanisms. For example, an apparent example of self-generated gradients to CCL19 was recently reported as "chemokinesis" [25] - where cells' speed is controlled by an agent, but not direction - but in hindsight, the cells are probably creating their own gradient [10]. Similarly, when macrophages migrate in liquid-walled devices [26] their migration can be followed in detail; the resulting pattern, with a wave of migration at the front [27], strongly suggests that the cells are locally amplifying the gradient.

Underlying mechanisms for cells to create or change steering gradients

Mechanisms that use either receptor-mediated uptake of chemoattractant, or enzymes to inactivate them, have been identified since the first descriptions of self-generated gradients. A newly described, and probably general, self-steering mechanism uses durotaxis [28]. Neural crest cells create a stiffness gradient in the tissue ahead of themselves, which steers them away from the neural crest and towards the placodes. This gradient is a

simple analogue of self-generated chemotactic gradients, with the dispersion of physical properties replacing diffusion of chemical, and is required for efficient migration. Emphasising the consistency of the underlying idea, self-generated durotaxis synergises with chemotaxis to SDF-1, itself also potentially self-generated.

Cells taking part in mechanical interaction that go on to steer them - feedback loops, that may be positive or negative - have been seen elsewhere, for example in the Drosophila nerve cord [29] and the zebrafish mesoderm during gastrulation [30]. It may also be seen in vitro, as a means of driving persistent migration in cell clusters [31]. Like self-generated chemotactic gradients, they are probably commonplace but only seen when specifically looked for.

One interesting additional mechanism is oxytaxis. Cells in a restricted environment deplete oxygen by aerobic respiration; chemotaxis towards fresh oxygen leads them away from the hypoxic conditions they are in. This has been shown for cancer cells [32] and, more recently and in more detail, *Dictyostelium* [33].

Making gradients using receptors and endocytosis

Previous work showed that signalling receptors [12] or scavenger receptors, receptor analogues that take up attractants but do not transduce a signal [34] are used to create self-generated gradients. Several new examples have emerged, again demonstrating the generality of the concept. Dendritic cells are extremely chemotactic towards the chemokine CCL19 [35], but it has never been completely clear how the gradients are formed. Now examination of migration in under-agarose assays, which provide a good 3D environment, shows that dendritic cells respond to CCL19 almost as well when it is provided uniformly in the agarose as in a preformed gradient [10]. The cells take up and degrade the CCL19 through its receptor, CCR7; the resulting gradient gives, if anything, more robust chemotaxis. As seen earlier for *Dictyostelium* and cancer [16], this also allows cells to navigate a branched path, and determine the most direct route to a CCL19 source, and thus mimics the steering problem faced in a lymph node. Similarly, the unusual signal Toddler/Apelin is the ligand for G-protein coupled receptors that are essential for directed cell movement in gastrulation, but it has not been clear how, or where the directional stimulus might arise [36]. This, too, has now been found to use a self-generated gradient - the receptor acts as both scavenger and transducer, making a gradient while it detects it [37].

Enzymes that degrade attractants

Using receptors to create gradients has advantages and disadvantages. One great advantage is simplicity - the system that is used to perceive the gradient also creates it. Fewer components are needed, and systems can evolve gradually (as presumably occurred with scavenger receptors). However, this system lacks robustness - receptors usually have a very high affinity, but as such can easily be saturated; and the cycle time for receptors to be endocytosed, then returned to the surface, is slow, perhaps 30′ or more. Together these limit receptors' ability to generate gradients out of high attractant concentrations. Enzymes, on the other hand, may each degrade tens or hundreds of molecules per second. Their Michaelis constants - representing the point at which they become saturated - are higher, typically in the region of 10 μM. They are thus much better placed to degrade high concentrations of attractant that saturate receptors, which typically have dissociation constants (the equivalent measure for receptors) more than 1000-fold lower, in the region of 0.1−10 nM.

Two classes of enzymes have been widely described in chemotaxis - peptidases, which trim small peptide attractants like chemokines, and phosphodiesterases. Leukocytes (in particular) are covered with an array of membrane-bound peptidases with their catalytic sites exposed to the medium, including specific N-terminal and C-terminal trimming enzymes and nonspecific proteases like MT1-MMP. Any peptide attractant that encounters such a cell will be subject to degradation and inactivation, so more self-generated gradients will likely soon be found. Phosphodiesterases attack signalling lipids like lysophosphatidic acid and sphingosine-1-phosphate and, in cells like *Dictyostelium*, extracellular cAMP. The best-described phosphodiesterases are *wunen* and *wunen2* in *Drosophila*, which are essential for germ cells to chemotax to the gonad [38,39]. Curiously, however, the lipid targets for wunens are not known, though there is a suggestion they may be related to isoprenoids like retinoic acid [40]. The components of the system - Tre1, the receptor, as well as wunens 1 and 2 - are also important in combination for *Drosophila* astrocyte steering [41], again implying a widespread role for self-generated chemotaxis in biology.

Cell-generated autoattractants

When cells generate their own chemoattractants - as opposed to breaking down chemoattractants made elsewhere - the results are yet more complex. Cells attract their neighbours, who in turn attract other cells near them. The resulting positive feedback loop tends to bring cells together, but the details can be unpredictable, depending on the precise dynamics of the feedback.

This mechanism, like self-generated chemotaxis by ligand breakdown, may be more common than we expect. Several classes of immune cells express both chemokines and their cognate receptors; neutrophils, for example, express both CXCL2 and CXCR2 so can

self-attract [42]. Similarly, neural crest cells both make and respond to complement C3 [43]. However, the most widely studied examples of self-attraction are *Dictyostelium* aggregation - using extracellular cAMP as an attractant, detected by the receptor cAR1 [44] - and neutrophil responses to leukotriene B4 (LTB4) [45]. The *Dictyostelium* system is very well understood, and consequently much picked over by mathematical biologists. It is the neutrophil system that has yielded the most recent progress. Activation of LTB4 pathways leads to "swarming", in which neutrophils move collectively and in large numbers to a single site [45]. One significant problem with swarming is stopping it from happening excessively, focusing all the body's defences in a single point. A recent paper confirms that stopping self-attraction by receptor inactivation [46] is essential to allow broad areas of tissue to be monitored.

Conclusions

The different methods of self-steering described here together provide the most efficient, robust, and informative ways for cells to migrate to a target. The underlying mechanisms — diffusible signalling molecules, physical interactions, and respiration — are diverse, but the idea is consistent. Cells and their surroundings together modulate signals, allowing complex and nuanced responses to neighbouring cells, larger collectives, and the environment. Complexity is thus a dominant feature in both self-generated gradients and self-stimulated chemotaxis. Outcomes are hard to predict, and often counterintuitive, because small changes are amplified. For example, if a large group of neutrophils receives a small stimulus, a single cell randomly secreting LTB4 completely changes the motility of the whole group. Likewise, the secondary processes that limit feedback (receptor saturation or substrate depletion, for example), which are rarely considered, can rapidly become very influential.

In general, this is a beneficial outcome. Biology is usually complex; the ability to explain complex outcomes with simple rules is a strong advantage. However, the resulting narrative often conflicts with the simple stories preferred in the biology literature. This is the primary reason why so few cases of self-steering have been described — if authors seek simplicity, self-steering will rarely be the answer.

Recent work has focused on revealing self-steering to be active in cells that were previously thought only to chemotax passively. We expect this will continue, and most chemotactic cells will be found to use at least a degree of self-steering, for example by reinforcing gradients that have already been externally set up. We also predict that complex combinations will increasingly emerge, in which cells use different combinations of receptors, enzymes, and self-stimulation to tune their responses. The range and importance of self-steering mechanisms will surely continue to grow.

Conflict of interest statement

Nothing declared.

Data availability

No data was used for the research described in the article.

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