

# Growth cone chemotaxis

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**Wiring up the nervous system depends on the precise guidance of axonal growth cones to their targets. A key mechanism underlying this guidance is chemotaxis, whereby growth cones detect and follow molecular gradients. Although recent work has uncovered many of the molecules involved in this process, the mechanisms underlying chemotactic axon guidance are still unclear. Here we compare growth cones with neutrophils and *Dictyostelium discoideum*, systems for which a clear conceptual framework for chemotaxis has recently emerged. This analogy suggests particular ways in which the three key steps of directional sensing, polarisation and motility might be implemented in chemotaxing growth cones.**

## Introduction

You take a break from the poster session, and wander into the unfamiliar street to find some dinner. Tonight you're craving Mexican, and soon you catch a tantalising whiff of guacamole and tortillas. But where did it come from? To be back in time for the plenary you now need to do three things. You have to decide the direction in which the strength of those spicy smells is increasing. You have to orient your body in that direction. And, not least, you have to put one foot in front of the other to take you there.

Wiring up the brain during neural development involves a series of similar challenges. Axons extend in search of their appropriate targets, often over long distances. A driving hypothesis in understanding how axons are guided *in vivo* is that they detect and follow concentration gradients of particular molecules, a process known more generally as chemotaxis. Over the past 15 years or so, spectacular progress has been made in identifying some of these molecules. They are organised into several families, most notably the Netrins, Semaphorins, Slits, Ephrins and some morphogens and neurotrophins [1–6]. These molecules are detected by growth cones, specialised sensory-motor structures at the extending axonal tip. Growth cones are highly motile, constantly changing their morphology by extending or retracting long, fingerlike filopodia to probe their surrounding environment. In the absence of instructive cues, growth cones progress along a relatively straight path, laying out the axon tract behind them, through a series of well-defined steps. First, the membrane extends, a process known as protrusion. This nascent region then becomes engorged as organelles from the central region of the growth cone flow forward. Finally,

molecular components at the rear of the growth cone consolidate to form a stabilised axon segment.

So, just like your body in the analogy above, growth cones are equipped with everything needed to detect the direction of the gradient and then move appropriately. However, progress in understanding exactly *how* they perform these feats has been rather slower than identifying the molecules involved. It is the 'how' question that we discuss in this review. In particular, we draw analogies with chemotaxis in neutrophils, a type of white blood cell, and the slime mold *Dictyostelium*. Although in these cases the whole cell moves in response to a molecular gradient, rather than just the growth cone, their behaviour is in many ways similar. Furthermore, similar molecules are involved in all these systems [7]. The relative ease of culturing and visualising the behaviour of neutrophils and *Dictyostelium* in quantitative gradient assays has resulted in a clear conceptual framework within which their chemotactic responses can be understood [8]. We suggest that this framework can provide a useful starting point for making sense of the complex signalling pathways that underlie neuronal growth cone guidance.

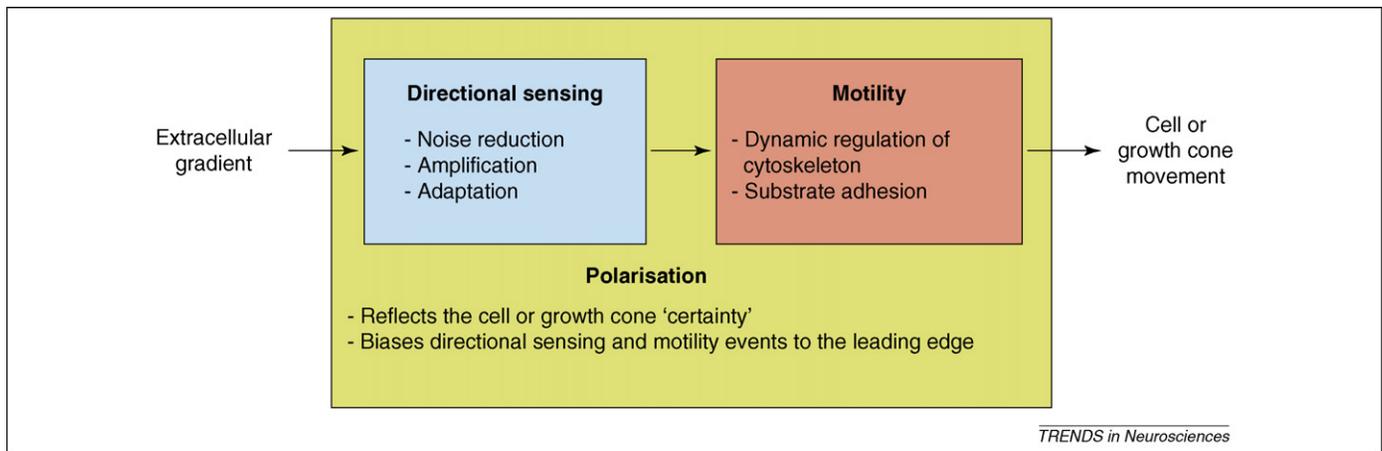
## A three-step program for chemotaxis

Effective chemotaxis in eukaryotic cells involves the coupling of three conceptually distinct phenomena: *directional sensing*, *motility* and *polarisation* [8,9] (Figure 1; Table 1). Directional sensing is the ability of a cell to transduce a shallow, externally presented gradient of chemotactic factor into a significantly steeper intracellular gradient of activity, to spatially bias its response. Motility is the generation of movement through the spatiotemporal coordination of cytoskeletal dynamics and substrate adhesion. Both of these processes occur within the context of polarisation, which is the ability of a cell to arrange key signalling components into persistent and distinct 'front' and 'rear' regions. It has been suggested that these processes are generically important in all eukaryotes exhibiting chemotaxis [2], and many of the signalling components and pathways are highly conserved, playing similar roles in, for instance, *Dictyostelium*, neutrophils, fibroblasts and axonal growth cones [7,8]. We now discuss these phenomena in more detail, and relate them to growth cone chemotaxis.

## Directional sensing

The directional sensing module in *Dictyostelium* and neutrophils transduces a shallow, possibly noisy external gradient into a significantly steeper intracellular gradient.

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**Figure 1.** A conceptual framework for chemotaxis. In the absence of guidance cues, cell motility is unregulated, leading to undirected motion. In the presence of an external guidance cue gradient, the direction of motility is biased by information transduced through directional sensing. Both directional sensing and motility occur in the context of polarisation. Unpolarised cells are sensitive to guidance cues uniformly on their periphery. By contrast, polarised cells are more sensitive at the leading edge. Similarly, unpolarised cells make frequent large changes in their direction of motion. A polarised cell, however, tends to continue moving in the same direction for an extended period of time.

To achieve this, the directional sensing mechanism must refine the external signal in three ways. First, at some point the shallow external gradient must be ‘amplified’ [10] into a stronger intracellular asymmetry. It is unknown which signalling component (the ‘chemotactic compass’ [11]) first displays this strong asymmetry. In *Dictyostelium* it occurs downstream of G protein activation but upstream of phosphoinositide signalling pathways [9]. By amplifying a shallow external gradient into an essentially binary internal response, the cell might be able to counteract the effects of noise [12] intrinsic to intracellular information transmission.

Second, in *Dictyostelium* and neutrophils, directional sensing functions over several orders of magnitude of background ligand concentrations, a property known as adaptation. This is achieved through a local-excitation, global-inhibition (LEGI) mechanism in which receptor-mediated signals generate two effects: a localised production or recruitment of ‘compass’ molecules, along with a global increase in the rate of degradation of these molecules [13]. As a result, the intracellular distribution of compass molecules reflects the steepness of the extracellular gradient, but not its absolute concentration (Figure 2a).

Finally, fluctuations inherent to receptor binding and ligand diffusion place important constraints on the ability of a cell to faithfully sense the gradient [14,15]. Although on average more chemoreceptors will be bound on the upgradient side rather than the downgradient side, this cannot be guaranteed at any particular instant because of this intrinsic noise. Indeed, estimates of the number of occupied receptors at the front and rear of chemotaxing *Dictyostelium* cells suggest that variation in the number of occupied receptors as a result of noise can be larger than the variation due to the gradient signal [15,16]. Thus, for reliable gradient detection, this noise must be reduced either before or during the amplification process. In *Dictyostelium*, this might be achieved through spatial and temporal averaging of signals from bound receptors through the production, diffusion and degradation of membrane phospholipids [15].

Axonal growth cones also face the challenges of noise reduction [17], amplification [18] and adaptation [7]. However, although *Dictyostelium* and neutrophils need to respond to rapidly changing gradients, for example, when hunting food or destroying bacteria, axon tracts develop in response to more stable gradients. Consequently, although morphological changes in both cases can occur rapidly, the timescale of the decision-making process differs between these organisms and thus the precise cellular mechanisms of amplification, adaptation and noise reduction probably also differ at several levels. Growth cones display robust guidance in 10% gradients [2], and a guidance response can be detected in gradients as shallow as 0.1% [19]. However, calcium gradients within the growth cone following receptor activation have been observed with higher steepnesses [20]. Thus, like *Dictyostelium*, growth cones can process receptor-mediated signals to emphasise an initially weak asymmetry. Indeed, it has been suggested that growth cones might implement LEGI for this purpose [21] (Figure 2b).

One mechanism by which a steep gradient could develop within a growth cone is through the microtubule-driven asymmetric clustering of activated receptors within lipid rafts [22]. An asymmetric distribution of activated receptors would in turn reinforce any asymmetric intracellular signals, presumably recruiting more active receptors in a positive feedback loop (Figure 2c). Lipid rafts are known to be important for signalling by several guidance cues, including NGF, BDNF, Netrin-1, Semaphorin 3a and reverse signalling by EphrinB [23–25]. Furthermore, this hypothesis is supported by direct observation of activated GABA receptors clustering on the upgradient side in response to a gradient of the neurotransmitter GABA, which can attract growth cones *in vitro* [26] (although is not known to play a guidance role *in vivo*). This redistribution occurs in a microtubule-dependent manner [22]. This mechanism is a form of LEGI, where trafficking of activated receptors away from regions of low activation acts as a form of inhibition. In addition, the slow dynamics of microtubule-based redistribution of receptors and receptor diffusion might allow this mechanism to function as a low-pass temporal filter to reduce noise [22].

**Table 1. Nonexhaustive comparison of directional sensing, motility and polarisation between eukaryotic cells and growth cones**

	<i>Dictyostelium</i> and/or neutrophils	Growth cones
<b>Directional sensing</b>		
Sensory apparatus	Various G-protein-coupled receptors, e.g. cytokine receptors, cAMP receptors [8,60]	Many receptor families, including G-protein-coupled receptors, receptor tyrosine kinases, growth factor receptors, morphogen receptors, classical guidance cue receptors, e.g. DCC, Robo, Neuropilin, Plexin, Ephrins and Eph receptors [3,6,61]
Noise reduction by temporal and spatial averaging	Noise filtering by production, diffusion and degradation of phospholipids; e.g. PtdIns(3,4,5)P3 [15,62]	Slow dynamics of microtubule-mediated receptor clustering possibly acts as a low-pass filter [22]
Adaptation through local-excitation, global-inhibition (LEGI) and amplification through positive feedback/cooperativity	LEGI-like mechanism in <i>Dictyostelium</i> might involve interactions between Rho GTPases [63]  Amplification through reciprocal localisation of PI3K and PTEN, and subsequent regulation of phosphatidylinositol lipids [9]	Local activation through microtubule-mediated clustering of activated receptors within lipid rafts; the trafficking of receptors away from other regions act as a form of global inhibition [22] Adaptation also involves receptor endocytosis and recycling, and protein synthesis [27,28]
<b>Motility</b>		
Leading edge dynamics	Pseudopodia form at regular intervals: ~60 s in <i>Dictyostelium</i> [30]; 30 s in neutrophils [29] Pseudopodia dynamics controlled by actin regulatory proteins such as Arp2/3, WASP, PAK and VASP [8,60]	Lamellipodial and filopodial dynamics controlled by actin regulatory proteins and complexes such as Arp2/3, Fascin, Ena/VASP, N-WASP, ADF/Cofilin, profilin, myosin [31]
Trailing edge retraction/consolidation	Myosin-driven contraction and stress fiber formation [60]	Consolidation involves microtubule bundling by Doublecortin [46] and myosin II-driven actin contraction and stress fiber formation [47]
Substrate adhesion	Leukocyte rolling adhesion moderated by chemokine regulation of integrin avidity [64]	Formation of point contacts at leading edge regulated by Rac and RhoA activity [65] Loss of adhesiveness and internalisation of adhesion molecules in the central zone, and recycling to the leading edge [45]
<b>Polarisation</b>		
Molecules localised to the leading edge; 'frontness' markers	Rac, PI3K, PtdIns(3,4,5)P3, WASP, CRAC, PhdA, Akt [8,60]	Rac, PI3K, Cdc42, actin bundling, adhesion
Molecules localised to the trailing edge or central zone; 'backness' markers	Myosin II, PTEN, PtdIns(4,5)P2, SHIP1, RhoA, ROCK, PAK [8,60]	Doublecortin [46], ROCK, Myosin II, RhoA [47], PTEN [9]
Molecules and processes involved in left-right polarisation	N/A	Left-right polarisation might be stabilised by microtubule capture and the subsequent delivery of actin-binding proteins [37] Toward the turning direction: Local protein synthesis (e.g. actin, cofilin); also involves the graded activation of eif4BP Autocrine signalling and local membrane modification through microtubule-directed exocytosis [53] Away from the turning direction: Actin retraction away from the turning direction might be driven by ROCK and Myosin II contraction [47]

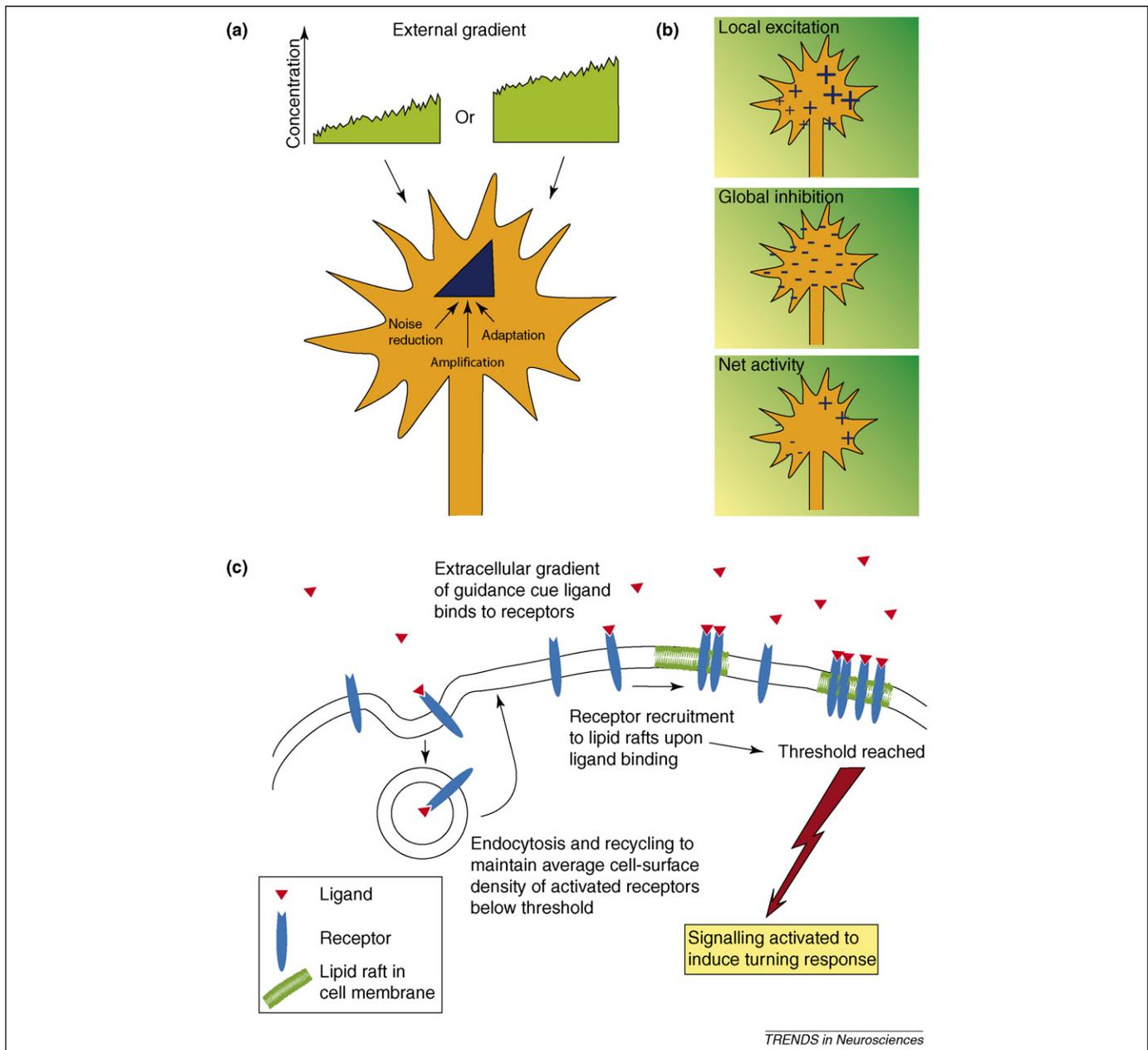
See text for further details. Also see Ref. [7] for a more exhaustive review. N/A, not applicable.

Adaptation of growth cones to changes in absolute ligand concentration has also been observed [27], and appears to involve receptor internalisation and local protein synthesis [28]. Incubation of *Xenopus* retinal growth cones with Sema3A or Netrin-1 results in rapid endocytosis-mediated removal of receptors from the plasma membrane, followed by resensitisation achieved by local protein synthesis and the recycling of receptors back to the membrane [28]. Receptor endocytosis might function as a form of global inhibition, whereas local excitation and amplification might be achieved by a 'switch' mechanism in which downstream signalling occurs only when the local density of activated receptors exceeds a specific threshold. Tuning receptor endocytosis and recycling such that the average density of bound receptors on the growth cone surface is maintained slightly below this

threshold might enhance the detection of a weak gradient (Figure 2c).

### Motility

In *Dictyostelium* and neutrophils, motility is driven by the extension of actin-based structures from the cell periphery, known as pseudopodia. These might then adhere to the substrate and eventually retract, pulling the cell forward. Simultaneously, myosin-driven contraction at the rear helps to push the cell forward into the extended region. This process occurs at fairly regular intervals – typically every 60 s or so in *Dictyostelium* [8]. Similarly, in neutrophils, pseudopod extension at the leading edge is driven by spreading waves of actin polymerisation, each with a lifetime of around 30 s [29]. Pseudopodia are self-organising structures [30]: once a pseudopod has been triggered



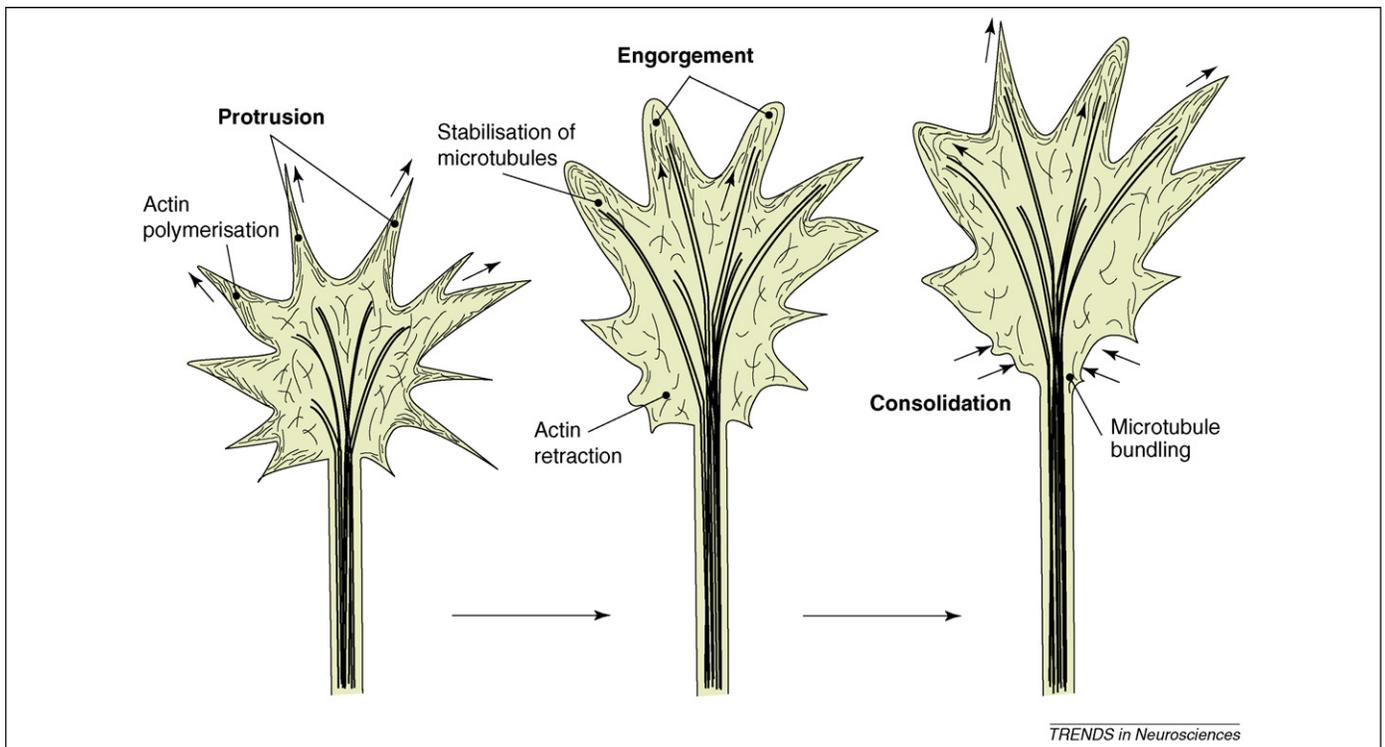
**Figure 2.** Directional sensing in the growth cone involves noise reduction, amplification and adaptation. **(a)** A shallow, noisy, external gradient of guidance cue (green) is transduced into a steeper, less noisy, internal gradient of activity (blue). We refer to an intracellular gradient of 'activity' because the specific nature is unknown at this stage; candidates include  $\text{Ca}^{2+}$ , phospholipids, actin polymerisation and mRNA pools. Adaptation ensures that the internal gradient of activity is independent of the basal concentration of the external guidance cue, thus allowing the growth cone to respond effectively over a range of external guidance cue concentrations. **(b)** A weak gradient, superimposed over a large background concentration, can be exaggerated through a LEGI mechanism. Under such a model, excitatory pathways are assumed to remain spatially confined and thus retain local information about the external gradient. By contrast, inhibitory pathways (whether through rapid diffusion of signalling components or global depletion) have a global influence, reflecting the average external concentration. The net signal obtained by combining the excitatory and inhibitory pathways thus mirrors the slope of the external gradient, but not its background concentration. **(c)** The clustering of bound receptors within lipid rafts might offer a mechanism for amplification: if a threshold density of bound receptors is required within a lipid raft before signalling commences, then a shallow gradient of ligand binding which crosses this threshold would be converted to a strong signalling gradient. For this mechanism to function over a range of concentrations, the average density of bound receptors would need to be maintained below the activation threshold. This might be regulated through endocytosis and receptor recycling.

downstream of receptor activation, it displays stereotypical behaviour, with highly reproducible dynamics and structure. This allows a partial separation of gradient interpretation machinery from that underlying motility, perhaps making for a more robust chemotactic response.

Growth cone motility is also driven by the coordinated regulation of cytoskeletal dynamics – specifically actin and tubulin – and substrate adhesion. The actin cytoskeleton is present in two primary structural forms: a meshwork of crosslinked, highly branched actin filaments that supports

the lamellipodia, and long bundles of fascin-linked actin filaments packed side by side, oriented in parallel, supporting filopodia [31].

Actin dynamics are regulated through various proteins that cleave or crosslink existing actin filaments or induce the nucleation of new filaments [31], and motor proteins that help to drive a rearward flow of polymerised actin [32]. Growth cone motility is partly driven by 'actin treadmilling,' in which actin subunits are incorporated into the filamentous actin (f-actin) cytoskeleton at the leading edge,



**Figure 3.** Growth cone motility proceeds by protrusion, engorgement and consolidation. Actin polymerisation exerts forces on the membrane, leading to growth cone protrusion. The stabilisation of microtubules along actin bundles facilitates the subsequent transport of material from the central zone of the growth cone into the periphery, leading to engorgement of the protruding region. Finally, a nascent axon segment becomes consolidated through the retraction of actin at the neck of the growth cone and the crosslinking of microtubules into a stable bundle.

and the f-actin meshwork undergoes continual retraction toward the growth cone center, where it depolymerises and is recycled to the leading edge [33]. Coupling f-actin to a permissive substrate through adhesion receptors allows the growth cone to develop a traction force, and leads to membrane protrusion (Figure 3).

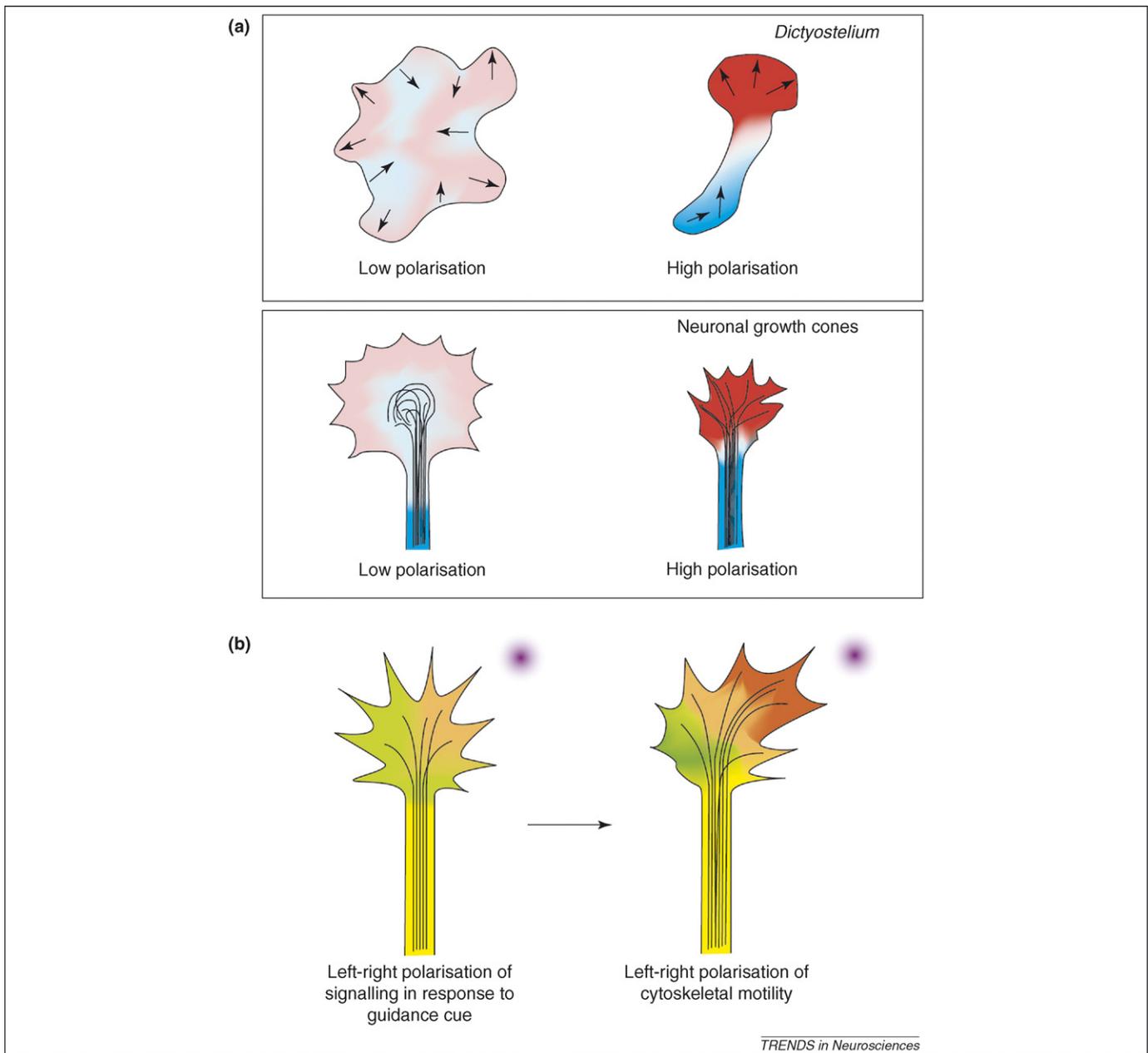
The growth cone is tethered by the trailing axon, so to make progress the axon shaft must lengthen. This involves the delivery of new microtubule segments to the growth cone and the consolidation of the loosely organised microtubules in the central zone into the tight bundle characteristic of the axon shaft (Figure 3). Microtubule polymerisation plays both a permissive role for axon extension and an instructive role in axon guidance [34]. Microtubules continually probe the peripheral region of the growth cone through dynamic instability, aided in resisting retrograde f-actin flow by the motor protein dynein [35]. During such excursions, they transiently associate with the radially directed actin bundles in filopodia [36]. Such associations in the peripheral zone can stabilise, leading to axon advance and turning [33].

Thus, in contrast to *Dictyostelium* and neutrophils, growth cone motility does not appear to be driven by triggering a single kind of self-organised motility event, such as pseudopod extension. Rather, growth cone motility involves a gradual integration of less definitive events that in turn inform the stabilisation and extension of microtubules. Feedback loops involving the delivery of actin-binding proteins via microtubules might then stabilise or destabilise interactions between the actin and tubulin cytoskeletal elements, or lead to the amplification of response in a particular region [37].

### Polarisation

To maintain forward motion, motility events must occur preferentially at the leading edge. In both neutrophils and *Dictyostelium*, several common ‘frontness’ and ‘backness’ molecules have been identified, with functions consistent with the promotion of pseudopod extension at the leading edge and inhibition at the trailing edge [8] (Table 1). In addition to facilitating efficient motility, polarisation also confers the cell’s leading edge with greater sensitivity to stimulation. Thus, polarised cells tend to execute smooth turns in response to changing gradients, rather than make sudden shifts in their direction of motion [8]. In polarised neutrophils, extensive membrane ruffling at the front of the cell might cause an effective increase in chemoreceptor concentration at the leading edge, contributing to this asymmetric sensitivity [38]. However, in polarised *Dictyostelium*, the receptors responsible for chemical sensing remain uniformly distributed, suggesting that processes downstream of receptor binding must also contribute [39,40].

Cells can display different degrees of polarisation (Figure 4a). This variation might reflect adaptation to the dynamics of the environment – cells that are highly polarised might be optimised for stable environments [41]. Such cells turn more slowly than weakly polarised cells [8]. Thus, they would be less capable of responding to rapidly changing gradients, but would also be less affected by noise and display stronger chemotaxis in stable gradients. The distinct segregation between front and rear regions suggests reciprocal negative feedback exists between frontness and backness pathways [42]. Also, polarisation typically persists for some time once established, which might



**Figure 4.** Polarised cells contain well-defined domains, which confer persistent motility and greater sensitivity at the leading edge. **(a)** Cells and growth cones can be polarised to different degrees. Weakly polarised *Dictyostelium* do not show a single distinct leading edge or trailing uropod. As a consequence, these cells display rapid, random changes in movement direction. By contrast, highly polarised *Dictyostelium* are elongated along a definite axis, and possess a single, well-defined leading edge and uropod. Pseudopodia form preferentially at the leading edge, resulting in persistent motion along the direction of polarisation. Similarly, whereas all growth cones show intrinsic front-back polarisation, they display this polarisation to varying degrees. Growth cones splay out at points where they must interpret complex guidance information. By comparison, growth cones travelling along a well-defined path become highly polarised (bullet shaped) and are less sensitive to guidance cues. **(b)** Turning requires the coordination of protrusion in the direction of, and retraction away from, the turn. This coordination is achieved through the development of polarisation along a 'left-right' axis, which ultimately biases cytoskeletal remodelling to effect the turn.

indicate positive feedback within the frontness and backness pathways [9].

Unlike *Dictyostelium* and neutrophils, which typically become polarised only upon exposure to a chemotactic cue, growth cones are conferred with an intrinsic front-back polarity by the trailing axon shaft. They can, however, display varying degrees of front-back polarisation [43]. 'Paused' growth cones take on more complex shapes, and display looped microtubules in their central region – such behaviour is thought to occur when the growth cone must make a complex guidance decision, for example, at a choice point [44]. However, when growing along a well-defined

pathway, growth cones become streamlined and bullet shaped, a morphology that correlates with reduced sensitivity and rapid growth [44].

Just as in *Dictyostelium* and neutrophils, growth cones appear to have a set of specific molecular components that mark their front and rear and maintain the appropriate regional cytoskeletal arrangement. The similarity also extends to some of the specific molecules involved (Table 1). The leading edge of the growth cone is enriched in molecules that promote actin polymerisation and bundling. To maintain anterograde motion a growth cone must also regulate its adhesion to the substrate, developing

stronger adhesion at the leading edge than in the central zone and axon shaft to extract motile force from treadmill actin. It has been demonstrated that the lipid raft-controlled endocytosis and trafficking of cell adhesion molecules from the central zone to the leading edge is crucial for axonal extension [45]. Analogous to the retraction of the uropod in cell motility, growth cone advance involves the process of axonal consolidation, the conversion of the rear of the growth cone into a well-defined axon segment. This requires the translocation and activation of proteins that induce microtubule bundling [46] and destabilise the actin cytoskeleton [47] (Table 1).

During a turn, growth cones develop a left–right polarity that coordinates cytoskeletal extension in the direction of the turn and retraction on the side away from the turn (Figure 4b). Local protein translation is required for many growth cone responses [48–50], and local translation of mRNAs inside the growth cone contributes directly to the turning process both for attractive and repulsive turning. Asymmetric stimulation of *Xenopus* growth cones by attractants such as Netrin or BDNF can cause a rapid recruitment of actin mRNA to the upgradient side of the growth cone, mediated through Vg1RBP [51,52]. These cues also induce the asymmetric activation of eIF4B-binding protein 1 (4EBP), a global transcription regulator, and new  $\beta$ -actin protein is rapidly transcribed in a spatially graded manner. This newly synthesized actin might be more effective in nucleating actin filament formation, subsequently promoting extension [51].

Autocrine signalling and focal addition of transmembrane proteins through targeted exocytosis might also play a role in left–right polarisation [53]. Attractive responses initiated by local uncaging of  $\text{Ca}^{2+}$  and calcium-induced calcium release in chick dorsal root ganglion neurons require the asymmetric, microtubule-dependent trafficking of vesicles to the leading edge of the growth cone, followed by exocytosis. Blocking exocytosis extinguishes the attractive response both to focal  $\text{Ca}^{2+}$  stimulation, and attraction by NGF [53]. Exocytosis occurs very soon after stimulation, observed before any detectable cytoskeletal modification. This suggests that it might be involved in ‘priming’ the growth cone to turn, by asymmetrically modifying membrane composition on the side to which the turn will later be made.

Left–right polarisation might be stabilised by the capture of dynamic microtubules by microtubule-associated proteins and the alignment of microtubules along actin bundles in the peripheral zone of the growth cone. The simultaneous microtubule-mediated delivery of actin-binding proteins, along with the positive regulation of microtubule stability by actin filaments, might act as a positive feedback loop to maintain left–right polarity while the growth cone completes a turn [37].

In addition to protruding toward the direction of the turn, a growth cone must also collapse on the side furthest from the turn. For example, filopodial contact with a nerve growth factor-coated bead usually leads to engorgement of the contacting filopodium with microtubules, followed by turning of the growth cone toward the bead. However, if contraction of actin around newly polymerised microtubule

bundles is inhibited, the growth cone fails to turn, even though the initial stages of filopodial contact, stabilisation and engorgement still occur [47].

### Chemorepulsion versus chemoattraction

Growth cones and eukaryotic cells can be attracted or repelled by guidance cues. Within the framework we have outlined, chemoattraction requires that the directional sensing mechanism transduces a noisy external gradient of chemoattractant into an amplified, cleaner internal gradient in the same direction. This internal gradient then biases intrinsic motility events so that membrane protrusion occurs more frequently on the upgradient side. However, for chemorepulsion, there are two possibilities: either the internal gradient itself must somehow be inverted, or the internal gradient must now act to suppress membrane protrusion on the upgradient side.

In *Dictyostelium* there is evidence for an early inversion of the internal gradient, just downstream of receptor activation, and before amplification, adaptation and noise reduction [54]. Here the chemoattractant cAMP signals through G-protein-coupled receptors (GPCRs) via the  $\text{G}\alpha 1$  G protein subunit to activate phospholipase  $\text{C}\gamma$  (PLC $\gamma$ ). By contrast, the chemorepellant cAMP analogue 8CPT-cAMP also signals through GPCRs, but activates the  $\text{G}\alpha 2$  subunit, which inhibits PLC. PLC in turn is an upstream regulator of the phosphoinositide signalling pathways, which in *Dictyostelium* play a key role in mediating amplification, adaptation and noise reduction.

For growth cones, however, the situation is less clear. For instance,  $\text{Ca}^{2+}$  gradients are downstream, but can induce attraction or repulsion despite having the same polarity as the external gradient [55]. As another example, in *Xenopus* spinal neurons, when bone morphogenic protein (BMP) acts as an attractant it signals through the LIM kinase pathway, ultimately inhibiting the actin depolymerising factor ADF/Cofilin, thus favouring actin polymerisation and membrane extension on the upgradient side [56]. However, when acting as a repellent, BMP instead activates the Slingshot phosphatase (SSH) which activates ADF/Cofilin, ultimately leading to repulsion. In this case, the intracellular gradient of activation is in the same direction as the external gradient; however, these pathways have opposite effects on the actin cytoskeleton. Thus, it appears that several different mechanisms for chemorepulsion have evolved, with less overlap between growth cones and other eukaryotic cells than for mechanisms of attraction.

### Chemotaxis under varying gradient conditions

Growth cones probably need to respond to a range of different gradient steepnesses. There is evidence in *Dictyostelium* that chemotaxis proceeds via different mechanisms depending on whether the gradient is shallow or steep [57]. Whereas in steep gradients these cells make small turns to correct their direction of motion, in shallow gradients *Dictyostelium* cells split their leading edge in two, to form two pseudopodia. One of these then becomes stable in a PI3K-independent manner.

Detailed comparisons of growth cone behaviour in shallow and steep gradients have not yet been performed.

However, migrating neurons, although known to use slightly different signalling pathways than chemotaxing growth cones, generate chemotaxis by extending multiple processes and then selecting one which appears best suited [58], similar to the strategy favoured by *Dictyostelium* in shallow gradients [57]. Why might different strategies be used depending on the gradient steepness? One possibility is that, in shallow gradients, it is difficult to maintain signal integrity over the integration time required to make reliable decisions in the 'turning' style strategy. Splitting the leading edge into two spatially segregated compartments might help to improve the signal-to-noise ratio by reducing correlations caused by diffusion of signalling components from one region of the growth cone to another.

### Concluding remarks and future directions

The challenge of detecting and responding to a chemical gradient is ubiquitous in biology. Hence, it is likely that functionally, if not molecularly, similar mechanisms might have evolved independently. Recently, the understanding of chemotaxis in eukaryotic cells has been propelled by the fruitful interaction between sophisticated theoretical models (see, e.g. Refs [41,42,59]) and experiments capable of determining not only which molecules interact but also where and when they interact within the cell [29,30,40]. Determining how molecular events are regulated temporally and spatially within the growth cone is crucial to furthering our understanding of growth cone chemotaxis. As we have discussed, we can draw inspiration for this endeavour from the parallels between growth cones and their better-understood cousins.

### Acknowledgements

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