Navigating Chemotaxis: an overview on cell's compass and movement.

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Abstract

Eukaryotic chemotaxis has various intracellular molecules that process spatial and temporal information. The relay of information is signaled throughout the cell to promote its movement towards the chemoattractant. This essay describes how cells behave differently in shallow and steep gradients based on the signal generated and the three processes involved in the chemotaxing movement. Interestingly, these external chemical cues may also have some involvement in memory formation for the directed movement of a cell in response to small external stimuli. Studies have shown intricate details on the signalling network of these processes and how each molecule has a role in it.

1. Introduction

Chemotaxis is characterized by the motion of the cell directed via its surrounding chemical gradient. Cell motility is an essential feature of systematic cell dynamics present in all organisms ranging from bacteria to humans (1). It is of great importance for the proper functioning of various cellular responses. In single- cell organisms, chemotaxis specifically controls motile behavior that is critical for locating food, avoiding predators or extreme conditions, all in order to have higher chances of survival. In multicellular organisms, migration is required for a plethora of fundamental functions – to guide cells of the immune system to the site of infection, to allow leukocytes to seek out site of inflammation and infection, for neurons to send projections to specific regions of the brain to find their synaptic partners, yeast cells to mate, fibroblasts to move into the wound space and amoeba- *Dictyostelium spp.* to aggregate during morphogenesis. Chemotaxis can also aid the spreading of cancer during metastasis, the process by which cells leave the primary tumor and seed new tumors in other parts of the body. Certain conditions exhibit unwanted immune cell chemotaxis, such as those which cause chronic inflammatory diseases such as asthma and arthritis (1–5).

The general mechanism of chemotaxis is conserved throughout all prokaryotic bacteria and archaea, which utilizes the temporal sampling mechanism to determine the direction of the gradient. More specifically, the motility is controlled by a 2- component system involving a histidine kinase for sensing the environment and a response regulator for signal transduction. Prokaryotes possess chemotactic proteins that can be classified into four groups – a signal recognition and transduction group, an excitation group, an adaptation group and a signal removal group (6, 7), and comprises of six proteins. These groups differentiate the chemoattractants via gradient sensing and transverse towards higher concentration. The adaption group helps in determining whether further movement is required and if needed, whether the direction is correct. This phenomenon is also known as temporal sensing. Direction is changed based on the probability of 'runs' and 'tumbles' that help in further sensing of the gradients, thus leading cells in the right direction (8).

The mechanism of chemotaxis in eukaryotic cells is different and more complex than that in prokaryotes, comprising of 100 proteins or more. Chemotaxis is characterized by three distinct processes: gradient sensing, cell polarization, and motility. Gradient sensing, also termed as the 'cell's compass', is the regulation of cell movement by detection and utilizing spatial or temporal sensing mechanisms. Cell polarization involves the detection of extracellular signals and progression through a series of cytoskeletal rearrangements that promote the protrusion of a leading edge of a cell. Finally, cell motility, which occurs irrespective of the presence or absence of an extracellular cue, is driven by a pseudopod (leading edge) protrusion that facilitates random and directed migration. For efficient translocation, motile activities need to be coordinated. This is achieved by cell polarization (7–10). In a eukaryotic cell, the three processes combine to provide the cell with a direction to move, and as a final step, actin assembly usually provides a major force for the cell movement – by driving the membrane protrusions that propel the motility (11).

2. How does the compass and forward movement work?

"Eukaryotic cells often devote much time and energy to business trips" (12). But how does the cell know where to move? The path chosen greatly depends on the chemotactic gradient and whether or not the cell is polarized. As mentioned earlier, eukaryotic cells detect and amplify the shallow external gradient of the attractant. They convey the signal into a steeper gradient via internal signalling which polarizes the cell. The polarization leads to a projection of pseudopods at the front, which points towards the external gradient. These protrusions are provided with driving force to move forward. In this essay, each method has been laid out

distinctly. However, since each signal can have multiple responses, each process is also well connected.

2.1 Gradient sensing

Benefitted by larger size, eukaryotes can detect the chemical gradient along their plasma membrane and sense the receptor occupancy to determine their response. The difference between activator and inactivator gives rise to spatially oriented and persistent signalling. Directional gradient sensing refers to the ability of the cell to detect an asymmetric extracellular signal and generate an internal amplified response (13). Eukaryotes effectively sense and respond to a spatial gradient. Cells are noted to be more sensitive at a constant mean of concentration levels or at increasing concentration with time. These cells respond to a wide variety of metabolites and peptides However, most cells are found to employ G-protein coupled receptors (GPCRs) to detect the external signals and provide feedback through small G-proteins (14, 15). Chemoattract receptors and their corresponding G-proteins remain uniformly distributed along the cell membrane which allows the cell to have a directional sense, even in shallow gradients with 2-10% chemoattractant concentration (16). The signalling molecules accumulate at the membrane adjacent to the higher concentration and initiate a downstream response by redistribution or activation of signalling lipids and proteins. A very small difference in the receptor occupancy from continuous external signal gradient can be actively amplified into highly polarized events, thus providing sensitivity to small alterations and giving rise to a dramatic redistribution of cytoskeletal machinery (17–19). Extension of the anterior pseudopod is carried out by the accumulation of F-actin and posterior accumulation of actomyosin. However, the directional sensing response does not always require the cell to be polarized. Unpolarized, immobilized cells can also detect gradients with a similar degree of signal amplification, a property observed by imaging fluorescently labelled proteins in immobilized cells (20). The cell's movement is dependent on its interpretation of the signal from the cell surface from the highly simulated regions, and this directional sensing is referred to as the cell's compass.

2.1.1 Chemoattractants and G-protein receptors

The binding of chemoattractants and gradient sensing by the receptors marks as the first event in a series that leads to polarization and, ultimately, results in cell movement/ migration. The chemotactic behavior presented by amoeba is very similar to that observed in mammalian phagocytic cells. The chemoattractant signalling pattern is also similar. Amoeba cells respond

to folic acid, platelet activating factors, lysophosphatidic acid (LPA) and cAMP, whereas mammalian cells respond to PAF, LPA and a wider variety of chemokines compared to that of amoeboid cells. Despite the wide spectrum of biochemical reactions elicited by the various attractants, most cells utilize G-protein linked pathways to carry out the signal transduction.

In *Dictyostelium*, two main forms of chemotaxis are observed. During their normal life cycle, folic acid - a bacterial byproduct, acts as a cue to direct the amoeboid cells towards their food source-bacteria (21, 22). In starvation conditions, these cells lose their ability to sense folic acid and instead enter a developmental program with the ability to produce and secrete cAMP (23). cAMP is used as a signalling molecule or an indicator across cells (Fig 1). Subsequently, acquiring the ability for directional movement to aggregate and for multicellular structures to resist harsh conditions (24).

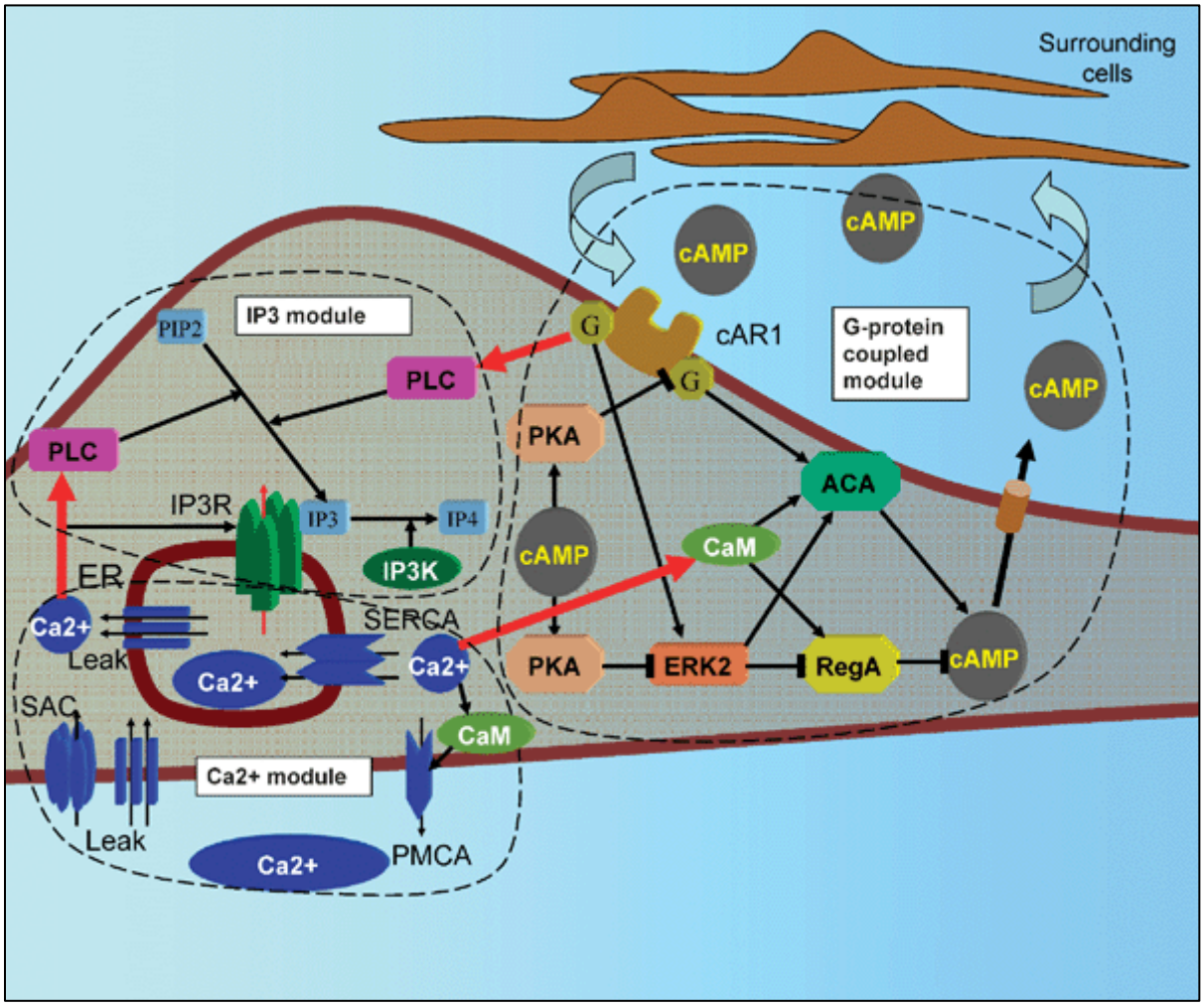

Figure 1 Chemotaxis signalling through Dictyostelium(25)

Both the chemoattractants belong to the G-protein coupled receptors family, each comprising of an extracellular N-terminal domain followed by seven transmembrane helices and a C-terminal tail. These receptors mediate most of their effects through heterotrimeric G proteins

(26, 27). The folic-acid receptor is yet to be well- studied on. However, four distinct cAMP receptors (cAR1 to cAR4), which are expressed at different times during *Dictyostelium* development are uniformly distributed around the cell periphery (28). Additionally, many responses have been noted from the binding of cAMP to cAR1 independent of G-proteins (13). Furthermore, two PH domain containing proteins, the *Dictyostelium* cytosolic regulator of adenylyl cyclase protein (CRAC) and the AkT/PKB (Protein Kinase B) were found to be regulators of chemotaxis.

Mammalian leukocytes couple to 20 types of chemoattractants and chemokines that signal through the G-proteins (Toxinsensitive Gi – coupled as well as G12 and G13 coupled GPCRs).

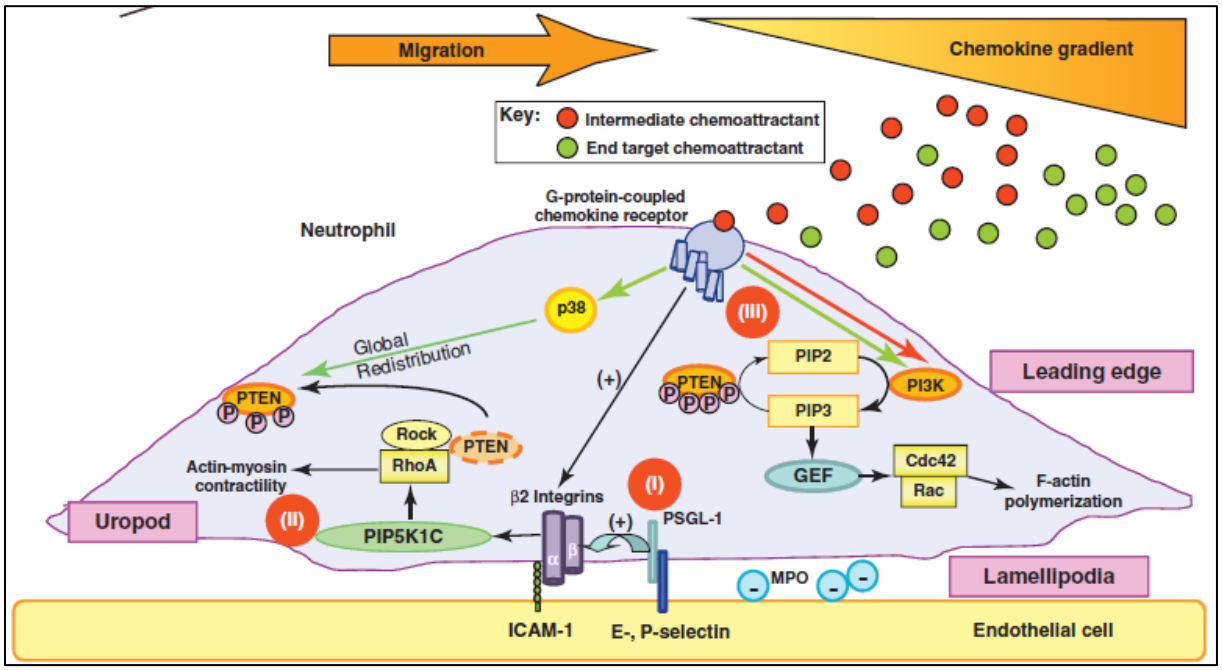

Figure 2 Chemotaxis signalling in Neutrophils (29)

Mammalians cells also respond to classical chemoattractants such as formylated peptides secreted by bacteria, N- formylmethionylleucyl-phenylalanine (fMLP), chemokines such as α, β, δ, γ, CC, CXC, CX3C, leukotrienes – paracrine and autocrine lipid mediators, phospholipid metabolites, cAMP, cGMP, inositol trisphosphate and Ca^{+2} (22, 30–33). Over the years, various other receptors were found for amino acids, insulin, and vasoactive peptides.

Most of these molecules can be divided into categories of intermediate and end-target chemoattractants. Chemokines and lipid mediators are classified as intermediate chemoattractant. Molecules generated by bacteria, like N-formylated peptides and complement 5a, are grouped under end-target chemoattractants. Intermediate

chemoattractants signal through Phosphoinositide 3-Kinase, PI3K pathway, whereas end-target chemoattractants signal via p38 mitogen activated protein kinase.

2.1.2 Relay of signals

In both, *Dictyostelium* and neutrophils, the binding of the chemoattractant to its receptor triggers the dissociation of proteins from the cell membrane. The signal transduction is carried out through the activation of GPCRs. This receptor couples to the heterotrimeric G protein and stimulates the exchange of GDP to GTP. This activation leads to the dissociation of α and βγ subunits (22, 34, 35). The βγ dimer acts as the main transducer of chemotactic signals and activated downstream receptors which also include adenyl guanylyl cyclase (AC), Ras and Rho proteins, and phospholipase C (PLC). Activation of AC increases the levels of cAMP whereas, PLC catalyzes the conversion of phosphatidylinositol 4,5- bisphophate to diacylglycerol (DAG) and inositol trisphosphate (IP3). Activation of Ras proteins is regulated by multiple guanine nucleotide exchange factos (GEFs) and GTPase-activating proteins (GAPs). GTP- dependent Ras protein and in certain cases, G-proteins can directly activate the local activator PI3K and global inactivator – phosphate and tensin homolog, PTEN (33, 36, 37). The PI3K converts/phosphorylates phosphatidylinositol-4,5-bisphosphate (PIP2) into phosphatidylinositol-3,4,5-trisphosphate (PIP3), initiating pseudopod formation at the leading edge. In *Dictyostelium*, the presence of PTEN at the sides and the back of the cell ensures spatial restriction of the PIP3 at the front (22). Locally generated PIP3 acts as a binding site for a subset of the pleckstrin homology (PH) domain containing proteins which are again translocated towards the leading edge, and act as nucleation factors to activate key effectors of migration. PI3K, in combination with PTEN, is involved as a regulator in chemotaxis by binding to two PH domain containing proteins – CRAC and PKB (38–41). PI3K and PTEN are mutually exclusive, while PI3K phosphorylates PIP2, the latter counteracts its action. The decrease in PI3K activity leads to a decrease in PIP3 and chemoattractant additions, subsequently reduced recruitment of CRAC to the plasma membrane (33, 42). Therefore, it can be concluded that cellular distribution of these two regulate the production of PIP3 at the leading edge of chemotaxing cells and serves to amplify external chemical gradients into internal polarized signals (7).

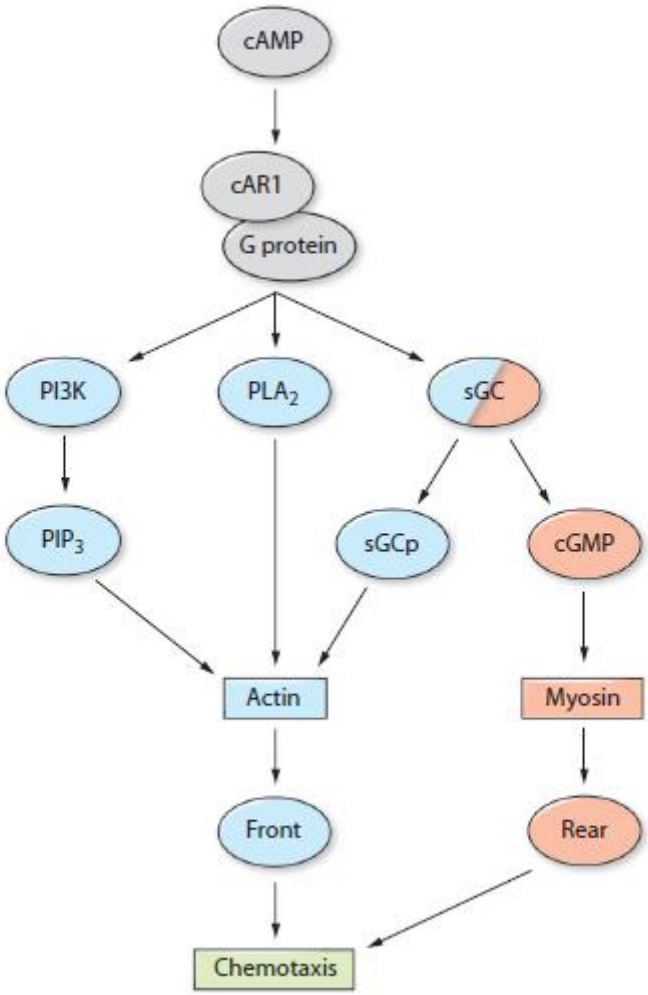

Figure 3 Relay of signal initiated by chemoattractant, cAMP (14)

Ras and PI3K are known key regulators of cell growth with an active role in signal transduction to regulate the actin cytoskeleton, cell polarity, and motility.

2.2 Cell Polarization

Cell polarization is a fundamentally necessary process for carrying out fundamental functions that range from neuronal signal transmission to ion transport across the epithelium to cell migration. It is a process widely conserved in eukaryotes from yeast to human and is said to maintain cell orientation by the anisotropic distribution of factors (43–46). Neutrophils and starved *D. discoideum* cells have been shown to have a strong polarity, which aids in their persistent motility. Chemotaxis, as noted earlier, is a dynamic process in which cells become polarized within a few minutes of being placed in a gradient, and often, can reverse direction when the gradient is altered. Chemotactic cells are morphologically polarized with a leading front and a trailing end. Polarity can be separable from directional sensing, as cells with

uniform chemoattractants can still be polarized. Although polarized cells move with more persistence than unpolarized cells, they do not move in a specific direction. Cells display various degrees of polarization that may also change with conditions. The relay of information generated as an outcome of the spatial gradient leads to a growth of actin-network known as the leading front/ pseudopods that pushes the membrane forward. The molecules associated with a leading edge, apart from the actin network, also include actin-binding proteins Scar, WASP, filopodin, cofilim and coronin. At the sides and back of the cell, the formation of errant pseudopods is suppressed by the cortical actin/myosin II complex. The cell's rear usually forms retracting uropods that help in the forward movement (47, 48). As mentioned earlier, cells can turn direction- however, the cells don't simply change their leading edge into a tailing edge. Instead, each cell follows a highly sensitive "noise", maintaining its original anterior instead of re-distributing PH domains and executes a U-turn. Determination of the cell front and back is a pre-requisite in understanding and organizing the machinery that gives rise to cell motility (49–51).

2.2.1 Symmetry breaking and responsiveness

Chemical stimuli such as cAMP for *D. discoideum*, fMLP for neutrophils and other known chemoattractants are universally known to lead cells into polarization. However, chemical gradients are not the only incentives. Other factors include membrane tension, especially in elongated cells, mechanical stimuli, electrical fields and substrate rigidity. What is also interesting is the continuous presence of polarity and formation of pseudopods in *D. discoideum* cells, which can be guided by the above-mentioned cues and can give the cell a direction in its motion. Unlike directional sensing, polarization depends critically on the actin cytoskeleton. Therefore, inhibitors of actin polymerization can convert a polarized cell to an unpolarized one. This treatment eliminates both polarized morphology and sensitivity, suggesting that an interaction of key signalling molecules with the cytoskeleton stabilizes the polarized state (51).

The asymmetric responsiveness can be explained through various mechanisms. The first focuses on positive feedback regulation of cell polarity by amplification of the response from the external signal at the front of the cell. This is carried out by the direct delivery and metabolism on PIP3. PIP3 has been shown to initiate signal transduction at the upstream level to exhibit asymmetry as well as, induce polarity and motility at downstream level. PIP3, in turn, activates Rho - GTPase. This family of proteins controls the protrusion and contraction of pseudopods. The activated GTPase, in turn, promotes actin polymerization in a pseudopod

at the leading edge, where the GTPases and new actin polymers together promote further PIP3 accumulation, amplifying the response to attractant at the front of the cell (Fig 4).

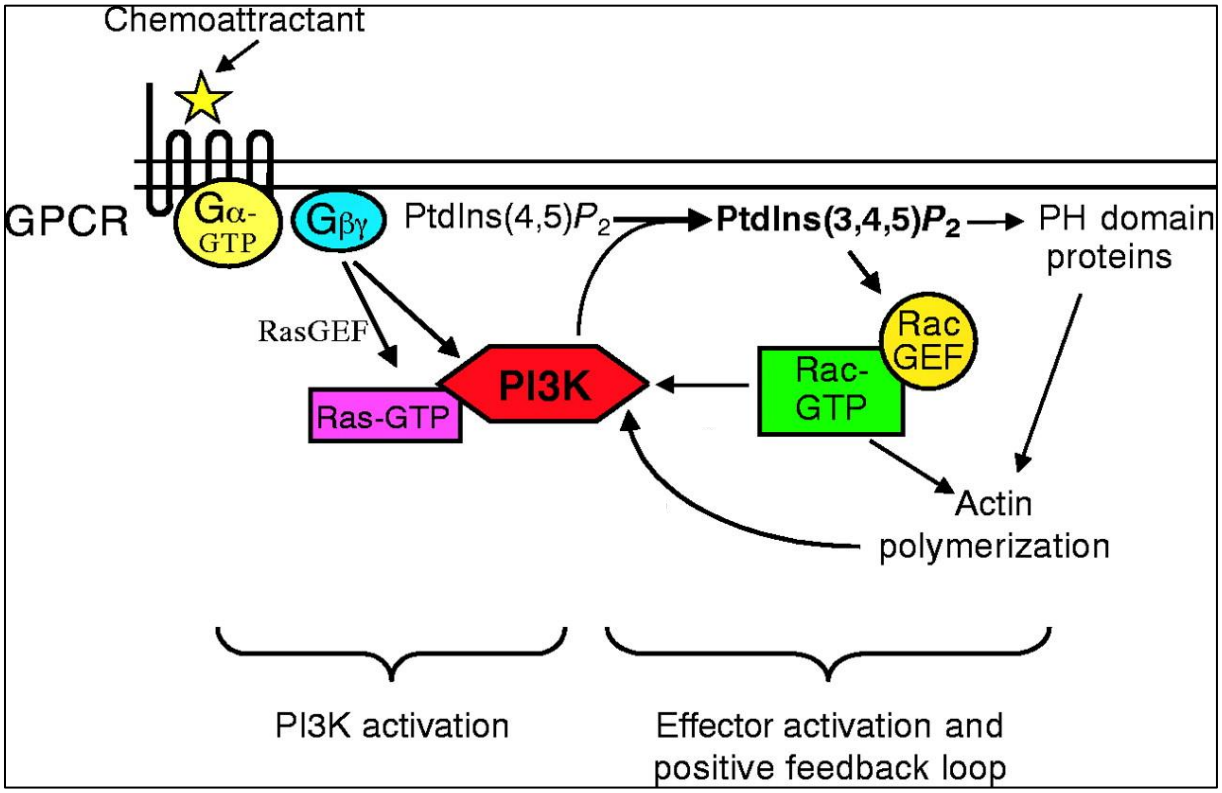

Figure 4 Chemotactic signal relay with a positive feedback loop (52)

Since PI3K is spatially distributed it can cause a disturbance in the presence of a small level of chemoattractants or short burst in the presence of a uniform gradient. Such accumulation can be downregulated by negative feedback. This includes inhibition or degradation of PIP3 and negative regulation of Rho-GTPase. 3′ lipid phosphatase, PTEN, and the 5′ lipid phosphatase SHIP are the two main components known to regulate phosphatase activity towards PIP3.

In both the machineries, components are controlled, so as to initiate an outward movement of the cell front (initial front protrusion) or a stable, inward movement of the cell rear (initial rear retraction), or both together to break cell symmetry and start the migration.

2.3 Cell motility

It has been known that a cell experiences several forces during migration, including the protrusion force, which overcomes hindrances caused by friction and viscosity, and drives the cell to move toward the stimulus. Other forces experienced by the cell are caused by adhesion

of the leading edge, de-adhesion at the cell body and rear, and cytoskeletal contraction (5, 15). The gradients of chemoattractants or chemorepellants modify the basal behavior of the cells. The absence of these molecules leads to uniform polarization of cell and pseudopods extension with random walks (53, 54). Presence of a gradient provides a direction for the movement. Extension of pseudopods at the leading edge is ensured by the uniform distribution of Gβγ and chemoattractant receptors. The uniform distribution selectively activates signalling pathways downstream of the receptors and G-protein and upstream of actin leading to actin polymerization and F-actin accumulation. As cells mostly require one pseudopod for its migration, it is also important to have a component to suppress the lateral pseudopod. This is achieved by the presence of PTEN, that promotes myosin II filament formation at the rear end providing it with a power to retract at the back. The pseudopod, composed of a dense dendritic network of actin filaments, not only pushes the plasma membrane forward but also serves as an intracellular scaffold favoring the appearance of links with the extracellular matrix (ECM). The ECM provides stability to the pseudopod and allows binding of the cell body together, hence can also be termed as adhesion complexes (55, 56). These links through intracellular cues can activate integrins which come together to form stable structure held together by talin and paxillin and strengthened with vinculin. The actin filaments apply force onto the adhesion complex and ability to migrate forward. Once they reach the gradient source, they begin to disassemble.

2.4 Cytoskeleton accumulation

Actin filament: To have a directional migration, rearrangements of the cytoskeleton are required. The redistribution is promoted by F-actin polymerization at the front and actomyosin at the back. Actin filaments have an inherent polarity, which is used to drive membrane protrusion. *In vitro* studies indicate that actin polymerization mediated by the Arp2/3 complex, the activity of which is controlled by adaptor protein WASP (Wiskott Aldrich syndrome protein), and SCAR/WAVE, suppressor of the cAMP receptor (57). SCAR/WAVE are localized in front of the cell and bind to Rac proteins, its activation leads to the production of the actin-filled pseudopod. Rac proteins belonging to the Rho GTPase, have been shown to activate the PI3K pathway leading to production and subsequent delivery of PIP3 to the membranes (Fig 4). The front actin- accumulation has been suggested to enhance the PI3K activity, generating a positive feedback to reinforce polarity and directional sensing. Another GTPase that might have a relation in the up-regulation of actin accumulation is Cdc42 (Fig 5B,5D), which is active towards the front of migrating neutrophils. A feedback

loop that could determine where pseudopodia are formed in neutrophils consists of p21-activated kinase-1, PAK1, a target of Cdc42, which, in turn, stimulates Cdc42 (14).

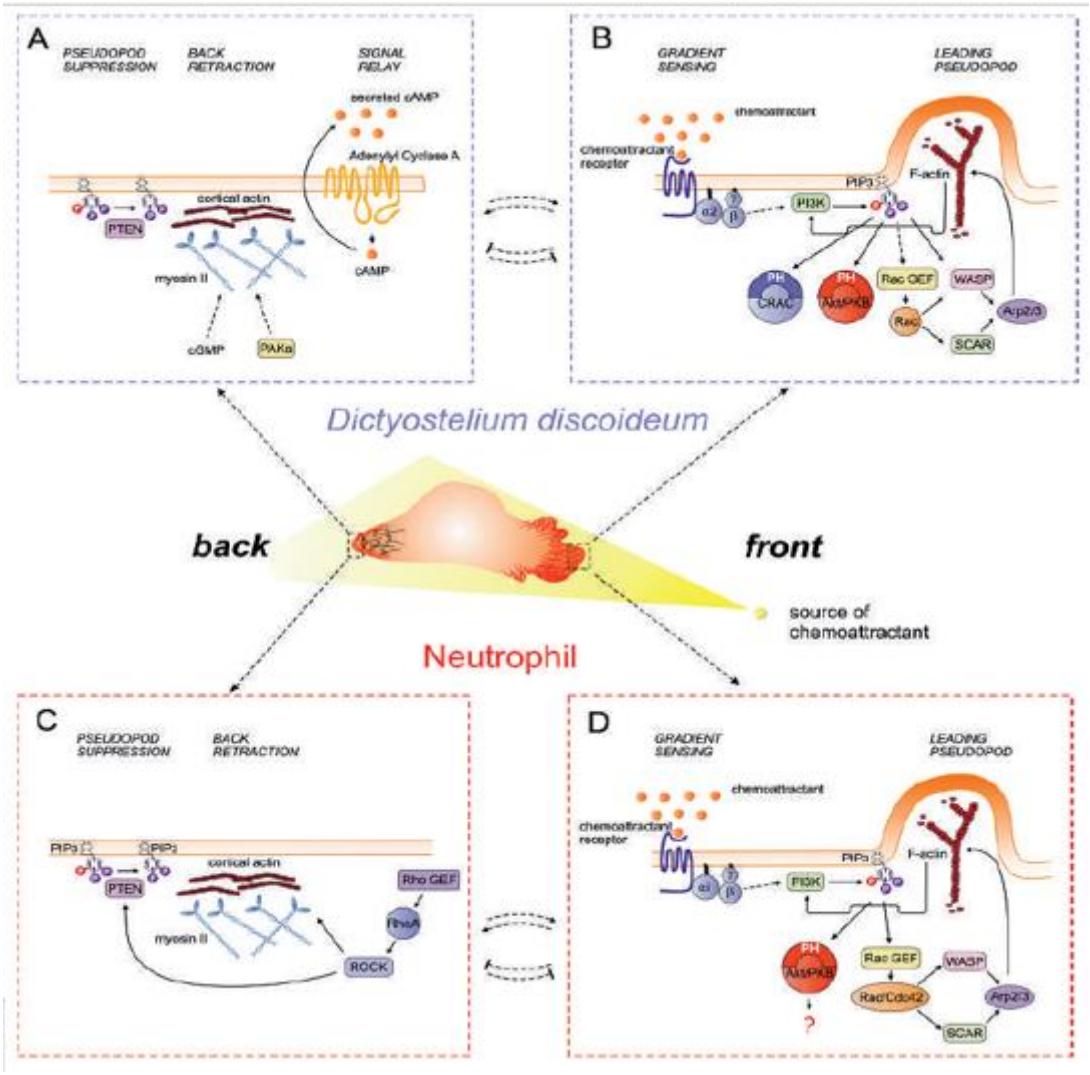

Figure 5 Representation of the chemical signals involved in pseudopod formation (22)

Myosin filament: The retraction occurring at the tailing edge is due to the myosin- II filament present at the back and sides of a cell. The retraction is mediated in neutrophils by RhoA, activated by $G\alpha_{12/13}$. RhoA along with its kinase, ROCK (RhoA and Rho- associated kinase) regulate the contractility of myosin-II which in turn in regulated by Rac and Cdc42 (Fig 5C). In *Dictyostelium* cells, RhoA and Cdc42 homologs are absent, instead, the intracellular cyclic GMP that is produced on chemotactic stimulation mediates the formation of myosin filaments (Fig 3). Like the pseudopods, myosin filled uropods are gradient dependent and are observed to spread more along the sides to the back to provide with a mechanism to suppress lateral

pseudopod (Fig 5A). The filaments provide the power to retract uropods and thus, more support in the motility (37, 48).

Summary box:

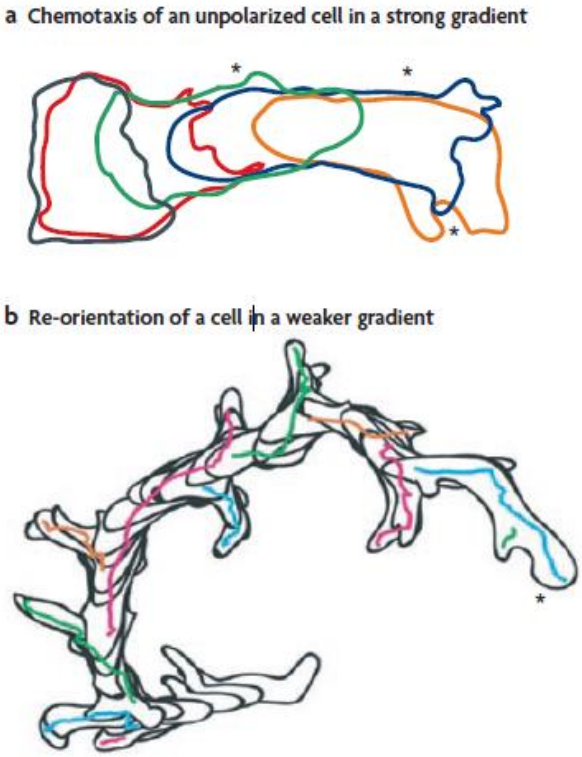

Figure 6:Movement of pseudopod based on gradient.(59)

a. Outline of cell chemotaxing in steep gradient and its ability to orient steadily
b. In shallow gradient, cell re-orients more frequently to determine its movement and process is more gradual
    - Marks the pipette position and couloured lines outline the movement of cell based on a timeline

As understood from the previous sections, the gradient strength and polarization of the cell elect the path chosen by it. Studies postulate that migration of cells with pseudopods occurs using one of the two models: splitting of an existing pseudopod or extension of a de novo pseudopod.

In the shallow gradient, new pseudopodia is produced by splitting from an existing one with little or no reference to the gradient. Cells then favor the pseudopod that is facing towards the higher gradients, retracting the unsuccessful daughter. These cells take up the biased random walks due to the background noise from asymmetry and fluctuations of receptor occupancy (59–61).

In steeper gradients, gradient and polarization each have strong roles. If the cells are unpolarized pseudopodia is produced directly facing the gradient, moving towards the source. And, in polarized cells, there is a tendency of to maintain the existing pseudopod even in opposing gradient (62, 63). That is, in cases of flipping of the gradient towards opposite direction instead of generation of a new pseudopod in that direction, a polarized cell makes a U-turn.

3. Memory

Over the years, a lot of research has been conducted to understand the chemotaxis mechanism in static gradient and its sensitivity to spatial cues. Chemotaxis in the natural environment, however, is much more dynamic and utilizes both spatial and temporal cues- the combination of which remains widely unexplored. For example, in the case of *Dictyostelium*, nutrient deprivation gives rise to a self organised chemoattractant gradient. A non-dissipating wave of gradient field transverses to guide the cells towards the wave, for aggregation. If cells were to react only to the spatial gradient, that would lead the cells to move forward in the front and backwards at the rear end, showcasing a wriggling movement at the same spot without any motility. Here, the temporal cue comes in – which senses increase in concentration in front with time and a decrease in the back, thus leading to a one-directional movement. In bacteria, temporal sensing provides with short term memory for an adapting system. The utilization of this cue in eukaryotes leads to the speculation of the presence of a similar memory in eukaryotic cells as well (58). Proof of this is believed to be shown by the Ras activation that can adapt on a short time scale. Additionally, polarized chemotactic cells have uniformly distributed receptors and signalling molecules that give rise to persistent random walk of 3-10 minutes. Presence of such migration in relation to polarity is hypothesized as an indication to a long-term memory. Long- term memory can enable a cell to remember the directional movement of the cell under the influence of the propagating wave. However, in symmetric wave propagation, the strength of the wave is similar at front and back of the cell, although in opposite direction. This raises the question of how the cell recognizes the origin of the wave and move towards it. Furthermore, it is unclear on how the memory helps in the determination of direction in such conditions and whether it can be employed as active sensing strategy to increase persistence and migration speed (11, 58).

4. Conclusion

In this essay, the chemotaxis system has been laid out in order to understand the mechanism individually and together, forming a complex system. The focus has been around the chemical signal transduction and its response-based results, however, various other methods have been utilized to understand chemotaxis in both *Dictyostelium* and neutrophils along with various other cells. Some of the most commonly used models are LEGI based.

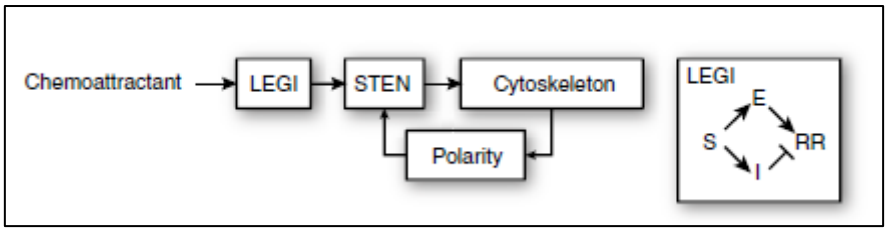

Figure 7 Chemotaxis signalling pathway based on LEGI (15)

The LEGI, local excitation- global inhibition, module senses chemoattractant stimuli and biases the activity of the STEN, which regulates the cytoskeleton. Polarity, which depends on the existence of an intact cytoskeleton, provides feedback by further biasing the STEN's activity. The LEGI module includes excitor (E) and inhibitor (I) elements that are regulated by receptor occupancy (S) and regulate the response regulator (RR) in complementary ways (Fig 7). This module allows to differentiate directional sensing from cellular polarization, it describes directional sensing by a rapid, local excitation which is balanced by a slower global-inhibition depending on the receptor occupancy. Differences in the local concentration of activator and inhibitor are then hypothesized to establish the front and back of the cell. Along with an explanation of the signalling pathway, this module, coupled with bi-stable memory module, can provide with a possible insight into the long term- memory of cell.

The LEGI model alone cannot explain the switch type behavior of PH- domain containing proteins. Therefore, additional modules were made to study, a balanced inactivation module for gradient sensing. Separate modules were also created to understand the polarization by studying it through various feedback loops or the noise and receptor occupancy or via symmetry breaking. Additionally, the spatio-temporal sensing is another module on which research is well focused on, however, it is not well understood. In Eukaryotes, it is not yet clear how cells have a fast rate of response and can respond to both spatial and temporal cues.

Furthermore, the focus on chemotaxis can also be understood through pathways not discussed above. Various other modules could also be based on connecting the gradient sensing and polarization to the memory of the cell for its motility. Finally, the detailed insight and understanding into the pathway and mechanism in eukaryotic cells is an open window to target specific pathways for various chemotaxis related diseases.

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