university of groningen

Dictyostelium discoideum: Memory in chemotaxis

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Table of contents

1 Abstract

The slime mold *Dictyostelium discoideum* is a eukaryotic amoeboid that can live in solitary, but also as multicellular organisms. Through chemotaxis, the movement of an organism in response to a chemical stimulus, cells can seek food and find other cells to survive and reproduce.
As a solitary amoeba, *Dictyostelium* feeds on bacteria in fertile soil and reproduces by binary fission. However, when food sources are depleted *Dictyostelium* cells aggregate and form a multicellular fruiting body which reproduces by releasing spores. To hunt down bacteria and find each other while aggregating, *Dictiostelium* cells need some sort of memory. Memory allows cells to have a sense of direction and determine efficient pathways to reach their destination. Since amoebas do not have brains like animals, memory is established in a vastly different way. Memory in amoebas like *Dictyostelium* is regulated on a molecular level by the use of specific signaling pathways. These signaling pathways are made up of proteins and molecules that form a cascade of chemical reactions and interactions, allowing cells to move persistently toward their destination, even if the direction of their destination is changed constantly. Research has shown that cells have distinguished short-term and long-term memory, similar to animals. This essay will discuss the details of short- and long-term memory in *Dictyostelium* cells and how these types of memory are established within the cell.

2 Introduction

Learning and memory are two of the most important things in life. Learning is the process of gaining new knowledge about the world, while memory enables one to store and retrieve this information. Stored information can be used to adapt mechanisms of behavior in new situations, based on previous experiences. Therefore organisms behave based on what they have learned, and what they remember and forget. Research has shown that the ability to learn and memorize is not only limited to animals but is also found in amoebas [1, 2]. However, memory in amoebas is not as sophisticated and mostly revolves around the memory of direction and spatial information, which is information obtained from the surrounding area.

The memory of spatial information is an important part of food-searching strategies in a heterogeneous environment [3]. Similar to animals spotting a food source in the distance, cells have to memorize the location of surrounding bacteria. The spatial information and memory of direction are used to chase after the food source. Even though the molecular mechanisms underlying memory in animals and amoebas are vastly different, some characteristics of memory are shared like having a distinct short- and long-term memory [2, 4].

The slime mold *Dictyostelium discoideum* is a eukaryotic amoeboid that lives in solitary but can be induced to form a multicellular organism. Therefore *Dictyistelium* has a rather interesting and multilayered life cycle. In the vegetative state, *Dictyostelium* lives as a solitary amoeba, feeding on bacteria and dividing by binary fission. When the food supply is depleted, *Dictyostelium* enters the aggregation stage, in which growth and vegetation stop [5-7]. Starved cells start secreting cAMP and forming aggregation centers from which pulsing cAMP waves are released and relayed by surrounding cells. The cAMP acts as a chemoattractant for other *Dictyostelium* cells and induces the cells to move toward the aggregation center to form a mound of cells [8]. The mound of aggregated cells starts a complex process of cell differentiation and cell movement in which cells differentiate into prestalk and prespore cells, forming a slug [9]. The slug acts as an intermediate stage and allows the aggregated cells to move to a new location exposed to light, using phototaxis instead of chemotaxis. Once the cells are fully differentiated and a new settling location has been found, the slime mold forms a fruiting body. The fruiting body resembles a tiny mushroom and consists of a stalk and cap filled with spores [10]. Finally, the spores are released and dispersed on fertile ground, containing food sources. At this point, new *Dictyostelium* cells emerge from the spores and the life cycle repeats.

During the vegetative and aggregation stage of *Dictyostelium*, a lot of movement and coordination of single cells is required. Eukaryotic amoeboid cells like *Dictyostelium* move through the extension of pseudopods, which are actin-filled protrusions of the cell surface that push the cell forward [11, 12]. The rear of the cell is contracted by a network of myosin II and actin filaments, called the uropod [13]. Instead of moving in random directions, cells have been shown to move with a correlated random walk, meaning that cells tend to move in a similar direction to their previous direction. [14, 15]. This correlated movement is called persistence and allows cells to move more effectively as long as cells are polarized. Polarized cells can retain persistent movement for several minutes, while unpolarized cells move persistently for less than a minute [14, 16]. The correlated walk found in amoebas implies that cells memorize their direction and spatial information, which are stored as part of short- and long-term memory.

The short-term memory of amoeboid cells remembers the position of their previous pseudopod, similar to an animal remembering which leg was previously put forward. Short-term memory allows cells to extend pseudopods alternatingly left and right in a zig-zag trajectory, which contributes to persistence [14, 16, 17]. The long-term memory of amoeboid cells is liked to polarity and remembers the overall direction of many previous pseudopods, comparable to an animal remembering the direction of its path. Polarized cells mainly extend pseudopods in the front and are less likely to extend pseudopods in the rear of the cell, thus having improved persistence [14]. Both short- and long-term memory in amoeboid cells are recognized to be related to persistence.

Navigating toward nearby food sources and finding aggregation centers through pulsing cAMP waves requires coordinated movement, making persistence important during the vegetative and aggregation stage of the *Dictyostelium* life cycle. Therefore *Dictyostelium* uses a combination of zig-zag trajectory and polarity to retain persistent movement. By studying the pseudopod formation in *Dictyostelium*, research has shown that cells show signs of both short-term and long-term memory during chemotaxis [2]. Both types of memory help *Dictyostelium* cells find food in heterogeneous environments and aggregate when food sources are depleted. Thus making memory an essential part of the survival of *Dictyostelium* cells.

How memory is established in cells, can be explained by the underlying molecular mechanisms of chemotaxis. The chemotaxis signaling pathways of *Dictyostelium* can be divided into front and rear pathways. As the name suggests, the front pathways regulate the front activity, and the rear pathways the rear activity of the cell. The front pathways are regulated by the signaling proteins sGC, TORC2, PI3K, and Rap1 [18-21]. The rear pathways are regulated by the signaling protein PLA2 and the second messenger molecule cGMP [18, 22]. A combination of these signaling proteins and their downstream effectors is responsible for the existence of memory in amoebas.

This essay discusses the purpose of memory in eukaryotic amoeboid cells, specifically *Dictyostelium*. How memory is established will be explained by discussing the Local Excitation, Global Inhibition (LEGI) model. Finally, short-term memory (memory of position) and long-term memory (memory of polarity) will be explained in detail as well as the underlying molecular mechanisms.

3 The purpose of memory

During the aggregation stage of *Dictyostelium*, non-dissipating waves of the chemoattractant cyclic AMP (cAMP) are sent out from the aggregation centers. Through signal relay, the cAMP waves can reach over a long range and cause the migration of cells toward the wave source [8]. Cells respond to the spatial gradient of the wave, which is the change of concentration chemicals with respect to the position coordinates. The spatial gradient of a symmetric traveling wave is of equal strength but in the opposite direction. Therefore, simply responding to the spatial information would cause the cells to move forward in the front of the wave and backward in the back of the wave. This problem is known as the back-of-the-wave paradox [23]. For cells to move efficiently toward the wave source, additional processing is required. *Dictyostelium* cells can do this by responding to the temporal gradient of the wave, which is the change of concentration chemicals over time. As time passes, the concentration of cAMP increases in the front of the wave and decreases in the back of the wave, making it possible for cells to distinguish between the front and back of the wave. Responding to the temporal gradient of a wave is likely achieved by the existing polarity within a moving cell.

Migrating *Dictyostelium* cells exhibit polarity, which elongates the cell shape and forms a defined front and rear of the cell through the distribution of signaling molecules. Polarization occurs after recognizing chemoattractant cues and subsequently directs the formed front toward the direction of the gradient. Upon polarization, cells can persistently migrate in the direction of the gradient. Therefore the persistence of migration is dependent on the time that polarity is maintained. Cells can also reverse their orientation by inverting their polarity upon reversal of the gradient [24]. However, the movement of *Dictyostelium* during aggregation remains persistent even when the gradient changes in the back of the wave. As the chemoattractant wave passes by, the cells can remember the gradient direction in the front of the wave and thus stay polarized. The persistent movement toward the aggregation center during the aggregation stage is therefore indicative of memory in *Dictyostelium*.

Research has given insight into the back-of-the-wave paradox. By varying the wave period, the response of *Dictyostelium* to the temporal gradient was measured [1]. It was shown that for natural wave periods (~6min), cells avoid reversing direction in the back of the wave and even continue to move toward the source for ~2min after the spatial gradient reverses (Fig. 1). Retained polarity leads to persistent forward movement in the back of the wave and suggests the presence of cellular memory. However, for longer wave periods, cells start to reverse direction in the back of the wave and thus are still capable of directional sensing in decreasing concentrations. These findings show that *Dictyostelium* responds to both spatial gradients by polarizing the cell in the front of the wave and temporal gradients by cellular memory, thus staying polarized during the back of the wave. The retained polarity in the back of the wave lasts for ~2min suggesting that memory of direction lasts for ~2min.

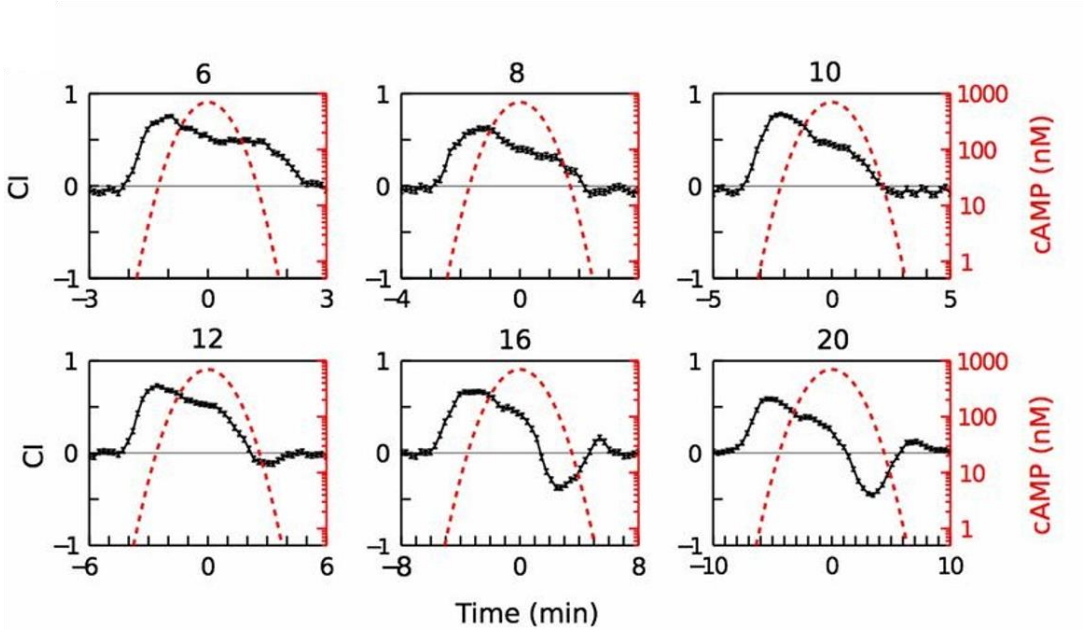

Figure 1| *Wave behavior of Dictyostelium.*
The movement of Dictyostelium in a cAMP wave was measured for different wave periods. The average chemotactic index (CI; black), is shown as a function of time for different wave periods (T; red). The front and back of the wave are depicted by the increase and decrease in cAMP concentration, respectively (Change from front to back of the wave at T= 0). The movement towards and away from the wave source is indicated as positive and negative CI, respectively. Wave periods in the range of T= 6-10min show maintained movement towards the source in the back of the wave for ~2min, indicating cellular memory. Wave periods in the range of T= 12-20min show a reversal in movement in the back of the wave. Figure taken from Skoge, Yue et al. 2014 [1].

Due to cellular memory, *Dictyostelium* can retain a persistent movement toward chemoattractant sources even when the spatial gradient changes. The purpose of memory is therefore efficient migration toward chemical cues. This includes migration toward aggregation centers in the aggregation stage or chasing bacteria in the vegetative state. Both processes require *Dictyostelium* to remember the original direction of the source for efficient movement.

4 Establishing memory in the cell

To explain how memory in *Dictyostelium* cells is established, it is important to understand the underlying mechanisms of chemotaxis. Within the cell, a cascade of signaling pathways is used for a combination of signal amplification, spatial sensing, and chemotaxis. This cascade consists of surface receptors, G-protein coupled receptors (GPRCs), small G-proteins, and signaling enzymes that lead to local excitation of the cytoskeleton. Proteins localized in the front of the cell regulate the front activity, while proteins throughout the cytosol regulate the rear activity of the cell. Therefore the signaling pathways are divided into front- and rear pathways.

The proteins sGC, TORC2, and PI3K are located at the front of the cell and regulate the front pathways during chemotaxis [18-20]. The front pathways are associated with the assembly of branched actin filament (bF-actin) and signal the formation of pseudopods. Another important front pathway is regulated by Rap1, which controls the disassembly of myosin II at the front of the cell [21, 25]. The rear pathways are regulated by the protein PLA2 and the second messenger molecule cGMP, which are distributed throughout the cytosol [18, 22]. These rear pathways are used for the assembly of myosin II with parallel actin filaments (pF-actin) and signal for the contraction of the uropod. The combination of front- and rear pathways cause polarity within the cell and therefore allows for directional movement during chemotaxis. A more in-depth schematic of the signaling cascade with the front- and rear pathways is displayed in figure 3.

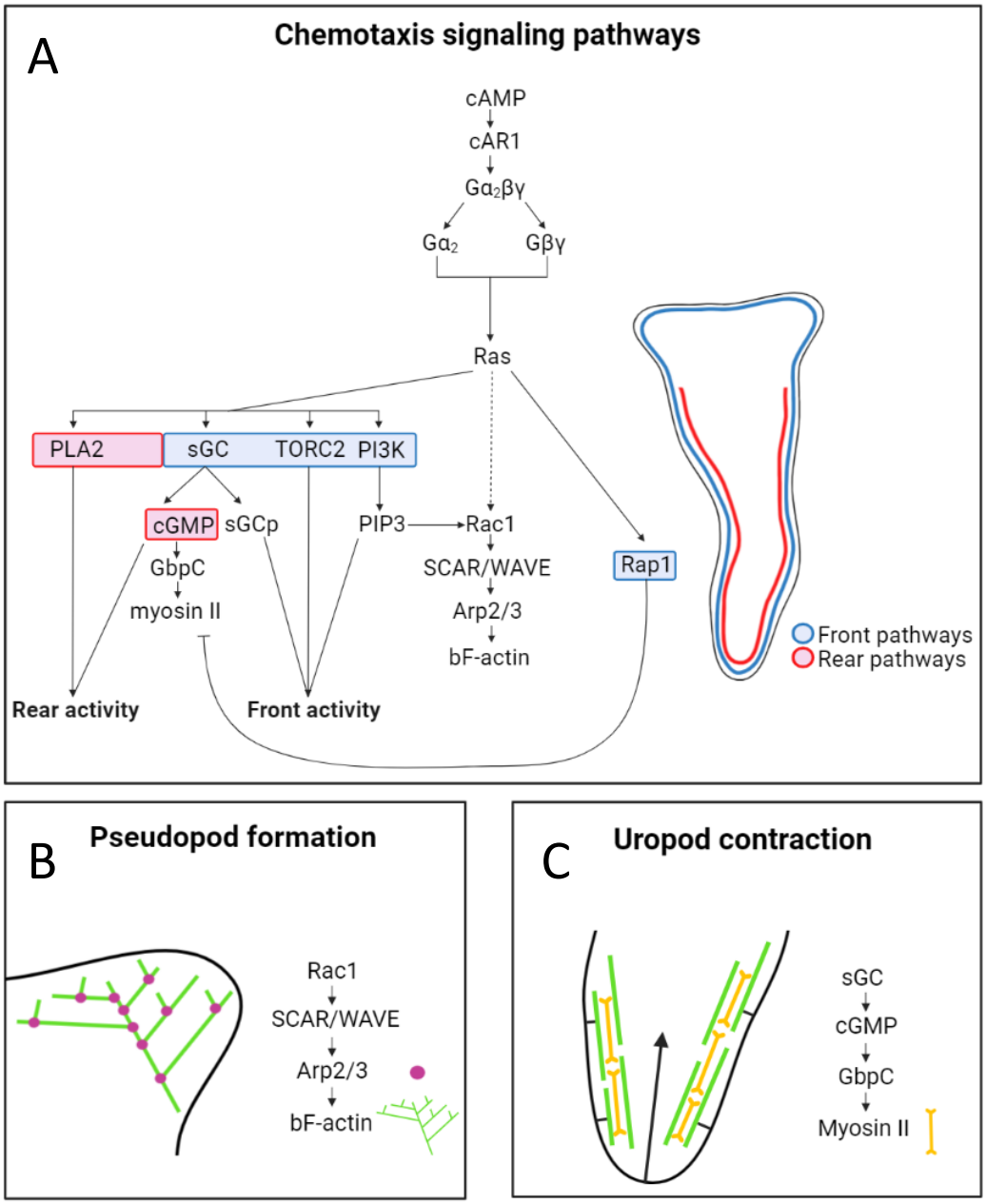

Figure 3| Chemotaxis signaling pathways in *Dictyostelium*

*The chemotaxis signaling pathways in Dictyostelium regulate the formation of pseudopods in the front of the cell and uropod contraction in the back of the cell. (**A**) Schematic representation of the chemotaxis signaling cascade. The cascade starts with binding chemotactic agent cAMP to the surface receptors cAR1. Next, the G-protein coupled receptor (GPRC) Gα₂βγ dissociates into the Gα₂ and Gβγ components upon activation by cAR1. This in turn causes the small G-protein Ras to be activated. Ras is involved in the activation of a multitude of pathways. The front pathways (blue) are mainly regulated by sGC, TORC2, and PI3K. These proteins regulate the pseudopod activity in the front of the cell by the formation of branched actin filaments (bF-actin). The rear pathways (red) are mainly regulated by the protein PLA2 and the second messenger molecule cGMP. Together they regulate the formation of myosin II filaments, which inhibits de novo pseudopod formation and causes uropod contraction. The protein Rap1 is activated by Ras and inhibits the formation of myosin II in the front of the cell. The combination of the rear pathways and Rap1 causes the formation of a polarity axis. (**B**) Schematic representation of a pseudopod. In Dictyostelium, pseudopod formation is activated by Rac1 and the SCAR/WAVE complex, which induces Arp2/3-mediated bF-actin nucleation and branching. (**C**) Schematic representation of a uropod. In Dictyostelium, a cGMP-based signaling pathway activates the interaction between myosin filaments and parallel actin filaments (pF-actin), leading to the contraction of the uropod. Figure created with BioRender.com, based on van Haastert, 2020 [26].*

Cells respond to the spatial gradient of passing chemoattractant concentrations through directional sensing. Upon sensing a chemoattractant, the signaling cascade starts, and cells begins to polarize and move toward the source. The response regulators for directional sensing in *Dictyostelium* can be explained by the Local Excitation, Global inhibition model. According to the LEGI model, cells respond to chemoattractant stimulus by a fast, local excitation and a slower, global inhibition. The response is controlled by receptor occupancy and therefore local excitation is higher toward the chemoattractant gradient, whereas inhibition is nearly uniform and intermediate in strength [27]. Therefore a surplus of excitation over inhibition yields a persistent response in the front, while inhibition exceeds excitation in the rear. This separation between response in the front and rear of the cell caused by LEGI, helps the cell to polarize.

An important part of directional sensing explained by the LEGI model is the enzymes regulating the number of membrane lipids phosphatidylinositol 4,5-bisphosphate (PIP2) and phosphatidylinositol 3,4,5-trisphosphate (PIP3). The conversion between these two lipids is facilitated by phosphoinositide 3-kinase (PI3K) and tensin homology protein (PTEN). Upon sensing a chemoattractant gradient, PI3K translocates from the cytosol to the plasma membrane at the front of the cell where it converts PIP2 into PIP3, which stimulates the formation of pseudopods [20]. PTEN on the other hand localizes at the sides and rear of the plasma membrane where it converts PIP3 into PIP2, making pseudopod formation at the sides and rear of the cell less likely [28]. The transient localization and complementary regulation of PI3K and PTEN are accounted for by the LEGI model. The model also explains the observed amplification of PIP3 and thus pseudopod formation in the front of the cell in a chemotactic gradient [29].

Soluble guanylyl cyclase (sGC) and its second messenger cyclic GMP (cGMP) are other components important for chemotaxis that function according to the LEGI model. The second messenger cGMP is a small soluble molecule with a high diffusion coefficient well above $100\mu m^2s^{-1}$ that mediates myosin II filament formation during chemotaxis in *Dictyostelium* [30-32]. It has been shown that for second messenger molecules with diffusion coefficients of $100\mu m^2s^{-1}$, the dispersion length is 10µm, which is similar to the average length of *Dictyostelium* [33]. Therefore upon formation of cGMP, the concentration throughout the cell becomes nearly instantly homogeneous. Because of this instant diffusion, cGMP is unsuitable for storing spatial information but excellent for transducing temporal information. The predominant source of cGMP is sGC, which localizes to the front of the cell upon stimulation with a cAMP gradient and associates with the membrane where it starts producing cGMP [34]. Because sGC is membrane-associated in its active state, it exhibits slow diffusion and is thus able to store spatial information and is also thought to stimulate Local pseudopod formation [35].

In conclusion, sGC and its produced second messenger cGMP have opposite properties and functions. sGC is involved with storing spatial information and promotes pseudopod formation at the front of the cell, in the proximity of the old pseudopod. cGMP on the other hand is involved with transducing temporal information and inhibits *de novo* pseudopod formation at the sides and rear of the cell by the formation of myosin II filaments [36]. Therefore this transduction pathway can also be described by the LEGI model.

The LEGI model is a relatively simple way to explain how memory is established within the cell. A schematic representation of how the LEGI model affects the cellular response in a chemotactic gradient is shown in figure 4. The signal transduction pathways described above help the cell form the memory of position and polarity. In particular, the PI3K/PTEN pathways seem to be involved with short-term memory, and the sGC/cGMP pathway with long-term memory. Together these types of memory help cells to retain persistent movement toward the chemoattractant source.

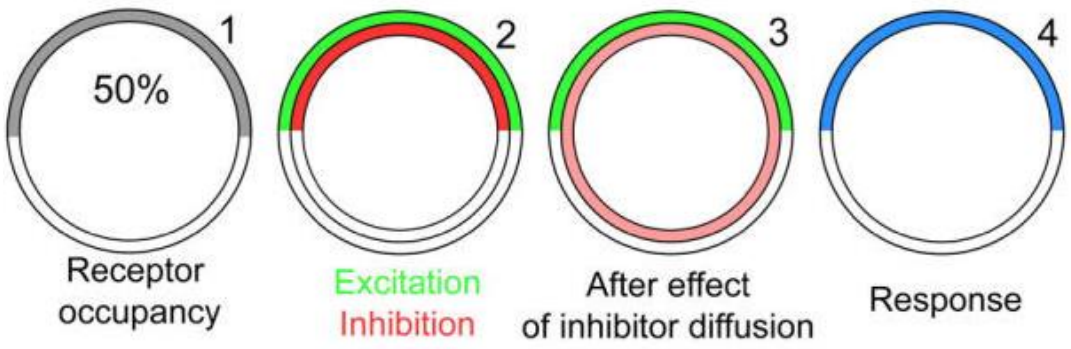

Figure 4| Schematic representation of the LEGI model
Local receptor occupation (gray band in column 1) activates the excitation and inhibition processes (green and red bands in column 2). Although the local excitation remains where it is produced, the inhibitor quickly diffuses evenly throughout the cell (column 3). Throughout the cell, the response is dictated by the balance between the excitation and inhibition processes (column 4). Wherever the receptor is occupied, a cellular response is observed, because the local excitation concentration is higher than the inhibition concentration. Therefore pseudopod formation is localized to the front of the cell. The figure is taken from Ma, Janetopoulos et al. 2004 [29]

5 Types of memory

Dictyostelium cells in the vegetative state and aggregation state mostly form pseudopods in the front of the cell. This localized formation of pseudopods helps cells to more efficiently chase bacteria or to move toward cAMP-releasing aggregation centers. Sometimes pseudopods are formed at the sides or back of the cell, known as *de novo* pseudopods. During the tracking of *Dictyostelium* cell movement and pseudopod extension, a distinction between splitting- and *de novo* pseudopods can be made. Splitting pseudopods are formed at the side of existing pseudopods, while *de novo* pseudopods are formed in an area of the cell without recent pseudopod activity [14]. Starved *Dictyostelium* cells are polarized and show high splitting pseudopod activity in the front 30% area of the cell and low *de novo* pseudopod activity in the rear 70% area of the cell (Fig. 2A).

For *Dictyostelium* to achieve strong persistent movement, two components have been recognized. As discussed previously, cells need to be polarized by having a defined front and rear. This polarization needs to be semi-stable and retained on a minute time scale. Next to polarization, *Dictyostelium* cells are observed to extend their pseudopods in a zig-zag trajectory [16]. This trajectory is created by the extension of subsequent splitting pseudopods alternating left and right, causing the cell to move forward in a straight path. In the human world, this movement is comparable to the sliding moves made during ice skating. The zig-zag movement has previously been explained by the formation of local memory in *Dictyostelium* [17]. The combination of cell polarization and the zig-zag trajectory causes the strong persistent movement observed in *Dictyostelium*.

A recent study on the molecular mechanism of memory for the persistence of pseudopod formation showed the existence of two types of memory [2]. The two types of memory were defined as the memory of position and the memory of polarity (Fig. 2B). The ***memory of position*** is related to the alternating left-right pseudopod extension and remembers the position of the previous pseudopod. The ***memory of polarity*** is the retained polarity axis within the cell by inhibition of *de novo* pseudopod formation in the sides and rear. Both types of memory are related to the two components needed for the strong persistent movement of *Dictyostelium* cells.

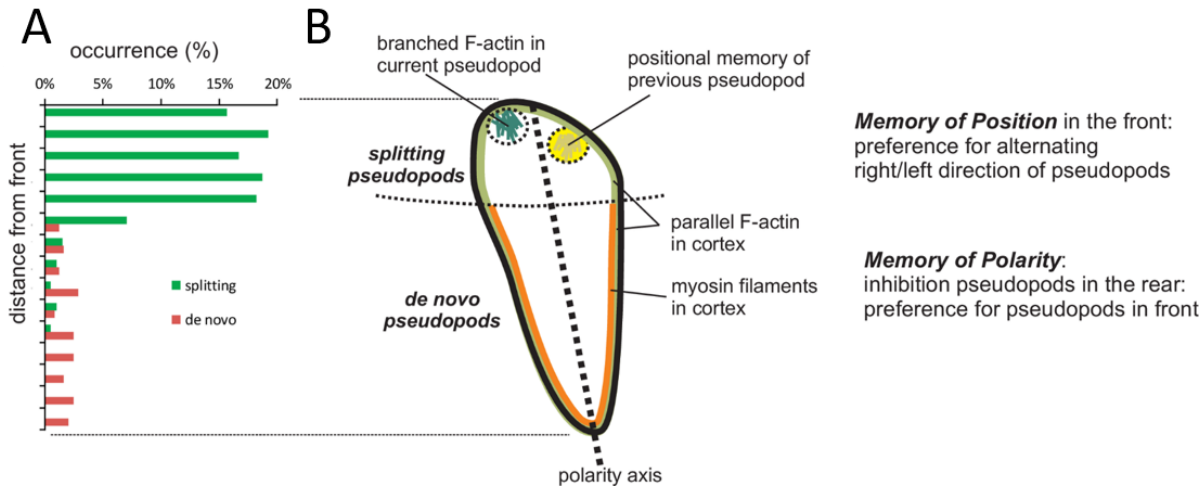

Figure 2| *Dictyostelium* has two types of memory.
*Depiction of splitting and de novo pseudopod occurrence in Dictyostelium related to cellular memory. (**A**) Cells frequently extend splitting pseudopods at the front ~30% of the cell and rarely extend de novo pseudopods from the side or the rear ~70% of the cell. This reveals that the cell has an axis of polarity leading to the persistence of direction. (**B**) Schematic of a cell showing that the source of persistence is a combination of memory of the polarity enhancing pseudopods to be made somewhere in the front, and memory of the position of the previous pseudopod enhancing the next pseudopod to be made at that position in the front. Figure taken from van Haastert 2021 [2].*

6 Memory of position (short-term memory)

6.1 Input signal

Dictyostelium cells were shown to actively remember positional information of the previous splitting pseudopod extension [2]. When tracing four consecutive pseudopods (P0 to P3) of a large number of moving *Dictyostelium* cells, it was shown that the average position of pseudopods P1 and P3 nearly coincide (Fig. 5). Therefore it seems that the start of pseudopod P3 depends on the position of P1. This was further confirmed by measuring the number of extending pseudopods in time. On average, cells extend one pseudopod every 15 seconds. The start of pseudopod extension in the same direction was determined to have a phase of 28.7± 1.4 seconds. Therefore the start of every even pseudopod (P2, P4, P6, etc.) and the start of every odd (P1, P3, P5, etc.) are correlated. From this data, it was concluded that splitting pseudopods extend alternately in a left/right motion and remember the extension of the previous pseudopod.

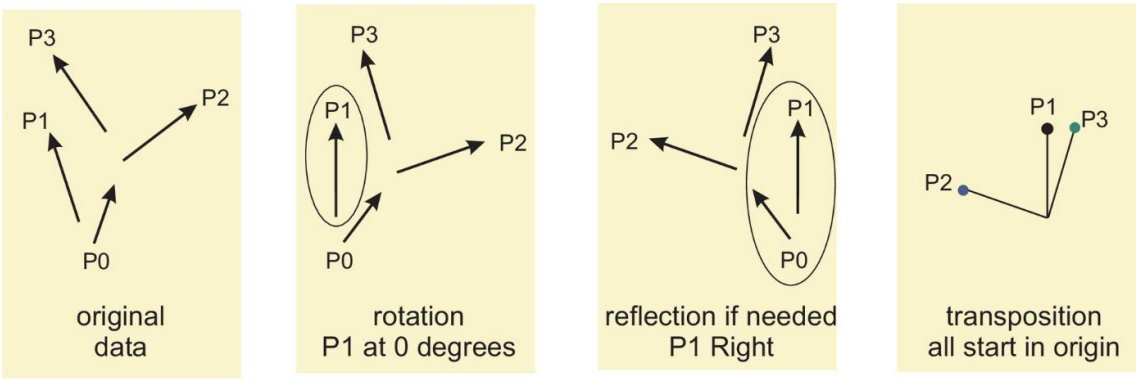

Figure 5| *Dictyostelium* cells remember the position of splitting pseudopods
Schematic for the analysis of pseudopod vectors with polar coordinates. Analysis of four consecutive pseudopods of which P1 to P3 are three splitting pseudopods. Pseudopod P0 can be a splitting or a de novo pseudopod. All four pseudopods are rotated such that P1 directs at 0 degrees, and if needed reflected around the Y-axis such that P1 directs to the right relative to P0; finally P1 to P3 are transposed so that they start in the origin. The average position of P1 and P3 nearly coincide, suggesting that the start of pseudopod P3 depends on the position of P1. Figure taken from van Haastert 2021 [2].

6.2 Kinetics

The kinetics of learning and forgetting the position of splitting pseudopods was determined by measuring the average angles between pseudopods P1 and P3 as a function of time [2]. During the extension of pseudopod P1, the positional memory is formed. The half-time of learning was determined to be 3.6 ± 0.3 seconds, starting almost immediately (0.3 ± 1.0 seconds) after the start of pseudopod P1 formation. Interestingly it was found that pseudopod P3 is less likely to extend from the same position as pseudopod P1 when P1 is extended for a shorter period. This indicates that it takes time to learn the position of the current extending pseudopod.

Forgetting the positional memory happens between the termination of pseudopod P1 and the start of pseudopod P3. The half-time of forgetting was determined to be 14.6 ± 1.1 seconds, starting at 28.1 ± 0.6 seconds after termination of pseudopod P1. The average growth time of pseudopods is 12 ± 6 seconds [14]. This means that the extension of pseudopod P3 starts while most of the positional memory of pseudopod P1 is still present. The formation of pseudopod P5 starts ~45 seconds after the extension of pseudopod P1 has stopped. Thus the position of pseudopod P5 is mostly directed by the positional memory of pseudopod P3 and only slightly by pseudopod P1. Therefore positional memory is short-term memory and only remembers the position of one pseudopod.

6.3 Signaling pathways involved

The signaling cascade proposed for the formation of pseudopods consists of activated Ras, PI3K, Rac1, SCAR/WAVE, and Arp2/3 which finally induces the formation of bF-actin filled protrusions [26, 37, 38]. A scheme of this cascade is included in figure 3. Not only is Ras known to induce the formation of bF-actin but also bF-actin was shown to induce local Ras activation, forming a positive feedback loop [39, 40]. Therefore bF-actin precedes Ras activation in left-right alternating splitting pseudopods. Using the F-actin inhibitor LatA, it was shown that spontaneous Ras patches were still able to form but cells lost the ability to alternate the Ras patches left and right [39]. This suggests that memory of position is formed downstream of Ras and is closely associated with mechanisms forming bF-actin filled protrusions. PI3K is activated downstream of Ras and together with PTEN regulates the local promotion of pseudopod formation at the front of the cell and global inhibition of *de novo* pseudopods according to the LEGI model [28]. Following is the activation of Rac1 by PI3K, which in turn activates the SCAR/WAVE complex, leading to the induction of pseudopods [38, 41].

Finally, the nucleation of bF-actin is facilitated by the SCAR/WAVE complex, which recruits and activates the Arp2/3 complex [42]. The Arp2/3 complex caps the ends of slow-growing actin filaments at a 70° angle and promotes the formation of new actin filaments, causing the actin networks to branch [43]. Specifically, the SCAR/WAVE complex was found to be important for retaining the positional memory of pseudopod formation. *Dictyostelium* cells expressing the phosphomimetic SCAR-S55D mutant were shown to have no alternating left/right bias [44]. Therefore the SCAR/WAVE complex is thought to remain present at the position of the previous pseudopod for some time, stimulating F-actin and Ras activation for the next-next pseudopod. In the SCAR-S55D mutant cell lines, pseudopods are extended in the entire front area of the cell without the restriction of alternating left/right movement, making these cells move slower than wild-type cells. Therefore the SCAR/WAVE complex seems to affect the memory of position and is thus linked to short-term memory.

7 Memory of polarity (long-term memory)

7.1 Input signal

Besides remembering the positional information of splitting pseudopods, *Dictyostelium* cells also remember the current front-rear polarity axis. The polarity axis is maintained through the suppression of *de novo* pseudopods at the sides and rear of the cell [14, 45]. However, a new polarity axis can be formed by extension of a *de novo* pseudopod. It has been shown that extension of a *de novo* pseudopod is more likely after pseudopod formation in the front of the cell is less frequent and when extension happens unusually slow [2]. Lower pseudopod activity in the front weakens the polarity axis, which in turn increases the probability of *de novo* pseudopod formation (Fig 6B). Therefore pseudopod activity in the front seems to be linked to memorizing and maintaining the polarity axis.

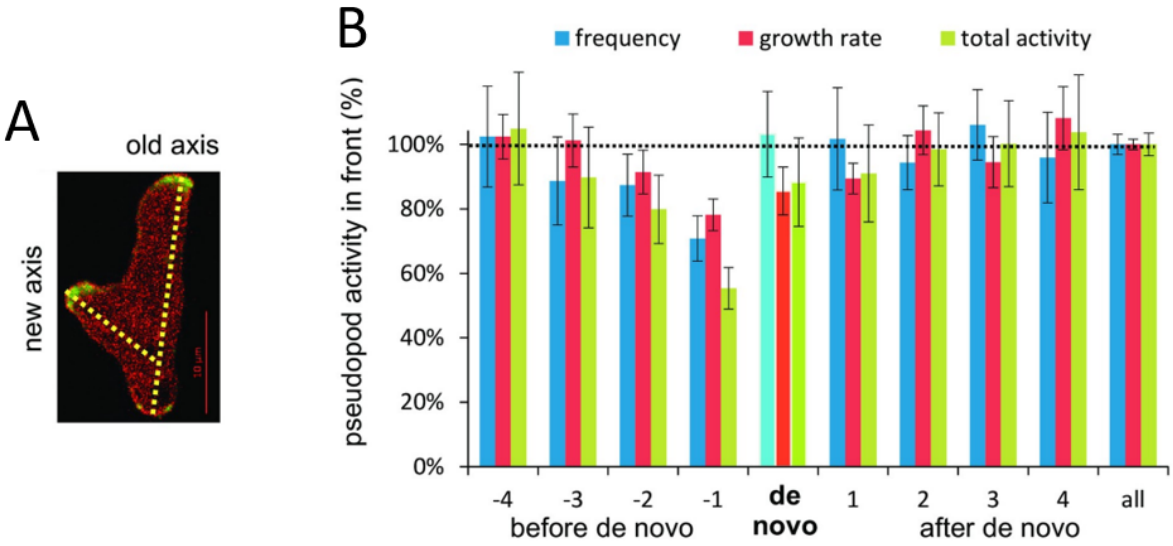

Figure 6| *Dictyostelium* cells memorize the polarity axis by pseudopod activity.
*The effects of de novo pseudopod formation on the polarity axis. (**A**) Wild-type cells expressing LimE-GFP (detecting F-actin; green) and myosin II-RFP (detecting myosin II; red). A de novo pseudopod can generate a new polarity axis. (**B**) Pseudopod activity in the front before and after the extension of a de novo pseudopod. The measured data were the pseudopod frequency (1/s) displayed in blue, the pseudopod growth rate (µm/s) in red, and their product (total activity; µm/s^2) in green. As the pseudopod activity in the front decreases, the polarity axis is weakened, and a de novo pseudopod forms, leading to a new polarity axis. Figure taken from van Haastert 2021 [2].*

7.2 Kinetics

After a *de novo* pseudopod is formed, a new front of the cell is formed. At this point, two fronts coexist within the cell, which leads to an unstable situation. Therefore either the old front or the new front is retracted at some point in time. This leads to either forming a new polarity axis or maintaining the old polarity axis. The kinetics of learning a new polarity axis and forgetting the old polarity axis was determined by observing pseudopod activity in combination with the retraction time of the old or newly formed front [2]. The half-time of learning a new polarity axis was determined to be 25.7 ± 4.6 seconds. The half-time of forgetting the old polarity axis was determined to be 88 ± 43 seconds. As previously mentioned, cells extend on average one pseudopod every 15 seconds. Therefore the half-maximal learning of a polarity axis requires an extension of 1 to 2 pseudopods. The half-maximal forgetting of the old axis requires the absence of 4 to 10 pseudopods. This shows that the memory of polarity is 4 to 8 times slower than the memory of position. The memory of polarity is thus considered to be a long-term memory.

7.3 Signaling pathways involved

The strength of the polarity axis is suggested to be dependent on the total pseudopod activity in the front of the cell [2]. The cGMP pathway functions according to the LEGI model and is linked to the formation of the polarity axis. The protein sGC is activated downstream of Ras and locally associates with the membrane where it produces cGMP and stimulates local pseudopod formation [35, 46]. The second messenger cGMP quickly diffuses throughout the cell and induces myosin II filament formation at the sides and rear of the cell, thus globally inhibiting the formation of *de novo* pseudopods [30, 31, 36]. Using the F-actin inhibitor LatA, it was shown that cGMP levels were reduced by 60% indicating that detection of the total pseudopod activity for memory occurs through the activity of membrane-associated sGC [2]. Since cGMP diffuses nearly instantaneously throughout the whole cell, myosin II filament is increased in both the front and rear of the cell [47]. Therefore it has been proposed that another signal has to be involved for the determination of the polarity axis. Active Rap1-GTP is broadly localized in the front of the cell and inhibits myosin II filament formation, causing Myosin II to only be formed at the sides and rear of the cell [21, 25]. The reduction of myosin II in the front allows for enhanced bF-actin assembly and thus increased pseudopod activity, thereby giving the polarity axis a direction. This forms a distinction between the front of the cell with enhanced pseudopod activity and the back of the cell which inhibits *de novo* pseudopod extension, forming the contractile cortex. As long as new pseudopods in the front are formed and cGMP levels are elevated, the polarity axis can be retained. When pseudopod activity in the front stalls, the polarity axis weakens and the chance of forming *de novo* pseudopods increases, allowing a new polarity axis to form. However, it was shown that Rap1 localization barely changes when a new pseudopod is formed with a large deviation from the polarity axis [2]. Therefore the polarity axis only changes slightly and causes the cells to make a small turn, allowing cells to retain their polarity axis. The cGMP and Rap1 pathways together thus allow cells to have a strong, directed polarity axis that can be memorized for a relatively long time, making the memory of polarity a long-term memory.

8 Discussion

Unicellular organisms like amoeba and bacteria are shown to have memory [48]. As discussed in this essay, one of these amoebas is *Dictyostelium discoideum*. By tracking the movement of *Dictyostelium* cells and recreating passing waves of cAMP, research has shown that these cells memorize the origin of a signal within a gradient [1]. A lot of information about the molecular mechanisms regulating chemotaxis in *Dictyostelium* has already been uncovered. Multiple pathways have been found, forming an intertwined signaling cascade that causes pseudopod extension and uropod contraction. Within this cascade, spatial and temporal information about chemical cues is memorized. This memory causes cells to move persistently toward chemical cues during food searching or aggregation. Recently it was found that *Dictyostelium* has two types of memory, short-term and long-term memory [2].

8.1 Memory of position (long-term memory)

The first type of memory in *Dictyostelium* entails the zig-zag movement of the cell's pseudopods. By alternatingly extending their pseudopods left and right, the cells can move in a straight line [16]. Remembering the position of the previous pseudopod, helps the cell to extend new pseudopods in a zig-zag pattern. This spatial type of memory is defined as the memory of position. Within a second time scale, information about the extended pseudopods is remembered and forgotten. The half-time of learning a pseudopod position is about 3.4s. The half-time of forgetting this position starts after a 28s refractory period and takes about 13s [2]. Since this type of memory lasts for a short period, it only influences the position of the next-next pseudopod making it a short-term memory. Therefore there is no integration of memory as it only lasts one pseudopod. The molecular mechanisms underlying the pseudopod extension are a cascade of activated Ras, PI3K, Rac1, SCAR/WAVE, and Arp2/3 which finally induces the formation of bF-actin [26, 37, 38]. Since these proteins are located at the front of the cell, the memory of position is precise and localized to a small part in the front. An important protein within the signaling cascade, linked to the positional memory is the SCAR/WAVE complex. Upon mutation of this protein, cells no longer have any preference for the zigzag movement and therefore lack persistence [44].

In summary, positional memory is a short-term memory that quickly recognizes and memorizes the local activity of a pseudopod. This memory is likely a local accumulation of an enhancer that promotes the formation of new pseudopods in the same position. The SCAR/WAVE complex seems to be an important part of this enhancer accumulation. The localized enhancer starts the formation of the next-next pseudopod accurately in the position of the remembered pseudopod.

8.2 Memory of polarity (long-term memory)

The second type of memory in *Dictyostelium* is linked to the formed polarity axis upon contact with a chemoattractant gradient. The formed polarity axis divides the cell into a defined front and rear. The front of the cell allows for pseudopod formation, while the rear causes uropod contraction. Pseudopod formation is limited to the front of the cell and causes persistent movement forward to the chemoattractant source. The time a cell can move in a certain direction is dependent on the longevity of the polarity axis. Therefore remembering the current polarity axis is important for persistent movement and is thus defined as the memory of polarity. The time a polarity axis is retained is determined by the pseudopod activity in the front. A decrease in pseudopod activity in the front increases the chance of *de novo* pseudopods forming thus changing the polarity axis. The half-time of learning a new polarity axis is about 25.7s. The half-time of forgetting the old polarity axis is about 88s [2]. This means that the memory of the polarity axis integrates the information of total pseudopod activity over the length of about 6 pseudopods. Therefore the memory of the polarity axis is a long-term memory, remembering the current polarity axis, and inhibiting pseudopod formation in the sides and rear of the cell. The molecular mechanisms underlying polarity axis

formation are a combination of the cGMP and Rap1 pathways. Through the fast diffusion of cGMP, myosin II is formed in the entire cell [47]. The myosin II formation in the front is inhibited by localized, activated Rap1-GTP [21, 25]. Together these pathways work globally and facilitate a polarity axis, which localizes pseudopod formation to the front 30% of the cell.

In summary, the memory of the polarity axis is a long-term memory that integrates the information of total pseudopod activity for about 2 minutes. It is facilitated by a combination of the cGMP and Rap1 pathways, which regulate myosin II formation. The memory leads to a polarization in which pseudopod formation is inhibited in the rear of the cell. This causes a global front where pseudopods are extended.

8.3 Synergy between the memory of position and polarity

The two different types of memory each contribute in their way to the efficient persistent cell movement of *Dictyostelium*, making them an important part of the cell's survival. Without memory, cells would lose the ability to move persistently and therefore have trouble seeking food and reproducing. The memory of position allows cells to move efficiently by alternating the extension of pseudopods left and right. Mutants having defects in the zig-zag motion, extend pseudopods in the entire front of the cell without coordination causing them to move slower than wild-type cells [44]. The memory of polarity gives cells a sense of direction and allows cells to move persistently in a certain direction by restricting pseudopod formation to the front of the cell through polarization. Both types of memory are the result of signaling pathways causing local excitation and global inhibition of pseudopod formation as described by the LEGI model. Even though each type of memory contributes to the persistent movement of cells, it is the synergistic effect of both memories that causes the observed efficient persistence. The memory of position causes the alternating left/right extension of pseudopods but only leads to persistence when the pseudopods are extended close to each other, which requires polarization of the cell. The memory of polarity causes cells to polarize and form a global front which is the front 30% area of the cell. Therefore memory of polarity is not very precise and requires the position of memory to achieve efficient persistent movement. The synergistic effect of memory of position and polarity allows *Dictyostelium* to have the persistence to chase food and aggregate for reproduction.

8.4 Summary and outlook

Many organisms can learn and memorize information, giving them the ability to survive and reproduce in heterogeneous environments. As shown in this essay, amoebas like *Dictyostelium* use directional memory to achieve persistent movement. This allows *Dictyostelium* cells to chase bacteria during the vegetative state and form fruiting bodies upon depletion of food sources in the aggregation state. Using combinations of molecular signaling pathways, cells regulate local excitation and global inhibition of pseudopod extension to have directed movement as described by the LEGI model. For cells to have persistent movement, pseudopods need to be extended with precise coordination, which is achieved through the memory of position and the memory of polarity. The memory of position causes pseudopods to extend in a left-right alternating pattern and lasts for ~20sec, making it a short-term memory of accurate positional information. The memory of polarity restricts pseudopod formation to the front of the cell and lasts for ~2min, making it a long-term memory of global pseudopod activity. Both types of memory work synergistically to achieve the high persistent movement of *Dictyostelium*, giving cells the ability to seek food and reproduce using chemotaxis.

Even though a lot of information about chemotaxis in *Dictyostelium* is already known, questions remain. The directional sensing of a gradient can be explained by the LEGI model. However, this simplified model does not account for long-term memory during the back of the wave [1]. Therefore improved models are still being researched and developed to better explain the mechanics behind gradient sensing [49, 50].

The contribution of each signaling pathway to the regulation of pseudopod formation is also not fully understood. Compared to sGC and PI3K, less is known about the exact involvement and workings of PLA2 and the TORC2 complex. For example, the TORC2 complex consists of multiple components and is known to be involved in chemotaxis, cAMP signal relay, and the development of the cell [19, 51]. Therefore each component's interactions and its effects on the signaling cascade are studied extensively through mutant knockouts.

The mechanisms underlying short-term memory are also unclear. Due to short-term memory, cells rarely form pseudopods at the position of the previous pseudopod. Instead, a pseudopod is formed left or right if this position, forming a zig-zag motion. However, the mechanism of knowing where a pseudopod recently stopped is still unclear. A likely explanation is that the mechanism or inhibitor that leads to stopping pseudopod formation is still present [14]. Further research using *Dictyostelium* mutants may uncover additional molecular mechanisms that might be able to explain these unanswered questions.

8.5 Memory in other organisms

It has been shown that learning and memory are not only of great importance for animals but also for amoebas. Learning and memorizing spatial information, helps amoebas efficiently navigate their surroundings while chasing food or finding other cells for reproduction. Besides *Dictyostelium*, other unicellular organisms like neutrophils, mesenchymal stem cells, and the fungus *Bd. chytrid* also shows signs of having memory during chemotaxis. The pseudopod extensions of the four different species have been extensively studied [26]. Using this data, it was shown that all 4 cell lines have a similar memory of polarity and memory of position [2]. Therefore memory in unicellular organisms is not only limited to *Dictyostelium* and is likely to be present in many more species. Other studies have shown that neutrophils in vivo have long-term memory by retaining their polarity [52, 53]. They are therefore less susceptible to polarity reversal and retain their direction of movement toward the gradient [52]. The networks regulating chemotaxis share many similarities between *Dictyostelium* and neutrophils [54]. Therefore understanding the underlying mechanisms of chemotaxis in *Dictyostelium* can give great insight into the behavior of human neutrophils. Understanding how neutrophils move and track down harmful microorganisms may be of great benefit to future healthcare.

9 References

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