A mechano-chemical compartment-based method to simulate the ameoboid movement

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Abstract

Keywords: *Dictiostelium discoiudium*, voxel-based simulation

Introduction   1

Motility is a survival evolutionary starategy in eukaryotes [1]. The ability requires the   2
coordination of complex mechanisms on the microscopic level, including transducing   3
information from the external environment to the interior of the cell and translating it   4
to a migratory response via a molecular signaling pathway based on enviromental cues.   5
One can classifies motile cells to swimmers and crawlers. Crawling cells , unlike   6
swimming ones, do not posses a segragated organel for their motility. Instead, they   7
employ an internal mechanism to push their membrane and in consequence the whole   8
body forward. Amoeboid locomotion is the most frequent mode of movement in   9
eukaryotic cells. *Dictyostelium discoideum* is a well-established model organism for   10
studying amoeboid like motility. The cell extend sucsessive pseudopodia, a temporary   11
actin-based protrusion of its surface in order to propel on the surface following the   12
signaling messenger cyclic Adenosin Mono Phosphate (cAMP) gradient.   13

    Chemotactic movement of *Dictyostelium discoideum* can be divided into three   14
different steps: (1) receptor binding (2) polarizing and directional sensing (3) and   15
migration response [2]. Experiments show that it takes less than 10 seconds for an   16
individual to respond an external chemical field [3]. When the starved cell exposed to a   17
gradient of the chemoattractant, extracellular cAMP stochasticly bindes to the cAR1   18
receptors distributed homogeneously around the cell membrane. This, in turn, triggers   19
internal pathway which leads to localization of different agents at the leading edge and   20
rear side of the cell. After the cell sensed its direction, the pathway must be linked to   21
the actin-machinery to generate forces in the cytoskeleton. Then, the cell starts to   22
migrate towards that direction.   23

    There are impressive studies trying to make theoretical grounds for different   24
aspects of chemotaxis in *Dictyostelium discoideum*. Local Excitation/Global Inhibition   25
(LEGI) models extended to explain gradient sensing in migrating cells. The ability of   26

living organism to produce adapted and amplified responses, traced back to a fast and local excitatory reply that accompanies with a slow and inhibitory signal [4–6]. In more recent versions of LEGI based models, parallel mechanism are introduced to regulate the accumulation of $PI3K$ and $PTEN$ proteins in front and back of the cell [7]. In polarization models, usually a diffusing inhibitor is present and positive feedback loops are used to amplify the response of the network [8,9]. One main feature of polarization models is a distinct differentiation between anterior and posterior regions results from positive feedback loops. It is beleived that a complete picture of chemotaxis achieves when coupled directional sensing and polarization mechanisms are taken into account. It is shown that chemoattractant activation of divergent pathways that inhibit each other by interaction with actin polymers, is sufficient for both gradient sensing and polarization [10]. Coupled exitable Ras and actin activation can mediate pseudopodia formations and directed movement [11]. A common feature in chemotaxis models is a chemical pathway activated by G-proteins coupled to receptors. The identity of these pathways and the way in which they interact, is critical for undrestanding of directed movement of cells in inhomogeneous environments.

Polarized regions contains distinct molecules with different functions and sensitivity to gradient of chemoattractants. The true mechanisms of polarity are still under debate, however, positive feedback loops containing actin polymers may be responsible for generation of local polarized regions inside the cell [12–14]. Observations shows that in polarized cells $PI3K$ molecules are localized in front and $PTEN$ molecules at the back of the cell [15,16]. Joining polarization and directional sensing results to co-ordinated migration up to the attractant gradient.

Cell migration is an ATP-consuming process associated with actin polymerization. The complex process, couples protrusion of the cell's leading edge with contraction of the cytoskeleton during a dynamically graded adhesion with substrate. Adhesion with the substrate is a requisite property without which crawlers are unmoveable. Dictyostelium cells, unlike other mammalian cells, enjoy of some innate Non-Specific cell substratum adhesion originally mediated by van der Waals interactions [17].

The polarized cells exhibit correlated deformations in their contour with a complex moleclar mechanism interacting with substrate via adhision forces. The sequential interaction leads to a persistant movement, even in the homogeneous medium. Ofcourse, eventually, on long runs the statistic of their locomotion in such field showes a diffusive behavior.This spomtanaous directional sensing and persistant random walk in the absence of environmental chemical cues is due to internal and external noises. When exposed to chemically inhomogeneous medium, the cells display a tendency to align with the local gradient of stimulants.

Previous works stands for modeling the trajectories of amoeboid cells by stochastic differential equations with parameters estimated from experimental data. Although the equations well-describe the cellular central mass dynamics, due to the large size of the cells ($nearly\ 10\mu m$) it comprises variant simplifications. The directional sensing models also presents more a generic (theoretical) scheme that could satisiy the above mentioned properties of directional sensing .

The living entities are not purpose-built artifacts, engeneered to consistantly perform an exact action in response to an incoming signal; rather a cell is more akin to a soup of ingreiedents coliding and aggreagting within cells. Local events in this animate soap should result in global actions, thus the key to decipher the behaviour of a living systems, is to understand how to collective effects of these local events can give rise to something that can be mistaken for the responce of an inanimate artifact with global roles.

One of the major shortfalls of a globalist view of a cell, is the fact that a cell,

especially of eukaryotic variety, is deeply compartmentalized, where the concentrations ₇₉
of different chemical species are drastically different from any two points in the cytocol. ₈₀
Here, to capture the compartmentalized nature of a living cell, we divide up our ₈₁
artificial cell into compartemnts of a fixed size, operting independently of each ₈₂
other.The size of these copmatemnts are such that a well-stirred environemnt for the ₈₃
chimical reactions occuring within them is availible. ₈₄

Materials and methods

Modeling the movement of a single-celled organism is seemingly an insurmountable task; ₈₇
given the complexity if such behavior, a faithful recreation would be computational ₈₈
cul-da-sac. Instead we have chosen to study this behavior at two different levels: ₈₉

1. *The chemical level*: at this level we simulate the chemical reaction in pre-defined ₉₀
   compartments, "voxels", using the Gillespie's method. The formation of ₉₁
   pseudopodia is net result of these reactions. ₉₂

2. *The mechanical level*: the collective outcome of the the chemical reactions is ₉₃
   translated into Newtonian equations in order to transform the chemical level into ₉₄
   the movement of a physical entity –i.e., the cell– on a surface. ₉₅

The interaction between the chemical and the physical levels results in a fascinating ₉₆
charactristic that neither levels nor their building blocks can muster: the ability to ₉₇
respond to chemotactic cues present in its envioerement. This emergenet feature ₉₈
separates the realm of goal-less, unguided, and mindless objects from the realm of living ₉₉
entities that change their behaviors vis-a'-vis their *Umwelt*. ₁₀₀

The chemical level

Directed migration of primitive or elaborated living systems, can be followed from the ₁₀₂
analysis of physio-chemical properties of chemotaxis process. From a hierarchical point ₁₀₃
of view, stimulation of cells with chemoattractants or intrinsic noise, triggers ₁₀₄
downstream chemical pathways induce polarization in motile cell. These complicated ₁₀₅
networks allows cells to detect external gradients. Difference in concentration of related ₁₀₆
agents, translates to physical forces at the outer level which are responsible for pushing ₁₀₇
the membrane forward and generating pseudopods. Any proposed model used to ₁₀₈
address chemotaxis problem throughly, should consider three steps: motility, directional ₁₀₉
sensing and polarity. Motility refers to propelling the cell in random directions probably ₁₁₀
due to external noise. Pseudopodia growth in arbitrary directions because of noisy ₁₁₁
behaviour of the environment. It seems the same chemical pathway works in the ₁₁₂
presence or absence of external stimuli. However, since noise exerts unidirectional forces, ₁₁₃
the movement of the cell is not biased in motile behaviour of cell. In directional sensing ₁₁₄
cells can differentiate between inhomogeneous distributions of chemoattractants. ₁₁₅
Directional sensing depends on the ability of cells to detect the change in the occupancy ₁₁₆
of receptors over time. It is known as temporal sensing provide cells to bias their ₁₁₇
movement by sensing the occupancy of receptors across the cell length. Sensing of ₁₁₈
gradients follows with the tendency of signals to reach a specific value during the ₁₁₉
saturation of receptors. It is said that responses *adapts* to the external stimulus. Then, ₁₂₀
directional sensing consist in reading the extracellular gradient and transmitting ₁₂₁
information about spatio-temporal properties of attractants inside the cell. Where the ₁₂₂
content of this information translates to change in cell morphology and induce motility ₁₂₃
in a directed fashion. Polarization is crucial for moving to the gradient after receiving ₁₂₄
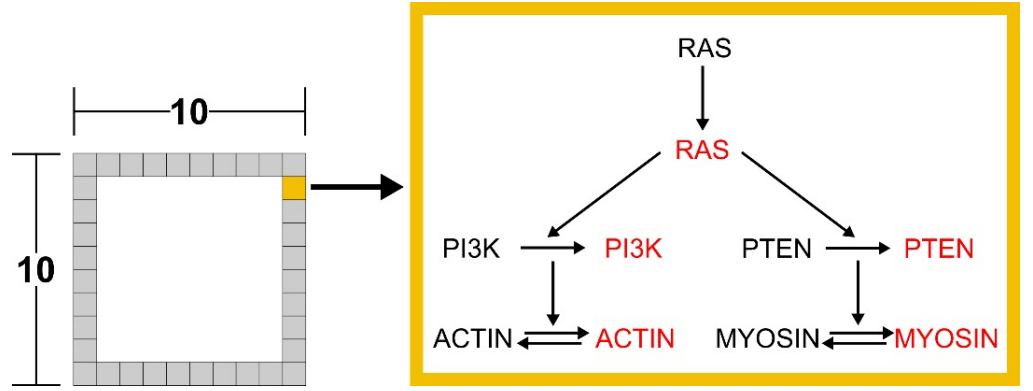
Fig 1. Chemical pathway for chemotaxis of *Dictyostelium discoideum.* Propensity functions for stochastic solution of kinetic equations are given for related reactions.

extracellular signals. In polarized cells spatial distribution of certain molecules are restricted to leading or lagging edge of the cell.

In our proposed pathway, stimulation of receptors with binding of cAMP, or in the absence of external stimuli, by fluctuations in the environment, activates transformation of Ras proteins into excited counterpart $Ras^\star$. $Ras^\star$ mediates transformation of deactivated $PI3K$ and $PTEN$ to their active forms $PI3K^\star$ and $PTEN^\star$. Activation passes through intermediates species $Ras^\star.PI3K$ and $Ras^\star.PTEN$ decomposed to active forms and deposit $PI3K$ and $PTEN$ enzymes intact. $PI3K^\star$ and $PTEN^\star$ has the same role of $Ras^\star$ for the polymerization reaction of actin monomers and contraction of at rest myosins, respectively. Polymerized actins ($Actin^\star$) and contracted myosins ($Myosin^\star$) can decompose to actin monomers and expanded myosins in backward reactions. $PI3K^\star.Actin$ and $PTEN^\star.Myosin$ faciliates the conversion of inactive actin-myosin molecules to their active forms.

We have used Gillespie algorithm for stochastic solution of chemical reactions. The main characteristics of the chemotaxis process, adaptation to signal, amplification in concentration of actin's activator and polarization of the cell due to difference in concentration of actin and myosin, are included in definitions of propensity functions of the reactions (see Fig.(1)). Following propensity functions, concentration of $PI3K^\star$ amplifies *linearly* with increase in concentration of cAMP and polymerized actin. Then, polymerization reaction can be considered as a positive feedback for activation of $PI3K$. The selected form of the hill-function $\dfrac{c(r)}{c(r)+k}$, shows that at least when the binding reaction of chemoattractants on the receptors is far from equilibrium, concentration of $PI3K^\star$ increases with $c(r)$. Since, the inverse of the hill-function appears in the propensity function of the activation reaction of $PTEN$, the spatial concentration of cAMP affects activation of $PI3K$ and $PTEN$ in opposite directions. It can be responsible for polarization of actins and myosin in front and back of the cell. We should emphasize that the spatial distributions are included with coordinate dependent concentration $c(r)$ without introducing diffusion of molecules inside the cell. Funtionality of hill-functions also demands that at steady-state, when $c(r) \gg k$, different levels of external stimuli, results in a same final adapted concentration of $PI3K$ which is proportional to constant k. Consequently, we expect that adaptation, amplification and polarization of chemotaxis process may be extracted from the suggested chemical pathway.

At physical level of explanation, we will show that Ras mediated actin polymerization and myosin contraction causes forces in leading and lagging edges of the cell which are responsible for rapid movements.....

Modeling

In order to capture the intricacies of a single-celled organism, we divide the cell contour 162
into 36 voxels. A voxel is defined as cube with a given volume in which a set of chemical 163
reactions relevant to actin polymerization. We utilize Gillespie's next reaction method 164
to simulate the occurrence of chemical reactions in each voxel (Table 2). 165

Table 1. Reactions in each voxel.

Reaction	*Propensity*	Parameters
1	$\#\text{RAS} \times k_1$	$k_1 = 1.$
2	k_2	$k_2 = 1.$
3	k_{-2}	$k_{-2} = 1.$
4	$k_3 \#\text{PI3K} \times \#\text{Actin}^* \times \frac{c(r)}{c(r)+i}$	$k_3 = 1, \#\text{PI3K} = 200, \#\text{Actin}^* = 200, i =?.$
5	k_4	$k_4 = 1.$
6	k_{-4}	$k_{-4} = 1.$
7	$k_5 \#\text{PTEN} \times \#\text{Myosin}^* \times \frac{c(r)+i}{c(r)}$	$k_5 = 1, \#\text{PTEN} = 200, \#\text{Myosin}^* = 200, i =?.$
8	k_6	$k_6 = 1.$
9	$k_7 \#\text{PI3K}^* \times \#\text{Actin}$	$k_7 = 1, \#\text{PI3K}^* = 200, \#\text{Actin} = 200.$
10	$k_{-7} \#\text{Myosin}^* \times \#\text{Actin}^*$	$k_{-7} = 1, \#\text{Myosin}^* = 200, \#\text{Actin}^* = 200.$
11	k_8	$k_8 = 1.$
12	$k_9 \#\text{PTEN}^* \times \#\text{Myosin}$	$k_7 = 1, \#\text{PTEN}^* = 200, \#\text{Myosin} = 200.$
13	$k_{-8} \#\text{Myosin}^*$	$k_{-8} = 1, \#\text{Myosin}^* = 200.$

The main hurdle in utilizing a voxel-based approach to simulate the behavior of a 166
single cell, is the fact the reactions in the Gillespie's algorithm occur randomly... 167

The mechanical level 168

The myriad of chemical reactions seething at the chimical level should be translated into 169
a physical force to move the cell in a givern direction. But this seemingly trivial step 170
requires further consideration, since the bioligical reality of ameaboid movement is far 171
from simple. To capture some of this apparent biological complexity in our model, the 172
mere act of locomotion is further divided into two steps: 173

1. *Deformationl*: To describe cellular deformation we apply sequential geometric 174
   transformations including translation, rotation and scaling. 175
   When the direction of polarization vector ascertained by chemical level, it is time 176
   to move.However, if the length of this ector would be less than a given threshold, 177
   howbeit the cells wiggles around, this can not lead to the movement of the 178
   centroid. Morover, if the distance of polarization vector and centroid would be 179
   significant, Traction forces provoke rotation of whole cell body around the 180
   centroid. Hence, the final direction of cell displacement deviates somehow from 181
   the polarization direction dictated from chemical reactions. The amount of this 182
   deviation equals to 183

$$\Delta\theta = \int_0^t \omega \, dt \tag{1}$$

   in which ω is anular velocity. As the movement occures in low Reinolds number 184
   regime it is convenient to ignore angular acceleration due to this rotation. 185
   Accordingly, one can assume that total external torque τ equals to rotational 186
   resistance 187

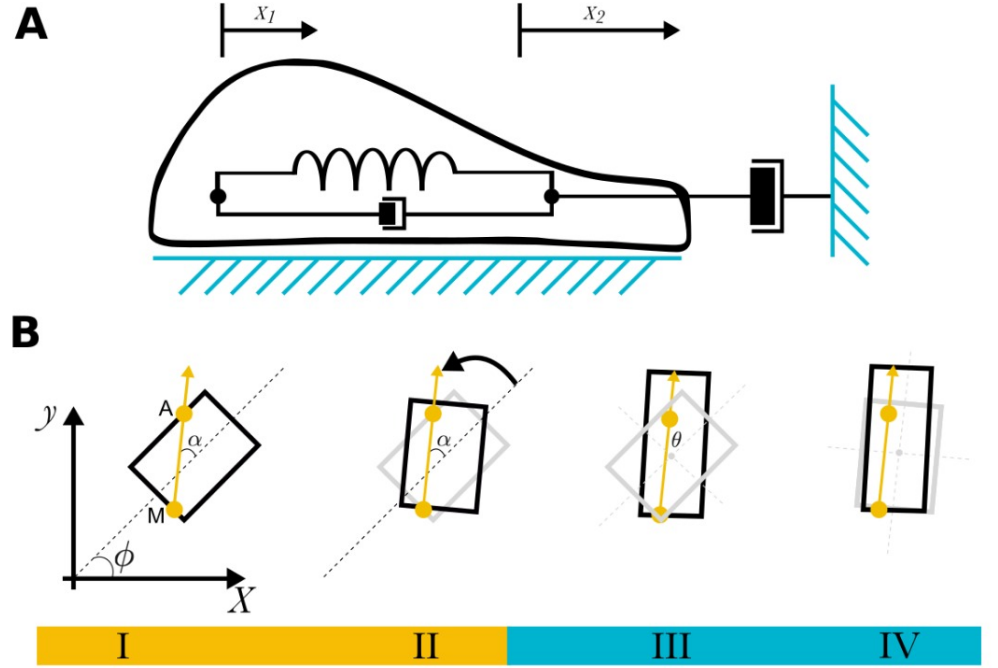$$\alpha\omega = \tau \tag{2}$$

Fig 2. The consecutive steps of cell motility.

where α is rotational friction coefficient. Assuming that $F = F_f - F_r$ whre f_f and f_r are frontal traction force and backward traction force, respectively and $r_\perp$ is the distance from centroid from plarization vector, then

$$\Delta\theta = \frac{1}{\alpha} r_\perp F t \qquad (3)$$

Here, t is a step time which is a exponential variable with 10 second mean. Now, the amorphous ameoboid cell can adjust itself along the new direction. The length of this step is determined by the interaction of adhesive forces between cell and substrate (see next subsection). In our model the cell readjustment in a polarized form along a new direction occurs by rotating the previous polarization vector around the meet point of new one (see 2-B-Panel II and supplementary material...). Afterwards, the whole cell rearranges around the new direction such that the area size of the rectangle remains constant (see supplementary material).

2. *Displacement*: The entire chain of intracellur chemical reactions lead us to two distinct loci, formally we named them the center of actins and the center of myosins. We assume that the center of actins specifies the leading edge of the cell while the center of myosins highlights the rear side of that. these luci represent polarization inside the cell and the arrow which points the rear edge to the leading one determines the orientation of the cell motility in the next step. Let us model a motile cell as a viscously damped two degrees of freedom spring-mass system moving forward interacting with substrate, as shown in Fig.(2-A). Since the ahesive forces with substrate is strong enough it is plausible that we assume that the dynamic of the system is over damped and under this condition one can ignore the masses and just follow dyanamics of the dashpots and the spring . The motion of the system can be describe by the coordinates $x_1(t)$ and $x_2(t)$ which defines the positions of Actin and Myosin centers, *i.e.* head

Table 2. Biologically meaningful ranges for the model parameters.

Parameters	Description	$Values$	Ref.
γ_{in}	Internal Friction Coefficient	$10^4\ \mu mpas$	ref
γ_{out}	External Friction Coefficient	$10\ \mu mpas$	ref
A	Cell Area	$100\ \mu m^2$	ref
η_{water}	Water Viscosity	$10^{-3}\ Pa.s$	ref
η_{cell}	Cell 's Viscosity	$10^3 - 10^5\ Pa.s$	ref
F_f	Frontal Traction Force	$150\ pN$	ref
F_r	Backward Traction Force	$120\ pN$	ref
ω	Rotational velocity	$4\ min^{-1}$	ref
α	Rotational Friction Coefficient	$25\ \mu mpNmin$	ref
k	Cell membrane elasticity	$10\ \mu mPa$	ref

and tail of the cell at any time t from the respective equilibrium positions. The traction forces exerted by the cell provides propulsion force to move forward. This motion is described by the set of coupled diffrential equations

$$-\gamma_{in}(\dot{x_2} - \dot{x_1}) - k(x_2 - x_1) = -F_r, \tag{4}$$

$$\gamma_{in}(\dot{x_2} - \dot{x_1}) + k(x_2 - x_1) + \gamma_{out}\dot{x_2} = F_f \tag{5}$$

where γ_{in} and γ_{out} are internal and external friction coefficients, respectively and k is the cell membrane elasticity.

Code availability

Results

0.1 Homogeneous Medium

0.2 Inhomogeneous Medium

Discussion

1. an step forward to have More realistic insight, merely focusing on circumference rather intricate crowded cytoplasm.

2. makes it possible to rebuild the mutation effects.

3. sensitivity of our model to minute assymetry of external concentration gradient

4. All the previous directional sensing models possess a diffusing communicator agent in cytosol medium of the cell which has not been identified yet, experimentally.

5. Our model assumes that at any instant the cells only grow one pseudopodium, which is not the case in very shallow gradients.

Supporting information

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Author contribution

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Additional information

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References

1. Brunet T,and Arendt D. 2016 From damage response to action potentials: early evolution of neural and contractile modules in stem eukaryotes. Phil. Trans. R. Soc. B 371:2050043

2. P. Devreotes and C. Janetopoulos. Eukaryotic chemotaxis: Distinctions between directional sensing and polarization. J. Biol. Chem., 278(23):20445–20448, 2003.

3. Meili R. , Ellsworth C. , Lee S. , Reddy T. B. , Ma H. , Firtel R. A. Chemoattractant-mediated transient activation and membrane localization of Akt/PKB is required for efficient chemotaxis to cAMP in Dictyostelium. (1999) EMBO J. 18:2092–2105

4. A. Levchenko, P. Iglesias, Models of Eukaryotic Gradient Sensing:Application to Chemotaxis of Amoebae and Neutrophils, 82, 50-63, 2002.

5. C. A. Parent, P. N. Devreotes, A cell's sense of direction, Science, 284, 765-770, 1999.

6. B. Kutscher, P. Devreotes, P. A. Iglesias, Local excitation, global inhibition mechanisms for gradient sensing:an interactive applet, Sci STKE, 2004.

7. H. Levine, D. A. Kessler, W. J. Rappel, Directional sensing in eukaryotic chemotaxis: a balanced inactivation model, Proc Natl Acad Sci USA, 103, 9761-9766, 2006.

8. M. Postma, P. J. Haastert, A diffusion-translocation model for gradient sensing by chemotactic cells, Biophys J, 81, 1314-1323, 2001.

9. A. Gamba, A. de Candia, S. Di. Talia, A. Coniglio, F. Bussolino, G. Serini, Diffusion-limited phase sepration in eukaryotic chemotaxis, Proc Natl Acad Sci USA, 102, 16927-16932, 2005.

10. J. Xu et al, Divergent signals and cytoskeletal assemblies regulate self-organizing polarity in neutrophils, Cell, 114, 201-214, 2003.

11. P. J. M. van Haastert, I. K. Gunnink, A. Kortholt, Coupled excitable Ras and F-actin activation mediates spontaneous pseudopod formation and directed cell movement, Mol Biol Cell, 28, 922-934, 2017.

12. R. Skupsky, W. Losert, R. J. Nossal, Distinguishing modes of eukaryotic gradient sensing, Biophys J, 89, 2806-2823, 2005.

13. X. Xu, M. Meier-Schellersheim, J. Yan, T. Jin, Locality controlled inhibitory mechanisms are involved in eukaryotic GPCR-mediated chemosensing, J. Cell Biol, 178, 141-153, 2007.

14. J. E. Ferrell, Self-perpetuating states in signal transduction: positive feedback, double-negative feedback and bistability, Curr Opin Cell Biol, 14, 140-148, 2002.

15. A. Narang, Spontaneous polarization in eukaryotic gradient sensing: a mathematical model based on mutual inhibition of frontness and backness pathway, J Theor Biol, 240, 538-553, 2006.

16. H. Meinhardt, Orientation of chemotactic cells and growth cones: models and mechanisms, J Cell Sci, 112, 2867-2874, 1999.

17. Loomis WF, Fuller D, Gutierrez E, Groisman A, Rappel W-J (2012) Innate Non-Specific Cell Substratum Adhesion. PLoS ONE 7(8): e42033. doi:10.1371/journal.pone.0042033