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A Computational Model of Cell Migration of Fish Keratocytes

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February 7, 2019

Contents

Chapter 1

Introduction

Amoeba proteus, a common ameba cell, migrates by stretching pseudopodia with changing its cell shape continuously. In contrast, fish keratocytes usually show a circular shape; however, they change their shape to a half-moon shape when they begin migration. This phenomenon suggests that the deformation of the cell shape is a key feature to realize cell migration of keratocytes.

As evidence that AP is important for cell deformation, it has been reported that a dense network of actin molecules regulates cell deformation[1]. Previous studies have reported that during cell migration actin molecules extend their head toward the cell membrane by actin polymerization (AP), which has been suggested as a source of the deformation of the cell membrane and the propulsive force of the cell. As evidence that AP is important for cell deformation, it has been reported that a dense network of actin molecules regulates cell deformation. The actin retrograde flow (ARF) that pulls the actin molecules back toward the stress fiber (SF), a bundle of actin fibers spreading from side to side of the rear part of the cell, has also been reported[2].

The purpose of this research is to clarify the mechanism that forms a half-moon shape by physical simulation experiments considering intracellular mechanism.

The structure of this paper is as follows. In Chapter 2, we introduce previous work on the molecular mechanism of cell migration of keratinocytes. In chapter 3 we will explain our method of simulation experiments. Section 4 shows the results of simulation experiments. In the last chapter, we will discuss conclusions and future prospects for the purpose.

Chapter 2

Keratocytes

2.1 Characteristics of Cell Migration of Keratocytes

A keratocytes, a migratory fish epidermal cell, is a wound healing cell about 70 μm in size. When a fish injures, keratocytes begin migration toward the injured position toward the wound by the speed of about one body length in one minute. The locomotion of keratocytes is a kind of amoeboid movements; however, it is different from typical amoeba movement at the point that they moves with keeping a half-moon shape.

The protruding events of cell migration are thought to be caused by actin polymerization (AP)[1]. AP is that actin molecules which are cytoskeletons overlap to form fibrous actin (F-actin). There is depolymerization in the opposite phenomenon of AP. The opposite of AP, the phenomenon that actin molecule separates from F-actin is called depolymerization. The actin molecule depolymerizes with AP and forms a dense network and pushes the cell membrane from the inside. There are not only actin molecules but also myosin molecules in the cell membrane. When actin molecule and myosin molecule are bound, it becomes actomyosin, and a bundle of actomyosin is called a stress fiber (SF). The actin retrograde flow (ARF) that pulls the actin molecules back toward the SF has also been reported [2]. Although details on ARF are not clarified, it is thought that SF attracts forward actin molecule towards SF [3]. The network of actin molecules moves by AP and ARF to promote cell membrane deformation. In addition, Okimura et.al reported that SF plays a role of wheels in cell migration [4]. In the cell migration when SF was removed, the migration speed decreased and the left and right balance disintegrated. This also indicates that SF is important for cell migration.

It has been reported that morphogenesis of morphology was observed even after removing the nucleus [5]. Hence, it was suggested that the cell migration of the keratocyte is due to a physical factor, not genetic information.

2.2 Molecular Mechanism of Cell Migration

A cell has a structure called a cytoskeleton. The cytoskeleton is like bones for humans, but in contrast to static human bones, the cytoskeleton is dynamic and is an important organelle that maintains the shape of the cell. The main component of the cytoskeleton is the actin molecule. As the actin molecule polymerizes and depolymerizes, the shape of the cell membrane changes. The actin molecule is elongated by polymerization in the cell membrane but retreats in the direction opposite to the elongation direction by the bundle of actomyosin called stress fiber. This phenomenon is called actin retrograde flow, and it is not clear what kind of role it plays in cell migration. The cell membrane extruded by

Figure 2.1: Keratocytes during cell migration.(Source: Takako Tanaka, Iwadate Lab).

polymerization of the actin molecule becomes the pseudopodia and adheres to the substrate at the advancing position. Thereafter, the adhesion between the substrates of the rear cell membrane is released and dragged forward. By repeating this cycle, cell migration mechanism is formed.

It is a common fact even in keratocytes that the cell membrane is deformed by actin molecule polymerization. In the simulation experiment described in the next chapter, we investigate how the cell membrane forms a half moon shape when actin molecule polymerization acts on the cell membrane.

Chapter 3

Simulation Methods

3.1 Simulation Methods of Cell Membrane

In the computer simulation of this study, the cell membrane was modeled by a network of simple particles interacting with each other and placed on a cylindrical surface as an initial condition. Each particle of the membrane was assumed to receive elastic force from neighboring particles and repulsive force from actin molecules. The equation of motion of the cell membrane molecule is assumed as follows.

$$m\frac{d^2\boldsymbol{x}_i}{dt^2} = \boldsymbol{F}_i^m + \boldsymbol{F}_i^a - \eta\frac{d\boldsymbol{x}_i}{dt} \tag{3.1}$$

where $\boldsymbol{x}_i$ is the position vector of the membrane molecule, m is the mass of the particle, $\eta = 8.9 \times 10^{-6}\,\mathrm{kg/s}$ is the viscous coefficient, $\boldsymbol{F}_i^m$ and $\boldsymbol{F}_i^a$ is the force received from the neighboring particles and the repulsive force from actin molecules. The force $\boldsymbol{F}_i^m$ was assumed as an elastic force:

$$\boldsymbol{F}_i^m = \sum_{i \in I_j} -k((\boldsymbol{x}_j - \boldsymbol{x}_i) - \boldsymbol{l}_{ij}) \tag{3.2}$$

where k is a spring constant, $\boldsymbol{l}_{ij}$ is the natural length between the i-th and j-th membrane particles, and I_j represents the set of particles that interacts with particle j. The natural length between particles were determined by the distance at the initial state. The force $\boldsymbol{F}_i^a$ received from an actin particle was assumed to be a repulsive force:

$$\boldsymbol{F}_i^a = \sum_{\{\forall i | \|\boldsymbol{x}_j - \boldsymbol{B}_i\| < D_2\}} \frac{s}{\|\boldsymbol{x}_j - \boldsymbol{B}_i\|}\frac{\boldsymbol{x}_j - \boldsymbol{B}_i}{\|\boldsymbol{x}_j - \boldsymbol{B}_i\|} \tag{3.3}$$

where s is a constant that determines the strength of the repulsive force, and D_2 is a distance range the force prepared to decrease the calculation amount.

3.2 Simulation Methods of Actin Molecules

3.2.1 Actin Polymerization

An actin molecule has polarity: one end which elongates by polymerization is called barbed-end, and the other end is called pointed-end. Since the frequency of polymerization is high

in a region where actin molecules are dense [1], the polymerization rate was assumed to be proportional to the actin concentration. The filament formed by actin polymerization is called F-actin. F-actin was expressed by a simple rod in the simulation. As an initial condition the length of each rod was zero and he direction of the actin polymerization $\boldsymbol{L}_i$ was randomly determined. The elongation of the F-actin by the polymerization is modeled by

$$\boldsymbol{B}_i \leftarrow \boldsymbol{P}_i + f^p(c)\boldsymbol{L}_i \cdot dt \tag{3.4}$$

where $\boldsymbol{B}_i$ and $\boldsymbol{P}_i$ are barbed end and pointed end of the F-actin, respectively, and $f^p(c) = 5.0 \cdot \exp\frac{c}{10.0}$ expresses the frequency of actin polymerization as a function of actin concentration c. c measures for each region obtained by dividing the plane of the simulation space into a lattice shape. The frequency of depolymerization decreases in an actin dense area and the depolymerization was expressed as follows.

$$\boldsymbol{P}_i \leftarrow \boldsymbol{P}_i + f^d(c)\boldsymbol{L}_i \cdot dt \tag{3.5}$$

where $f^d(c) = \frac{5.0}{c}$ represents the frequency of depolymerization. The actin polymerization and depolymerization was simulated by a probability

$$p_i(c) = c_i = \frac{a_i}{N}$$

at each step, where $p_i(c)$ is the probability that depends on the concentration c_i in i-th region, a_i is the number of actin in i-th region, and N is the total number of actin. In this simulation, the space where a virtual cell was simulated was divided into 3600 regions ($i = 1, ...3600$).

As actin molecules continue to polymerize in random directions, the actin molecule region continues to spread monotonically. The actin molecule in the region of low actin molecule density disappears and regenerates near the cell membrane. The new polymerization direction of the actin molecule is randomly determined.

3.2.2 Actin Retrograde Flow

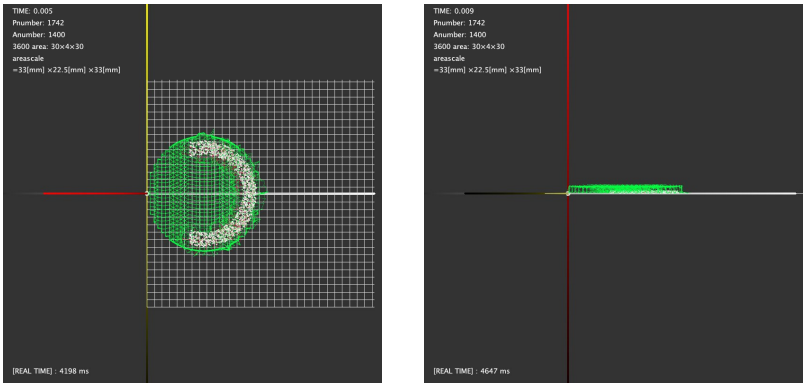
Because the function and underlying mechanism of the ARF have not been known in detail, we simulated the ARF under some assumptions. The ARF is a movement of F-actins toward the stress fiber which is a bundle of actin fibers aligning from side to side in the rear side of keratocytes.

At first, we assumed the ARF as the retraction of the actin molecules toward the leftmost point of the cell, i.e., the position of the membrane particle with the smallest x coordinate, which we call the reference point. The retraction of the actin molecule by the ARF was expressed by the following equation:

$$\begin{cases} \boldsymbol{B}_i \leftarrow \boldsymbol{B}_i - \alpha \dfrac{\boldsymbol{B}_i - \boldsymbol{E}_0}{\|\boldsymbol{B}_i - \boldsymbol{E}_0\|^w} & (3.6) \\ \boldsymbol{P}_i \leftarrow \boldsymbol{P}_i - \beta \dfrac{\boldsymbol{P}_i - \boldsymbol{E}_0}{\|\boldsymbol{P}_i - \boldsymbol{E}_0\|^w} & (3.7) \end{cases}$$

where $\boldsymbol{E}_0$ is the reference point, and α and β are constants that determines the strength of the ARF. w is a parameter indicating the effect of distance between actin molecule and cell membrane molecule on retraction strength of the ARF. When $w = 2$, the closer the intermolecular distance is, the stronger is pulled. When $w = 1$, all actin molecules are equally pulled irrespective of the intermolecular distance.

When $\alpha < \beta$, the AP direction is adjusted by pulling back the pointed-end of F-actin stronger than barbed-end. When $\alpha = \beta$, the orientation effect is ineffective and it can be pulled back without changing the direction of AP.



(a) (b)

Figure 3.1: Initial state. (a) Top view. Green lines and white dots show actin molecules and membrane particles, respectively. (b) Side view.

Second, we assumed that another reference points $\boldsymbol{E}_1$ and $\boldsymbol{E}_2$ are proposed. Hence, two pattern experiments are performed with one and two ARF reference points. As shown in the figure, the reference points $\boldsymbol{E}_1$ and $\boldsymbol{E}_2$ are positioned at 1/5 of the cell membrane. The reference points $\boldsymbol{E}_1$ and $\boldsymbol{E}_2$ are located 1/5 rearward of the cell membrane. ARF was expressed by contraction of actin molecule to the two points. In this case the retraction by the ARF was expressed as follows:

$$\begin{cases} \boldsymbol{B}_i \leftarrow \boldsymbol{B}_i - \gamma \left(\dfrac{\boldsymbol{B}_i - \boldsymbol{E}_1}{\|\boldsymbol{B}_i - \boldsymbol{E}_1\|^w} + \dfrac{\boldsymbol{B}_i - \boldsymbol{E}_2}{\|\boldsymbol{B}_i - \boldsymbol{E}_2\|^w} \right) & (3.8) \\ \boldsymbol{P}_i \leftarrow \boldsymbol{P}_i - \delta \left(\dfrac{\boldsymbol{P}_i - \boldsymbol{E}_1}{\|\boldsymbol{P}_i - \boldsymbol{E}_1\|^w} + \dfrac{\boldsymbol{P}_i - \boldsymbol{E}_2}{\|\boldsymbol{P}_i - \boldsymbol{E}_2\|^w} \right) & (3.9) \end{cases}$$

where γ and δ are constants that determines the strength of the ARF. As in eq. 3.7, the orientation effect of ARF can be controlled by the magnitude relation of γ and δ.

3.3 Initial Condition

The number of actin molecules and membrane particles were 3000 and 2000, respectively, in the computer simulation. The positions of membrane particles were updated every step of the numerical computation and the positions of actin molecules were updated every 10 time steps.

Fig. 3.1 shows the initial state of the virtual cell of the computer simulation. The initial arrangement of the actin molecule is a U-shaped area excluding the rear of the cell (Fig. 3.1). The membrane molecules are placed on the surface of the cylinder and not inside.

Chapter 4

Results and Discussion

4.1 Simulation Results

4.1.1 Role of Actin Retrograde Flow

Fig. 4.1 showed the simulation result when two reference points of the ARF were prepared (eq. 3.9) and the effect of the ARF was independent of the distance (w=1). In this case, the actin molecule aggregated in a half-moon shape.

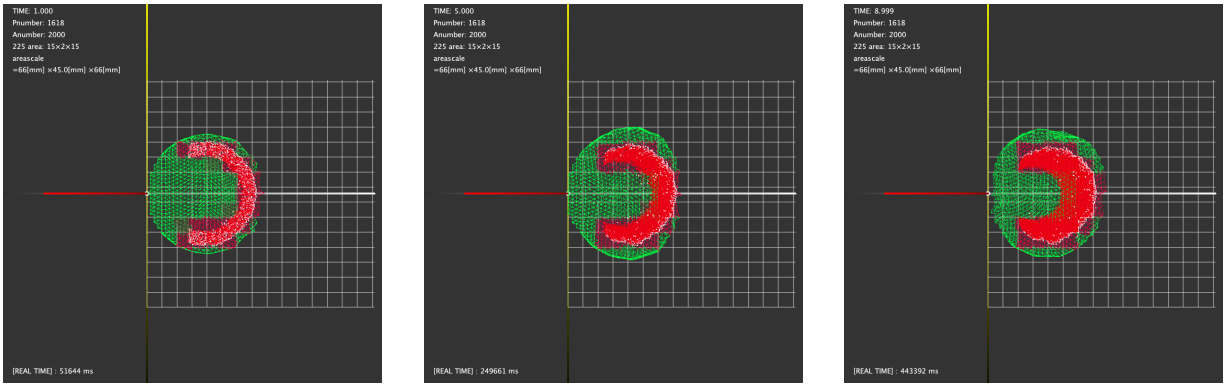
Fig. 4.2 showed the simulation results under the same condition as Fig. 4.1 except that distance-dependent ARF was used (w=2). Since actin molecules that polymerize in the transverse direction close to SF are strongly pulled back, actin molecules aggregate vertically. As a result of the continuation of the AP, the actin molecule region continued to spread beyond the cell membrane. Since the AP in the lateral direction is indispensable for forming a half-moon shape, the condition of this ARF is inappropriate.

Fig. 4.3 showed simulation results assuming the case where there is one ARF reference point (eq. 3.7). The actin molecule migrated towards the center of the cell rather than when pulled from the two reference points and circular aggregation occurred. By this, the effect of AP in the lateral direction on the cell membrane was alleviated. As in the case where a distance-dependent ARF is used, in this situation the formation of a half-moon shape is not done. That is, the actin molecules aggregate in a U-shaped area due to the influence from two points at both ends of SF rather than from one point.

Fig. 4.4 showed the simulation results when the effect of the ARF was ignored. Since there is no ARF which is the orientation mechanism of AP, actin molecule continued polymerization in random directions and did not aggregate. Compared to the other results, it can be seen that the actin molecule elongation in all directions and stresses the cell membrane.

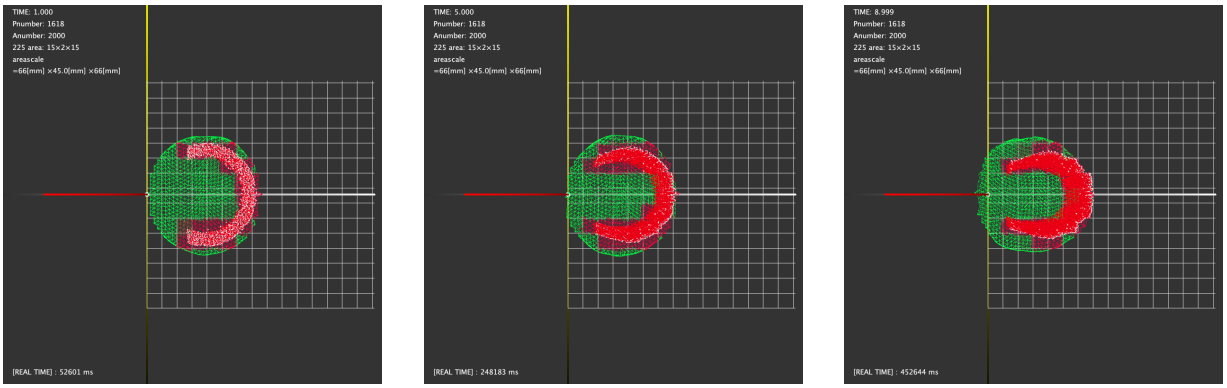
Fig. 4.5 showed the simulation result when ignoring the orientation effect of ARF. It seems an agglutination form of actin molecule similar to Fig. 4.1, but when focusing on the head of actin molecule, it is found that it is polymerizing in various directions. Without the orientation effect of AP, this aggregate of actin molecules will expand in this shape. Furthermore, this aggregate stagnates on the spot because only ARF retraction works. It is understood that a mechanism for aligning the directions is necessary for cell migration.

As indicated above, changing each condition associated with ARF affects the aggregated shape of the actin molecule.



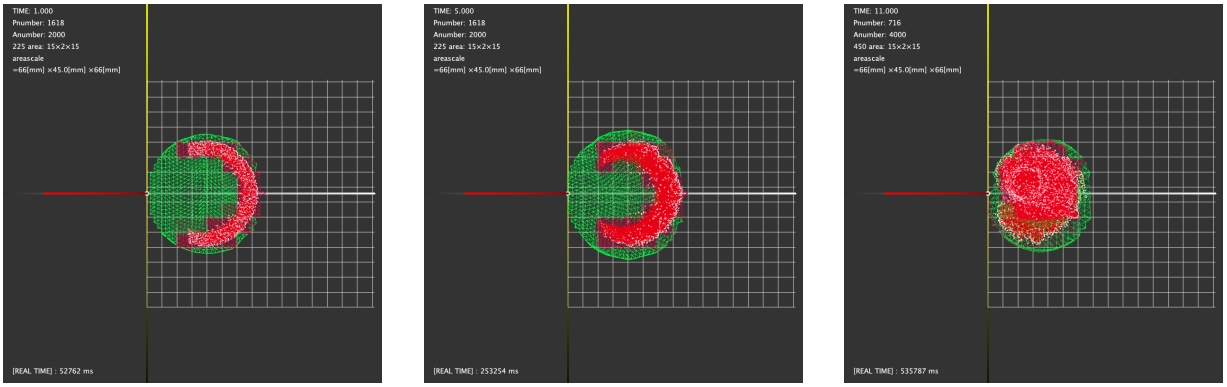
(a) (b) (c)

Figure 4.1: Simulation results when the amplitude of the retraction by the ARF is independent of the distance. time $t = 1.0, 6.0, 11.0[s]$. Green lines show the cell membrane, white dots show the head of F-actin and red dots show F-actin.



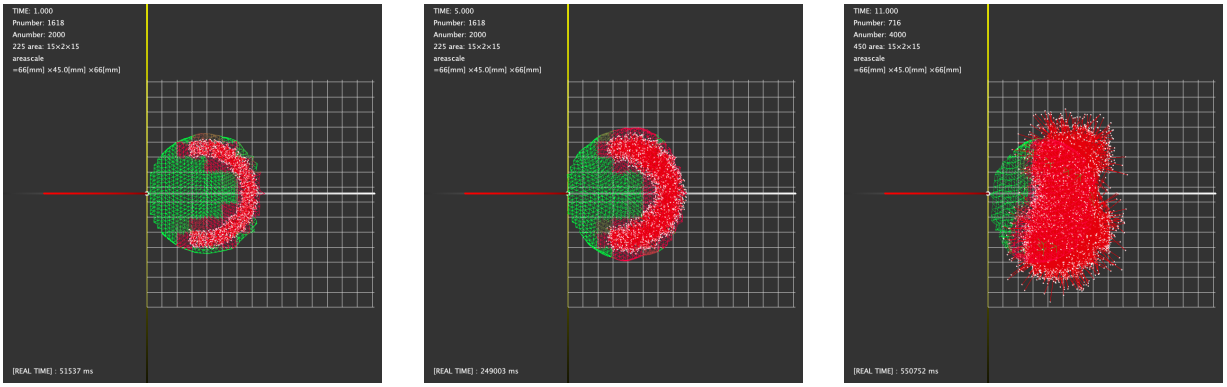
(a) (b) (c)

Figure 4.2: A simulation result of ARF when $w = 2$ in Equation 3.9.



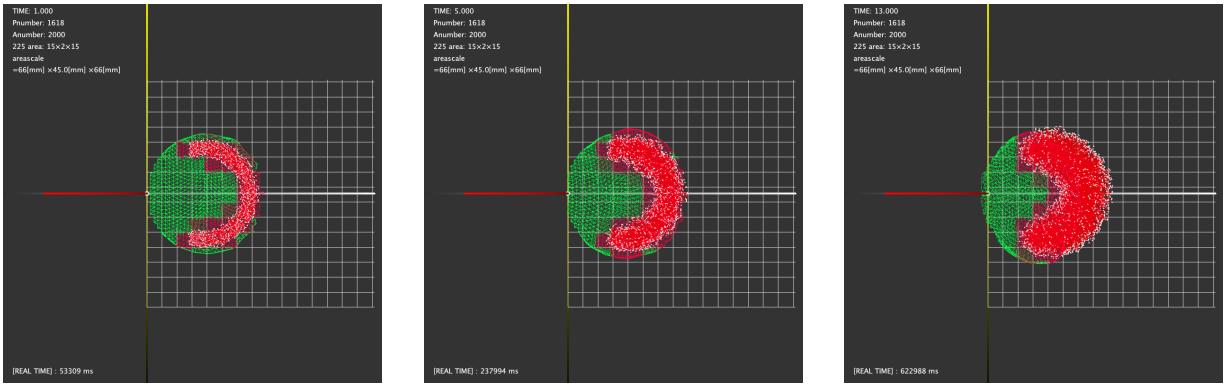
(a) (b) (c)

Figure 4.3: Simulation results when the reference point of the ARF is assumed as the leftmost membrane molecule of the cell.



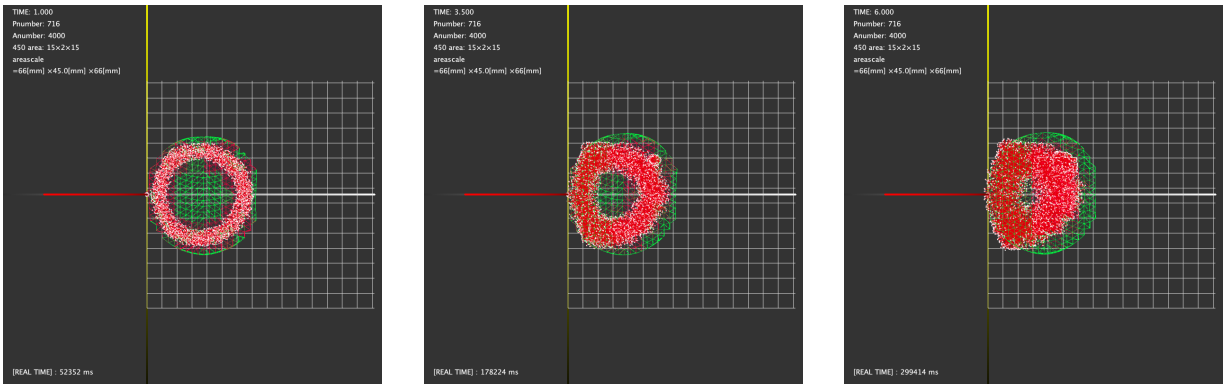
(a) (b) (c)

Figure 4.4: Simulation Results without ARF.



(a) (b) (c)

Figure 4.5: Simulation results when the orientation effect of ARF is invalidated.



(a) (b) (c)

Figure 4.6: Simulation results when actin molecules are distributed in a circular region as an initial condition.

4.1.2 Effect of Initial Distribution of Actin Molecules

In the simulation experiments in the previous section, it was assumed that the actin molecules were randomly distributed in a U-shaped region as an initial condition. Fig. 4.6 shows the simulation results of actin molecules were distributed in a donut shape. The other simulation conditions were the same as those in Fig. 4.1. The actin molecule did not aggregate in a half-moon shape but aggregated around the SF. When ARF acts in the state where actin molecules are arranged in a donut shape, agglutination around the SF before the actin molecule forms a half moon shape ahead of the cell. In the state where the actin molecule is arranged in the U-shaped region, it can be explained that some mechanical stimulation is given to the donut-shaped state and the arrangement of actin molecules is biased. If the arrangement of actin molecules is biased into a U-shaped region, the propulsive direction of cell migration is determined. Hence, cell migration is impossible when actin molecules are arranged in a donut shape.

4.2 Discussion

If the ARF condition is $w = 1$ (eq. 3.9), there is an orientation effect in the SF direction, and when the reference point is two points, the actin molecule aggregated in a half-moon shape (Fig. 4.1). Under the other conditions, the actin molecule did not aggregate in a half-moon shape.

The membrane particles receive force from actin molecules and neighboring membrane particles simultaneously. Cell membrane is deformed by the repulsive force by AP exceeding the binding force between cell membranes. As the AP becomes more active, the cell membrane deforms significantly. However, simply lengthening the actin molecule only increases the size of the cell. There are two problems of "deforming" and " keeping" of a half-moon form for cell migration of keratocyte by polymerization of actin molecule. The presence of inhibiting the expansion of the aggregating region of actin molecule is the ARF. The ARF deforms the actin molecule in aggregated form from a U-shape shape to a half-moon shape by pulling back the actin molecule in the direction of SF. Even if we look at the simulation results in the absence of AP (Fig. 4.4), the importance of the ARF is obvious. However, it is impossible to form a half-moon shape simply by attracting the actin molecule backwards. Since actin molecules are pulled back while stretching in the same direction, they stop on the spot. Traction of the actin molecule contributes to maintaining the aggregated form of the actin molecule.

Changing the direction of AP by orientation effect gives the change of aggregation form of actin molecules. As the orientation of the AP changes while being pulled back, the arrangement of actin molecules changes. The fact that the orientation of the AP aligns in the direction of SF means that all actin molecules polymerize radially to the cell membrane. The condition that the actin molecule is pulled back independently of the distance also contributes to the formation of a half-moon shape.

The above results indicate that ARF is orienting and retracting of actin molecular polymerization plays an important role in deforming and keeping the keratocyte in a half-moon shape.

Chapter 5

Conclusion

5.1 Conclusion

Actin molecules played an important role in migrating cells. The actin molecule is attracted in the direction opposite to the polymerization direction by ARF caused by SF while pushing the cell membrane while approaching the cell membrane while repeating polymerization and depolymerization. AP and ARF are intracellular mechanisms acting in opposite directions, respectively. However, if either mechanism is lacking, a half-moon shaped form is not formed. Cell migration is a complicated combination of these two elements.

The ARF changes the direction of polymerization when drawing actin molecules towards SF. It is presumed that the form formed differs depending on how much the angle changes at this time. It was suggested that the effect of aligning the orientation of the AP to the direction of the ARF is important for the formation of the half-moon shape and that the contraction effect of the actin molecule prevents excessive expansion of the cell membrane.

In this study, it is assumed that both ends of SF are pulling actin molecules, but it is considered that the form formed is different under other conditions. Therefore, the form formed when precondition is combined with AP condition and the ARF condition is a half-moon shape.

5.2 Future Works

Regarding ARF, many conditions remain to be tried. For example, when the force pulled back by time or the reference point changes, or the direction to be oriented differs from the position to be pulled back. In this cell model, it is possible to know the importance of ARF by trying various ARFs. There is room to investigate also when changing the strength and rigidity of the cell membrane. It is interesting to see experiments that carefully model the SF as well as AP and ARF, or what results are obtained when a completely new intracellular mechanism is introduced.

In the cell membrane molecular simulation, it could not reflect well on the movement of actin molecule. Even if the actin molecule polymerized at the front pushes the cell membrane, the displacement of the membrane molecule is slow to propagate to the back. It is unknown whether the cause is a bug in the program or the condition that the model can not be found. It is also important how actin molecules aggregate, but the most important thing is how actin molecules transform cell membranes.

Acknowledgements

I would like to thank Prof. Nishii whose enormous support and insightful comments were invaluable during the course of my study. I also owe a very important debt to Prof. Urakami, Prof. Iwadate and the members of the laboratory whose comments made an enormous contribution to my work.

Bibliography

[1] Tatyana M. Svitkina, Alexander B. Verkhovsky, Kyle M. McQuade, and Gary G. Borisy. Analysis of the actin–myosin II system in fish epidermal keratocytes: mechanism of cell body translocation. *The Journal of Cell Biology*, Vol. 139, No. 2, pp. 397–415, 1997.

[2] Vinay Swaminathan, Joseph Mathew Kalappurakkal, Shalin B Mehta, Pontus Nordenfelt, Travis I Moore, Nobuyasu Koga, David A Baker, Rudolf Oldenbourg, Tomomi Tani, and Satyajit Mayor. Actin retrograde flow actively aligns and orients ligand-engaged integrins in focal adhesions. *Proceedings of the National Academy of Sciences*, Vol. 114, No. 40, pp. 10648–10653, 2017.

[3] Takako Nakata, Chika Okimura, Takafumi Mizuno, and Yoshiaki Iwadate. The role of stress fibers in the shape determination mechanism of fish keratocytes. *Biophysical Journal*, Vol. 110, No. 2, pp. 481–492, 2016.

[4] Chika Okimura, Atsushi Taniguchi, Shigenori Nonaka, and Yoshiaki Iwadate. Rotation of stress fibers as a single wheel in migrating fish keratocytes. *Scientific Reports*, Vol. 8, No. 1, p. 10615, 2018.

[5] Y. Asano, T. Mizuno, T. Kon, A. Nagasaki, K. Sutoh, and T. Q. Uyeda. Keratocyte-like locomotion in amiB-null dictyostelium cells. *Cell Motility and the Cytoskeleton*, Vol. 59, No. 1, pp. 17–27, 2004.