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A Computational Model of Cell Migration of Fish Keratocytes

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Chapter 1

Introduction

1.1 Introduction

Amoeba proteus, a common ameba cell, migrates by stretching pseudopodia with changing its cell shape continuously. In contrast, keratocytes usually take a circular shape; however it deforms in a half-moon shape and migrates with keeping the shape. In order to understand the cell migration mechanism of keratocytes, it is important to clarify why a half-moon shape is formed. It is not clear why keratocytes form a half-moon shape and migrate without changing its shape.

Migrating cells including keratocytes perform cell migration by the action of cytoskeleton called actin molecule. Actin polymerization (AP) is a phenomenon in which actin molecules overlap each other to form F-actin. F-actin is shortened in length by depolymerization or lengthened by AP. F-actin that repeats elasticity pushes the cell membrane and deforms the cell. If the AP is too active, the cell membrane will swell up.

It is reported that cell migration is promoted by a dense network formed by actin molecule as evidence that AP is important for cell deformation [1]. It can be seen that it is difficult to keep the shape of cells during cell migration even by paying attention to one element called AP.

The purpose of this research is to clarify what kind of mechanism a half-moon shape is formed 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 experiment. 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 [4]. 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.

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 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,

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

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 Molecules

In the cell membrane model, each cell membrane molecule moves under the resultant of force and velocity from the actin molecule and resistance force proportional to force between the cell membrane molecules. The equation of motion of the cell membrane molecule is 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 m is the mass, $\eta = 8.9 \times 10^{-6}\,\mathrm{kg/s}$ is the viscous coefficient, and $\boldsymbol{x}_i$ is the position vector of the membrane molecule. The membrane molecule has the force $\boldsymbol{F}_i^m$ received from the membrane molecule and the force $\boldsymbol{F}_i^a$ received from the actin molecule. It is assumed that the force $\boldsymbol{F}_i^m$ acting between the membrane molecules can be represented by 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 the spring constant, and $\boldsymbol{l}_{ij}$ is the natural length between the membrane molecules $i - j$. Molecule i belongs to the set of index I adjacent to molecule j. However, this natural length measures the distance between the membrane molecules in the initial state. The force $\boldsymbol{F}_i^a$ that a membrane molecule receives from an actin molecule is assumed to be the 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 to receive a force.

3.2 Simulation Methods of Actin Molecules

3.2.1 Actin Polymerazation

The polymerization of actin has polarity, the end where polymerization is carried out is called barbed-end, and the end which is not done is called pointed-end. Since polymerization is carried out well in a region where many actin molecules are present , the polymerization rate is proportional to the actin concentration in the vicinity. The filament formed by actin polymerization is called F-actin. F-actin on the simulation is treated as a rod. F-actin is

defined as the position vector at both ends of the rod. Here, the position vector of pointed-end of particle i is $\boldsymbol{P}_i$, and the position vector of barbed-end of particle i is $\boldsymbol{B}_i$. $\boldsymbol{P}_i$ and $\boldsymbol{B}_i$ are updated step by step to control polymerization and depolymerization.

As described above, the polymerization direction $\boldsymbol{L}_i$ is determined at the initial stage of cell migration.Therefore, the formula for updating the polymerization is

$$\boldsymbol{B}_i \leftarrow \boldsymbol{P}_i + \boldsymbol{L}_i$$

. However, considering the surrounding actin concentration and time step, the formula can be rewritten as follows.

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

where $f^p(c) = 5.0 \cdot \exp \frac{c}{10.0}$ is a function that increases exponentially with actin concentration c and dt is the time step.

Regarding depolymerization, the update formula is as follows.

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

where $f^d(c) = \frac{5.0}{c}$ is a function inversely proportional to c. However, these formulas are not done every time, but are done with probability

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

where $p_i(c)$ is the probability that depends on the concentration c_i in region i, a_i is the number of actin in region i, and N is the total number of actin. In this simulation, the original space was divided into 3600 spaces($i = 3600$).

3.2.2 Actin Retrograde Flow

Because ARF is often unknown, we simulate with some assumptions. Although the element that causes ARF is a stress fiber, since the cell model of this paper does not introduce a stress fiber, it substitutes the membrane molecule at the end of the cell membrane. Since it is not clear how much the effect on actin molecule also works, it was assumed that the closer to the membrane molecule the stronger the effect.

The cell membrane molecule with the smallest x coordinate is the reference point $\boldsymbol{E}_0$. The calculation formula for implementing ARF is shown below.

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

where the vector $\boldsymbol{B}_i$ is barbed-end, the vector $\boldsymbol{P}_i$ is pointed-end, and α and β are constants that determines the strength of the ARF. This equation means ARF which does not depend on distance when w = 1 and ARF depending on distance when w = 2.

Since there is a possibility that the shape of the cell membrane may change at the position of the reference point $\boldsymbol{E}_0$, 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 A and B are positioned at 1/5 of the cell membrane. The position update formula of ARF when two reference points $\boldsymbol{E}_1$ and $\boldsymbol{E}_2$ are used is shown below.

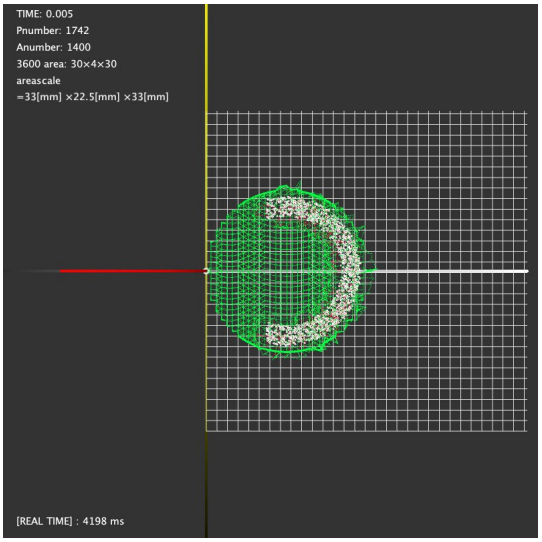


Figure 3.1: Top view of keratocyte model at time $t = 0$.

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

where γ and δ are constants that determines the strength of the ARF.

In the above equation, the denominator is squared taking into consideration the distance dependency between the particles. If we compare distance-dependent ARF and distance-independent ARF, the square of the denominator of the above equation is deleted.

3.3 Initial Condition and Relocation Condition

The total number of actin is 3000, the number of membrane molecules is 2000. When updating the position of F-actin every step, deformation of the cell membrane can not catch up. Therefore, the position of the cell membrane molecule is updated each time, and the position of actin molecule is updated once every 10 times.

The direction of polymerization is randomly determined. Some actins invade areas where they can not normally invade due to the progress of polymerization. For example, it is outside the cell membrane and in the nucleus. In such a case, relocation processing is applied to the actin. F-actin escaping from the cell membrane disappears and moves to a random place close to the cell membrane. The region to be repositioned is outside of the cell membrane and regions where the concentration of actin molecule is low.

The initial state in the simulation experiment is shown in Fig. 3.1 and Fig. 3.2. The actin molecule is indicated by a green line, and the cell membrane is indicated by a white dot. This is the initial placement of actin molecules because the nucleus is present in the center of the cell and the actin molecule is distributed a lot in the anterior direction. The membrane molecules are placed on the surface of the cylinder and not inside.

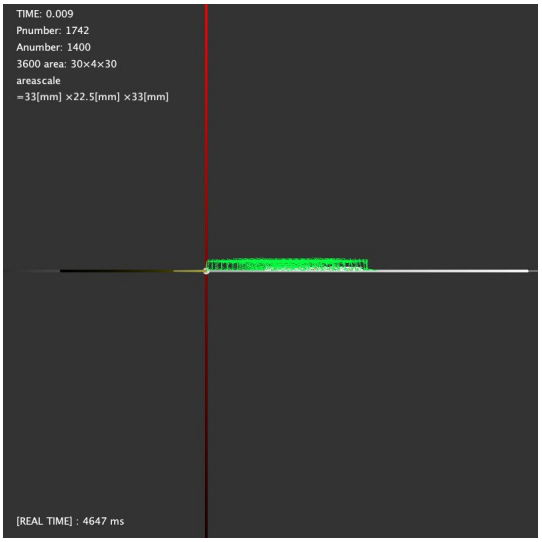


Figure 3.2: Side view of keratocyte model at time $t = 0$.

3.4 Reduction in Calculation Volume

Calculating the distance between membrane molecules in all steps increases the computational complexity. Therefore, when calculating the cell membrane model, the distance between the cell membrane molecules stored in advance was referred to. In the initial state, the index information of the adjacent cell membrane molecules was stored. This method can be adopted because the position information adjacent does not change regardless of how much the interparticle distance changes.

Likewise, if the distance between the cell membrane molecule and the actin molecule is calculated in all steps, the process becomes heavy. Unlike the case of intermolecular molecules, the index information of nearby molecules changes in the case of actin molecule and cell membrane molecules. For this reason, the simulation space was divided into areas in mesh form, and index information of molecules in the same area was saved.

Chapter 4

Results and Discussion

4.1 Simulation Results

4.1.1 Role of Actin Retrograde Flow

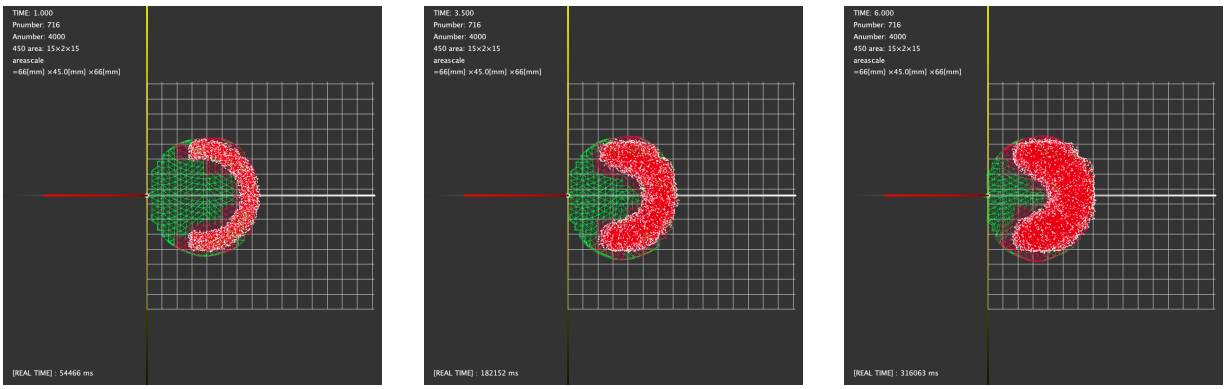
Fig.4.1 showed the simulation result with the ARF reference point being SF expressed by two points and ARF being distance independent type. The actin molecule aggregated in a half-moon shape with time. Fig.4.2 showed simulation results under the same condition as Fig.4.1 except that distance-dependent ARF is used. In Fig.4.1, the actin molecule protruded from the cell membrane as a result of continuing AP. Fig.4.3 showed simulation results assuming that the ARF reference point is the last cell membrane molecule, not SF. Deformation occurred towards the center of the cell rather than pulling at two points. Fig.4.4 showed the simulation results when ARF is excluded. The actin molecule protruded from the cell membrane as in the case of Fig.4.2. From the experimental results, the conditions under which the actin molecule is most stable are the conditions shown in Fig.4.1.

4.1.2 Effect of Initial Placement

The initial arrangement of actin molecules was half donut-shaped, but simulations were carried out for other shapes (Fig.4.5). Since it is not a half donut type, since the distribution of the actin molecule is not biased, the direction of cell movement was not determined. Moreover, since the effect of ARF affects actin molecule, the aggregation occurs near SF.

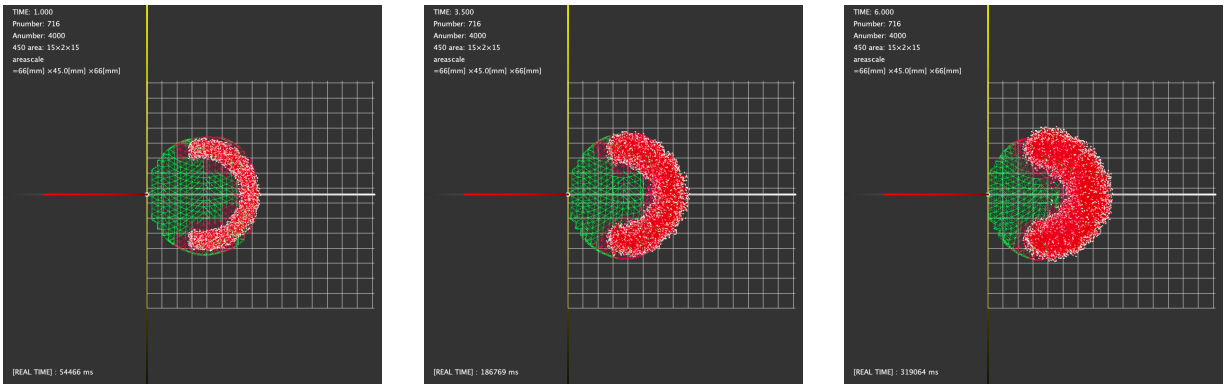
4.2 Discussion

The cell membrane molecule undergoes both the force from the actin molecule and the force of the nearby membrane molecule simultaneously. Therefore, when the actin molecule is excessively polymerized, the cell membrane can not be flexibly deformed, so it ruptures. This result suggests that ARF alleviates the excessive load on the cell membrane by regressing actin molecule. However, as ARF continually pulls actin molecules, it eventually inhibits the effect on cell membranes. As can be seen from Fig.4.5, in the case where ARF exists from a state in which the distribution of actin molecules is unbiased, a half-moon shape is not formed. The ARF is not constant, and it is presumed that the intensity of influence will change as time passes. As cell migration progresses SF is gradually formed and ARF is considered to occur. As can be seen from comparison between Fig.4.1 and Fig.4.3, actin molecules aggregate in a half-moon shape when ARF occurs from SF. SF is an actomyosin bundle



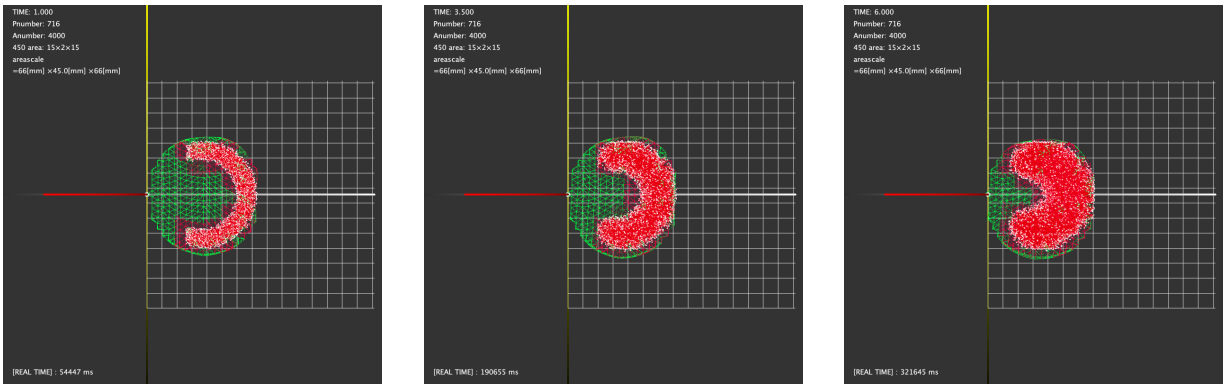
(a) t=1.0 (b) t=3.5 (c) t=6.0

Figure 4.1: Simulation results. This is a picture of the keratocyte model taken at time $t = 1.0, 3.5, 6.0[s]$. The top is the top view and the bottom is the side view pictures of the same time zone. A green line indicates a cell membrane, and a red dot indicates F-actin polymerized. Distance independent ARF is used, and the setting value of relocation condition is 2.5%.



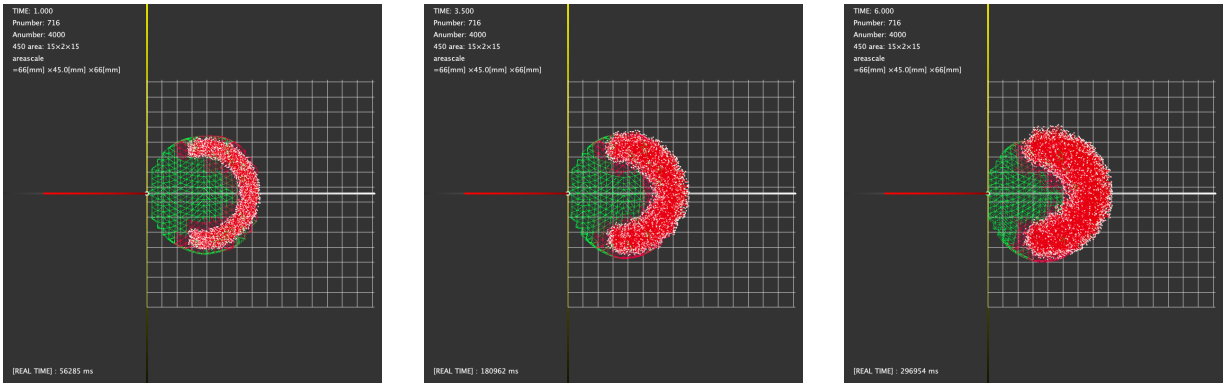
(a) t=1.0 (b) t=3.5 (c) t=6.0

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



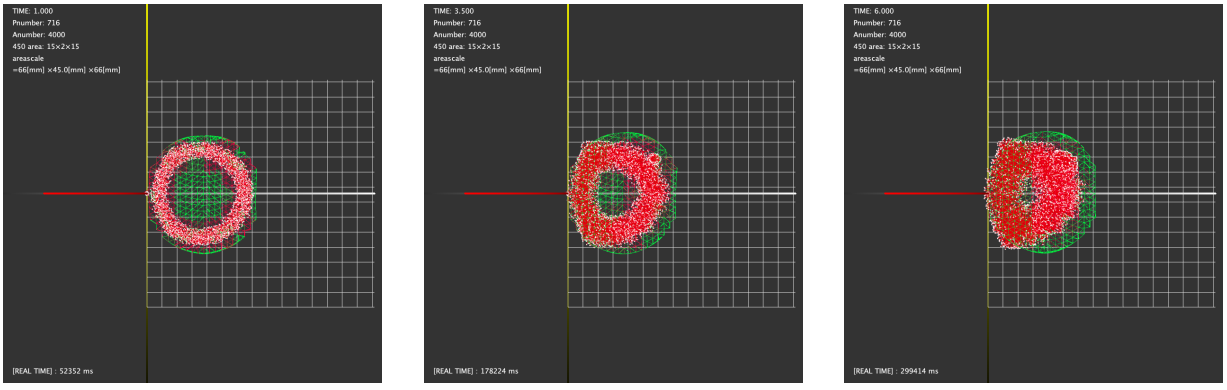
(a) t=1.0 (b) t=3.5 (c) t=6.0

Figure 4.3: Simulation results in the case of using ARF with reference to the membrane molecule at the end rather than SF.



(a) t=1.0 (b) t=3.5 (c) t=6.0

Figure 4.4: Simulation Results without ARF.



(a) t=1.0 (b) t=3.5 (c) t=6.0

Figure 4.5: Simulation results when the initial arrangement of actin molecules is circular.

formed by depolymerized actin molecule accumulating in the posterior part and combining with myosin molecule. As SF does not exist in the initial state, it is presumed that ARF probably also does not exist at the initial stage of cell migration.

If the actin molecule is located far away from the SF, there may be a mechanism that weakens or inhibit ARF. This is because if the strength of ARF is constant, the influence of AP does not pass well to the cell membrane.

Chapter 5

Conclusions and Future Work

5.1 Half-Moon Shape for Keratocytes

Actin molecules played a 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.

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. 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 ARF condition is half moon shape.

5.2 Future Work

In cell membrane molecular simulation, we could not cope with the movement of actin molecule well. 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.

Acknowledgements

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