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A Computational Model of Cell Migration of Fish Keratocytes

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Chapter 1

Introduction

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

Actin proteins compose cytoskeleton of eukaryotic cells [1], and in keratocytes, actin molecules have been reported to 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. 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. Chapter 2 introduces previous works on the molecular mechanism of cell migration of keratinocytes. Chapter 3 explains the method of physical simulation experiments. Chapter 4 shows the simulation results, and the last chapter concludes this study and discusses the future prospects of this study.

Chapter 2

Keratocytes

2.1 Characteristics of Cell Migration of Keratocytes

A keratocyte, a migratory fish epidermal cell, is a wound healing cell about 70 μm in size. When a fish is injured, 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 move with keeping a half-moon shape.

The cell migration of keratocytes has been suggested to be caused by actin polymerization (AP)[1]. AP is a phenomenon in which cytoskeletal actin molecules linearly bind to form filamentous actin (F-actin). AP occurs only at one end of F-actin, depolymerization occurs at the opposite end. The actin molecules polymerize to form a dense network and push the cell membrane from the inside. It has been reported that the actin molecules are polymerized more frequently in the higher density region of actin molecules and depolymerized less in a lower density region[3]. There is a stress fiber (SF) at the rear of the cell. SF is a bundle of actomyosin to which actin molecule and myosin molecule are bound. The actin retrograde flow (ARF) which pulls the actin molecules back toward the SF has also been reported [4]. Although details on ARF are not clarified, SF is considered to orient the polymerization direction in that direction when attracting actin molecules towards SF[5]. When SF was removed, the left and right balance of the cells collapsed and the moving speed decreased. This also indicates the importance of SF for cell migration.

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. In addition, Okimura et.al reported that SF plays a role of wheels in cell migration [6]. It has been reported that morphogenesis of morphology was observed even after removing the nucleus [7]. Therefore, it was suggested that the cell migration of keratinocytes is not a nuclear part, and other parts where SF and F-actin exist are important.

2.2 Molecular Mechanism of Cell Migration

A cell has a structure called a cytoskeleton. The cytoskeleton is like bones of humans and forms a cell shape, however, it shows dynamic behavior in contrast to the rigid nature of human bones. One of the main components of the cytoskeleton is actin molecules. The polymerization and depolymerization of actin molecules change the cell shape. The actin molecule is elongated toward the cell membrane by polymerization and is retracted to the bundle of actomyosin called stress fiber which is formed in a rear part of a keratocyte. Such retraction of actin fibers is called actin retrograde flow; however, the role of the retrograde

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

flow has not been known. The membrane of amoeboid cells extruded by the polymerization of actin molecules forms a pseudopodia which adheres to the substrate under the pseudopodia. Thereafter, the adhesion to the substrates of the rear part of the cell is released and the cell is dragged forward. By repeating this cycle, cell migration is realized. The cell membrane of keratocytes is also deformed by actin molecule polymerization. By the simulation experiment described in the next chapter, we investigate how the cell membrane forms a half-moon shape by the actin molecule polymerization.

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 was 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 $\boldsymbol{F}_i^a$ are the forces 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 interact with particle j. The natural length between particles was 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 of the effect of 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 the 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 [3], 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 the 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 the barbed end and pointed end of the F-actin, respectively, and $f^p(c) = 5.0 \ \exp \frac{c}{10.0}$ expresses the frequency of actin polymerization as a function of actin concentration c which was computed for each region by dividing the simulation space into a grid. 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 simulation space was divided into $3,600$ regions ($i = 1, ...3600$).

The actin molecules in the region with low density of actin molecules were assumed to disappear and the same number of new actin molecules were put near the cell membrane. The direction of polymerization of each actin was randomly determined at the initial state.

3.2.2 Actin Retrograde Flow

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 a keratocyte.

We assumed the ARF as the retraction of the actin molecules toward the leftmost point of the cell in Fig.3.2, i.e., the position of the membrane particle with the smallest x coordinate which we call the reference point. The retraction of the actin molecules by the ARF was expressed by the following equation:

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

where $\boldsymbol{E}_0$ is the reference point, and α and β are constants that determine the strength of the ARF. w is a parameter indicating the effect of distance between the actin molecule and cell membrane molecule on the retraction strength of the ARF. When $w = 2$, the closer the intermolecular distance is, the stronger the retraction is. When $w = 1$, all actin molecules are equally pulled irrespective of the intermolecular distance. When $\alpha = \beta$, the orientation effect is ineffective, therefore, the actin molecule is retracted without changing the direction.

We also investigated the case that two reference points $\boldsymbol{E}_1$ and $\boldsymbol{E}_2$ are prepared in order to express the retraction of actin molecules toward the SF. In this case, assuming that SF spreads linearly at a distance of 1/5 from the left end of the cell (Fig. 3.1) and the reference points $\boldsymbol{E}_1$ and $\boldsymbol{E}_2$ were put at both ends. The ARF was expressed by the retraction of actin

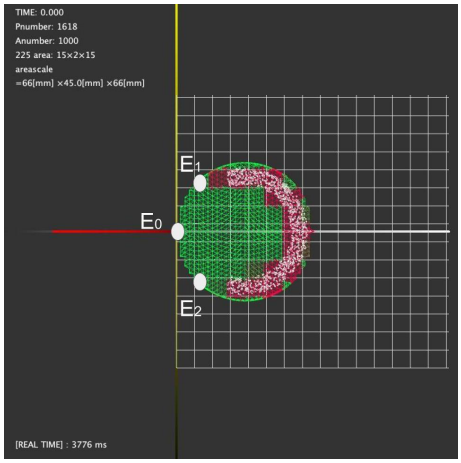


Figure 3.1: Location of the ARF reference point, $\boldsymbol{E}_0$, $\boldsymbol{E}_1$ and $\boldsymbol{E}_2$.

molecules toward the direction of the sum of vectors from the actin molecules to the reference points:

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

where γ and δ are constants that determine 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. Table 3.1 shows the values of α, β, γ and δ.

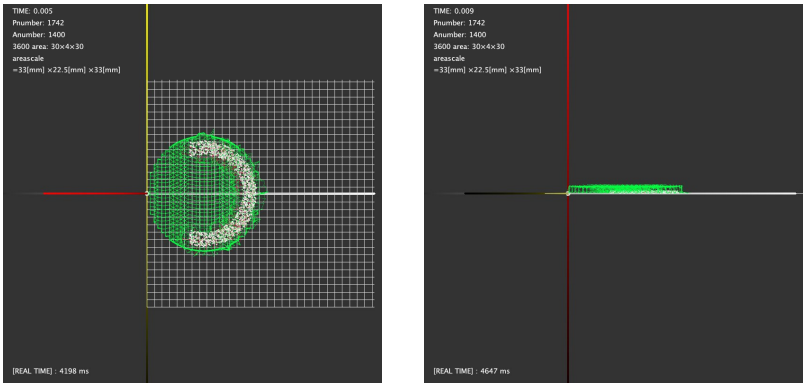
Table 3.1: The value of each parameter.

	$w = 1$	$w = 2$	$w = 1$ (No orientation)	$w = 2$ (No orientation)
α	5.0	0.02	20.0	0.1
β	50.0	0.2	20.0	0.1
γ	2.5	0.01	10.0	0.05
δ	25.0	0.1	10.0	0.05

3.3 Initial Condition

The number of actin molecules and membrane particles were 3,000 and 2,000, 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 steps.

Fig. 3.2 shows the initial state of the virtual cell of the computer simulation. The actin molecule are in a U-shaped area excluding the rear of the cell (Fig. 3.2). The membrane molecules were placed on the surface of the cylinder.



(a) (b)

Figure 3.2: Initial state. (a) Top view. Green lines and white dots show actin molecules and membrane particles, respectively. (b) Side view.

Chapter 4

Results and Discussion

4.1 Simulation Results

4.1.1 Role of Actin Retrograde Flow

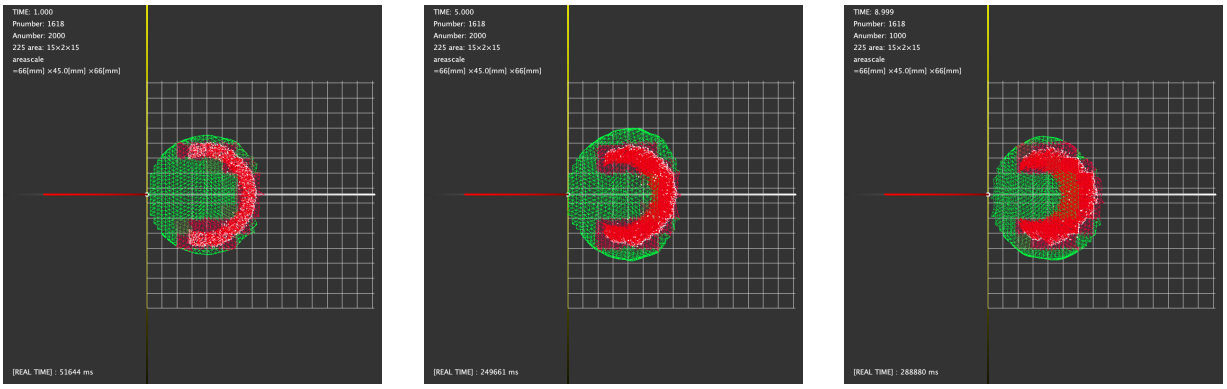
Fig. 4.1 shows the simulation result when two reference points of the ARF is prepared (eq. (3.9)) and the effect of the ARF is independent of the distance (w=1 in eq. (3.9)). In this case, the actin molecules aggregated in a half-moon shape and the shape was kept for a long time even after the time shown in Fig.4.1 (c).

Fig. 4.2 shows the results under the same condition as Fig. 4.1 except that distance-dependent ARF was assumed (w=2 in eq. (3.9)). Since actin molecules close to the SF are strongly pulled back in the case, actin molecules aggregated. As a result the AP, the region of actin molecules spread only in one direction pushing only the cell membrane front end. Since the repulsive force from the actin molecule concentrated in one place, the cell membrane was broken. As the convergence of repulsive forces at destroys the cell membranes in this way, ARF should not retract actin molecules that polymerize in the lateral direction.

Fig. 4.3 shows simulation results when one reference point of the ARF was prepared (eq. 3.7). The actin molecules migrated toward the center of the cell and the half-moon shape was not achieved. When the actin molecule aggregates in a circular shape, the direction of movement of the cell is not determined because it pushes the entire cell membrane. These results suggest that the retraction of actin molecules by the ARF toward not a point but the SF is an important phenomenon to make the cell shape the half moon.

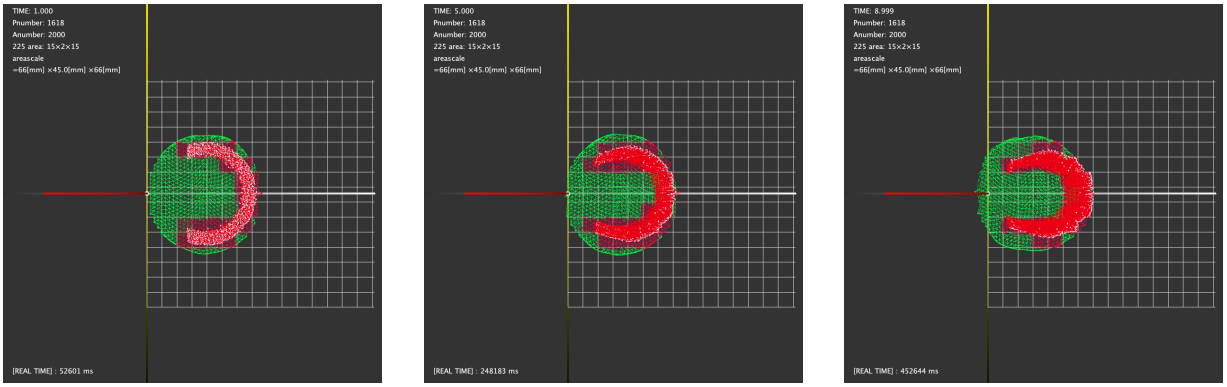
Fig. 4.4 shows the simulation results when the retraction of actin molecules by the ARF was ignored. Actin molecules were continuously elongated by polymerization toward random directions. This result shows that the retraction by the ARF is important to keep specific cell size against actin polymerization.

Fig. 4.5 showed the simulation result when ignoring the orientation effect by the ARF. The actin molecules aggregate into a V-shape and break the cell membrane. Paying attention to the white dots which is barbed-end, when compared with Fig.4.1, no alignment of the direction of the actin molecules was not observed. The shapes in Fig.4.5 and Fig.4.1 are similar, but since the barbed-end of the actin molecule is oriented in all directions, the shape when a certain time elapses differs (Fig.4.7).



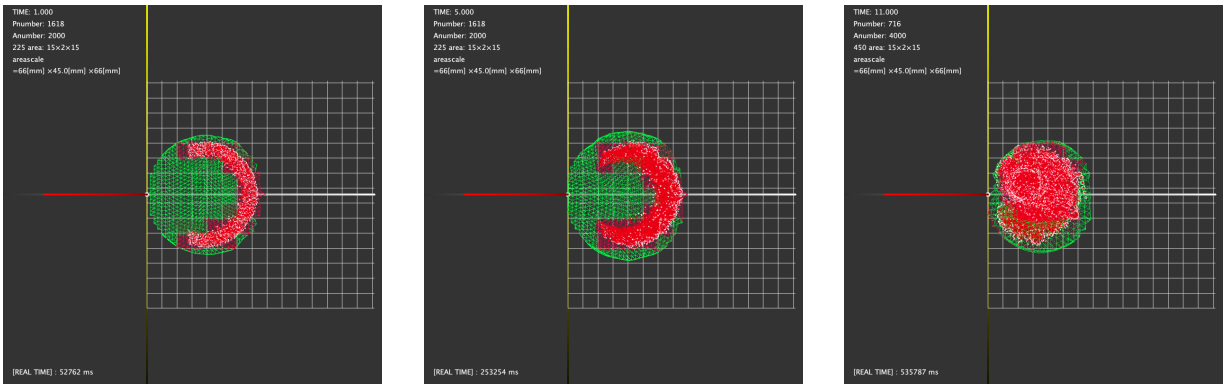
(a) (b) (c)

Figure 4.1: Simulation results when the amplitude of the retraction by the ARF is independent of the distance. (a), (b), and (c) show the results at $t = 1.0, 6.0, 11.0$ [s]. Green lines show the cell membrane, and white and red dots show the barbed-end of the F-actin, respectively.



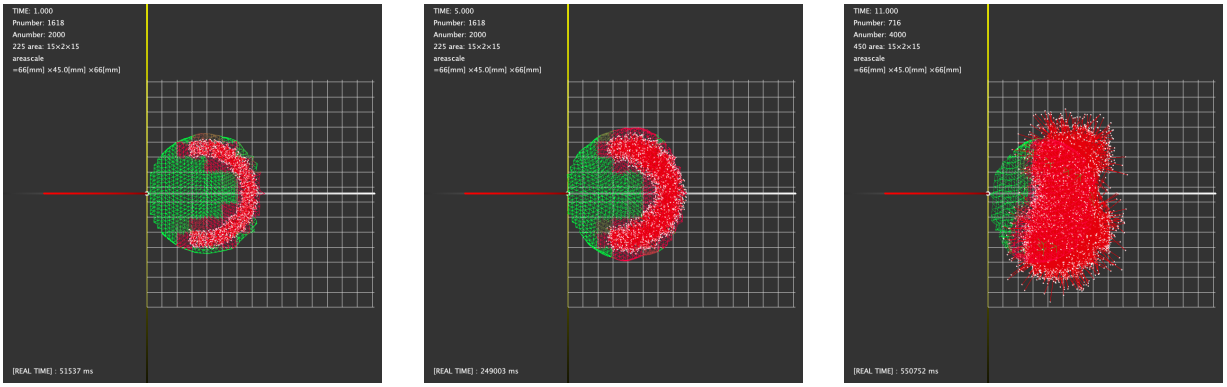
(a) (b) (c)

Figure 4.2: Simulation results under the same codition as those in Fig. 4.1 except $w = 2$ in eq. 3.9. The subfigures show the results at different timings as in Fig. 4.1.



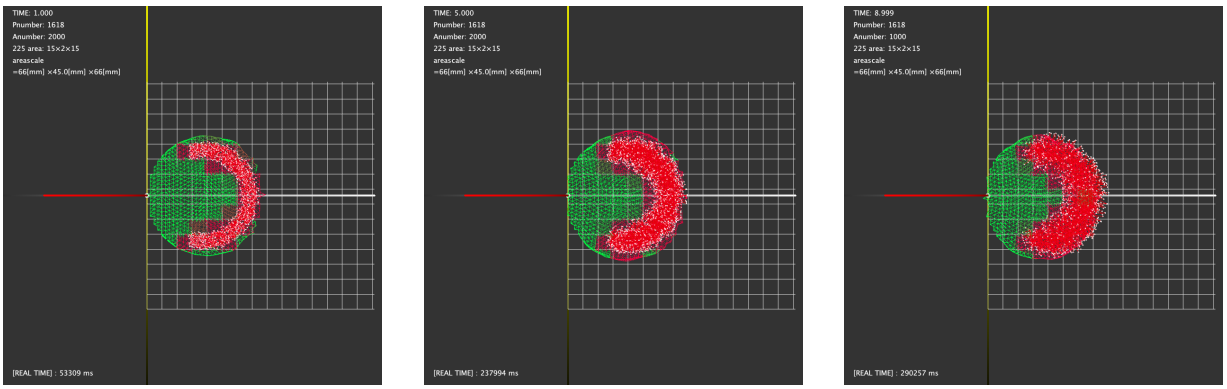
(a) (b) (c)

Figure 4.3: Simulation results when the reference point of the ARF was assumed at the position of the leftmost membrane molecule of the cell. The subfigures show the results at different timings as in Fig. 4.1.



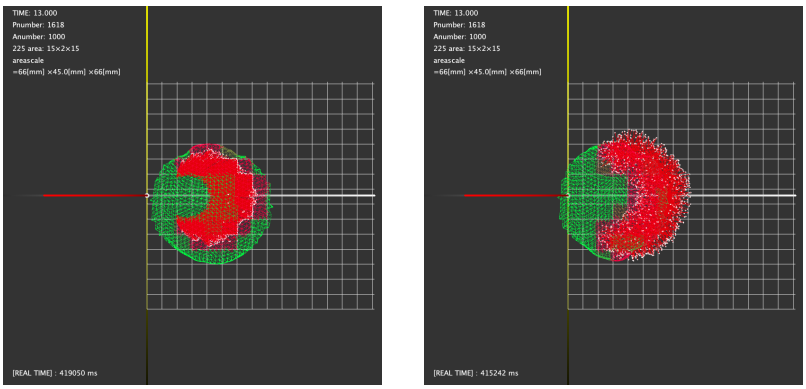
(a) (b) (c)

Figure 4.4: Simulation Results without ARF. The subfigures show the results at different timings as in Fig. 4.1.



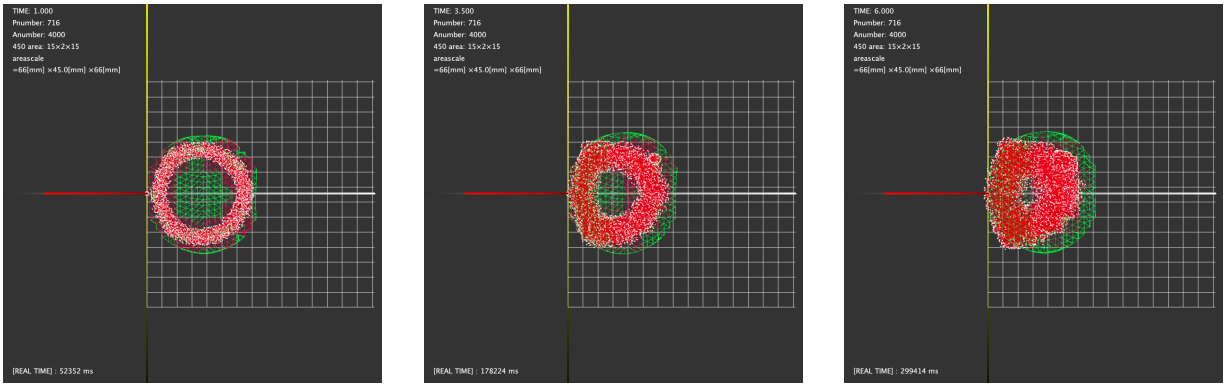
(a) (b) (c)

Figure 4.5: Simulation results without the orientation effect on the actin molecules by the ARF. The subfigures show the results at different timings as in Fig. 4.1.



(a) (b)

Figure 4.6: Simulation results at $t = 13$ [s]. (a) When there is orientation. (a) When there is no orientation.



(a) (b) (c)

Figure 4.7: Simulation results when actin molecules are distributed in a circular region as an initial condition. The subfigures show the results at different timings as in Fig. 4.1.

4.1.2 Effect of Initial Distribution of Actin Molecules

In the simulation experiments in the previous section, the actin molecules were randomly distributed in a U-shaped region as an initial condition. Fig. 4.7 shows the simulation results when 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 around the SF. This is due to the presence of actin molecules already in the vicinity of SF when ARF acts with actin molecules arranged in a donut shape. From the results in Fig.4.1 and Fig.4.7, it is clear that the actin molecules aggregate in a U-shaped rather than a donut shape to form a half-moon shape. In keratocytes, the number of actin molecules decreases at the rear part of the cell when migration starts [8]. Hence, such a break of uniform distribution of the actin is a key to the formation of the half-moon shape.

4.2 Discussion

The simulation results showed that the retraction and orientation of actin molecules by the ARF contribute to the regulation of the cell size and the formation of the half-moon shape, respectively.

The membrane particles receive force from actin molecules and neighboring membrane particles simultaneously. When the AP is 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 simulation experiment of this study, actin molecules often stretched its head by the AP to the outside of the cell. One reason for this problem was the slow propagation of the displacement of membrane particles and actin molecules often go through the position of the membrane before the membrane deforms against the actin movement. Solving this problem is important to simulate the formation of the cell shape for longer duration.

Acknowledgements

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