ISSN 1672-9145 **Acta Biochimica et Biophysica Sinica** 2005, **37**(9): 643–648 **CN** 31-1940/Q

Membrane Phospholipid Redistribution in Cytokinesis: A Theoretical Model

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Abstract In cell mitosis, cytokinesis is a major deformation process, during which the site of the contractile ring is determined by the biochemical stimulus from asters of the mitotic apparatus, actin and myosin assembly is related to the motion of membrane phospholipids, and local distribution and arrangement of the microfilament cytoskeleton are different at different cytokinesis stages. Based on the Zinemanas-Nir model, a new model is proposed in this study to simulate the entire process by coupling the biochemical stimulus with the mechanical actions. There were three assumptions in this model: the movements of phospholipid proteins are driven by gradients of biochemical stimulus on the membrane surface; the local assembly of actin and myosin filament depends on the amount of phospholipid proteins at the same location; and the surface tension includes membrane tensions due to both the passive deformation of the membrane and the active contraction of actin filament, which is determined by microfilament redistribution and rearrangement. This model could explain the dynamic movement of microfilaments during cytokinesis and predict cell deformation. The calculated results from this model demonstrated that the reorientation of phospholipid proteins and the redistribution and reorientation of microfilaments may play a crucial role in cell division. This model may better represent the cytokinesis process by the introduction of biochemical stimulus.

Key words cytokinesis; biochemical stimulus; phospholipid; actomyosin microfilament

Cytokinesis is a physical process during which a cell is divided into two after the completion of mitosis. In most eukaryotic cells, cytokinesis is accomplished by the assembly of an actomyosin ring that contracts to divide the cell midway between the poles of the mitotic spindle [1]. In many cell components, actin and myosin play important roles in controlling the placement of the contractile ring, the movement of the mitotic spindle and the segregation of chromosomes during cell cleavage. Experimental results showed that microfilaments and microtubules were uniformly distributed and randomly oriented at the initial stage of cytokinesis. After a period of time, the microfilament orientation changed in accordance with its position. Microfilaments under the leading furrow edge in the contractile ring were aligned parallel to the cleavage plane, while microfilaments at other positions were oriented along the tangential direction of cytoplasm flow [2,3]. These findings indicated that the completion of cytokinesis depended on the assembly and redistribution of microfilament. Results of other related experiments [4] have also shown that the phospholipids in biological membranes are arranged symmetrically between the bilayer leaflets under normal conditions. Only during the late telophase of mitosis, phosphatidylethanolamine (PE) is exposed on the cell surface specifically at the cleavage furrow. There is no alteration in the distribution of the plasma membrane-bound peptide during cytokinesis. Cell surface immobilization of PE induced by cyclic peptide coupled with streptavidin at prometaphase could effectively block cytokinesis at late telophase. The peptide-streptavidin complex could specifically bind to the cleavage furrow and inhibit both actin filament assembly at the cleavage furrow and subsequent plasma membrane fusion. These results suggest that the surface exposure of PE reflects the enhanced transbilayer movement of PE at the cleavage furrow and that PE's movement is important in the actin and myosin filament assembly. The position of the cleavage furrow [5] and the

Received: November 23, 2004 Accepted: June 10, 2005

This work was supported by a grant from the National Natural Science Foundation of China (No. 10332060)

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DOI: 10.1111/j.1745-7270.2005.00090.x

contractile ring is related to the asters of the mitotic apparatus (MA), which consist of a pair of asters and a spindle. The function of MA in cytokinesis seems to be purely stimulatory [6]. Because several related experiments showed that the absence of MA would result in the disappearance of furrow formation, leading to no cell division [7]. Cells cleaved normally when MA was disrupted or eliminated between anaphase and the onset of furrowing. If MA was disrupted or eliminated before anaphase, the furrow would sometimes still be formed but cleavage was only partial [8,9]. These results may suggest that the biochemical stimulus originates from asters and diverges along the microtubules. To explain how the contracting force of the contractile ring drives the mother cell to cleave, many models have been proposed. These models can be grouped into two. In the first group [10–12], the plasma membrane is regarded as Mooney-Rivilin material and the deformation of the membrane is caused by an equatorial constricting force. These models could satisfactorily predict the cell deformation but the predicted constricting force and the internal pressure were not consistant with the experimental results described by Hiramoto [13]. In the second group [14–16], the main concern was the effect of cytoplasm flow and microfilament redistribution on surface tension. These models were successful in predicting the formation of the contractile ring and parameters such as surface force, surface concentration, internal pressure and flow fields [8,17]. But the biochemical stimulus was not involved in the mathematical equations of the model.

In order to get a better understanding of the cytokinesis process and a better explanation of the experimental findings, a new model was proposed. The model is based on Zinemanas-Nir hydrodynamic model, and incorporates biochemical processes from asters stimulus to actin and myosin filaments assembly.

Biomechanics Model

The model proposed in this paper is based on the following assumptions.

(1) The movement of phospholipids on the membrane surface is driven by the biochemical stimulus from asters [2].

(2) The velocity of PE at a surface point P is determined by the amount of biochemical stimulus received at this point P. The aster microtubule is supposed to be straight, and the center of each aster transmits the biochemical stimulus in all directions through a microtubule at the same frequency. Thus the amount of biochemical stimulus transmitted through each aster microtubule is equal, and the amount of biochemical stimulus received at a certain part of the cell surface depends on microtubule density. Here the probability of the length of the microtubule is assumed normal distribution [5].

(3) The distribution of the phospholipid molecules on the cell membrane during the mitosis telophase determines the amount of actin and myosin assembly, namely the microfilament, at the beginning of cytokinesis [4].

(4) During cytokinesis, the microfilament is linked to the membrane through macromolecules, and changes in microfilament density are related only to membrane deformation [4].

(5) The microfilament endures a time-dependent single-direction force parallel to its symmetry axis only. The membrane is a uniform and isotropic material that can endure uniform surface tension. Therefore, the cortex tension consists of two parts: one is the surface tension of the membrane caused by the active contraction of the microfilaments; the other is the surface tension of the membrane caused by the passive deformation of the cell membrane [14].

(6) The assembly of microfilament at the equator plane initially forms the contractile ring. The active contraction of the microfilament causes the flow of cytoplast. Simultaneously, the viscous flow of the cytoplast results in microfilament reorientation. The active contraction of the contractile ring and the flow of the cytoplast together deform the cell, and the membrane is also deformed. The movement of each point of the membrane drives the adherent microfilament to move along the membrane by way of the macromolecules. The surface motion and the microfilament reorientation lead to the anisotropy of cortex tension.

(7) The membrane is regarded as Mooney-Rivlin material.

(8) Because the volume change during division is negligible, the cell is regarded as an incompressible body.

Governing Equations

Redistribution of phospholipid molecules determines the initial distribution of microfilaments

Under the above assumptions, the probability of the aster microtubule's length obeys the normal distribution. When the distance between the aster center and a point P on the cell surface is d and the microtubule length is larger than or equal to d, the biochemical stimulus can reach the point P. The microtubule density at the point P is directly proportional to $1–F(d)$ [5], but inversely proportional to

d^2. In general, the aster microtubule that reaches P is not perpendicular to the cell surface, and it does not span the spindle to arrive at the opposite side. Therefore, the microtubule density is calculated as:

$$f(P)=\begin{cases}\sum|\cos\alpha|S(d),\theta>H\\ \sum|\cos\alpha|S(d)(\frac{\theta}{H})^n,0\le\theta\le H\end{cases} \quad 1$$

$$S(d)=2\mu^2S(\mu)[1-F(d)]d^{-2} \quad 2$$

where $f(P)$ represents the microtubule density at the point P; $S(d)$ represents the microtubule density on the surface of the imaginary sphere with radius d; α is the angle between the microtubule and the normal direction of the surface; θ is the angle between the microtubule and the spindle axis; H and n are the parameters set for eliminating the effect of the microtubule from an aster to the opposite cell surface over the spindle area: H is the angle between the spindle tangent line and the spindle axis, and the value is 0.45π and n is constant 15; μ and σ represent normal distribution with mean and variance corresponding to the values of 60 μm and 20 μm respectively, $F(d)$ is the probability function.

Microtubule density is proportional to biochemical stimulus. The gradient of biochemical stimulus drives the PE molecule to move along the membrane surface from a high-density area to a low-density one, and the diffusion of the PE molecule is one of the causes of molecular movement. The distribution function C at point P on the membrane surface is computed as **Equation 3**, and at the poles as **Euqation 4**.

$$\frac{\partial C}{\partial t}=\frac{1}{\sqrt{1+[r']^2}}(2kC\frac{d^2f}{dz^2}+2D\frac{\partial^2C}{\partial z^2}) \quad 4$$

On the basis of assumption (3), the concentration of PE molecules determines the assembly amount of the microfilament at the same place, hence the function C can also express the initial distribution of the microfilament.

Cytokinesis model

In the Zinemanas-Nir model [14], the cell membrane is considered as a 2-D elastic membrane, and the cytoplasm and the cell environment as Newtonian fluid with viscosity μ and μ' respectively. Because the cytoplasm flows very slowly, the effect of gravity and inertia can be neglected. The motion equations depend on velocity υ, stress σ and pressure p.

$$\nabla\cdot\sigma=0 \quad 5$$

$$\nabla\cdot\upsilon=0 \quad 6$$

$$\sigma=-pI+\nabla\cdot\upsilon+\nabla\cdot\upsilon^+ \quad 7$$

The boundary conditions are:

$$\Delta\upsilon=0 \quad 8$$

$$\Delta(\sigma\cdot n)=f \quad 9$$

where Δ represents the difference across the surface (outer minus inner), f is interface force, n is the unit vector on the surface pointing to outer (from B to B*).

The surface velocity is:

$$\upsilon=\frac{dR}{dt} \quad 10$$

Surface stress tensor

Owing to the neglect of the inertial force and the surface momentum, the surface force is only related to the surface stress tensor $\gamma^{\alpha\beta}$. According to assumption (5), the stress tensor includes active and passive components that can be described by the following expression:

$$\gamma^{\alpha\beta}=\gamma^{\alpha\beta}_{(a)}(C,N)+\gamma^{\alpha\beta}_{(p)}(\varepsilon_{\alpha\beta},\dot{\varepsilon}_{\alpha\beta}) \quad 11$$

where $\gamma^{\alpha\beta}_{(a)}$ represents the active component caused by the active contraction of the microfilaments, which depends on the local distribution function C and orientation function N of the microfilaments. $\gamma^{\alpha\beta}_{(p)}$ represents the passive component caused by the passive surface deformation, which depends on the strain $\varepsilon_{\alpha\beta}$ and the rate of strain $\dot{\varepsilon}_{\alpha\beta}$ of the cell membrane.

By means of assumption (4), the local function C depends on the stretch deformation of the membrane only, and the material derivative is zero:

$$\frac{DC}{Dt}=0 \quad 12$$

The microfilament orientation N follows the orientation balance and relates to translation, rotation and diffusion:

$$\frac{DN}{Dt}=D_R\nabla^2_{(d)}N+\nabla_{(d)}\cdot(wN)+\frac{D_T}{C}(C_{,\alpha}N_{,\alpha}) \quad 13$$

where w is the microfilament angular velocity, d is the unit vector along the microfilament axis, D_R is the rotational

$$\frac{\partial C}{\partial t}=\frac{k}{1+[r'(z)]^2}\{\frac{C}{L}\frac{\partial L}{\partial z}\frac{df}{dz}+\frac{\partial C}{\partial z}\frac{df}{dz}-\frac{r'(z)r''(z)}{1+[r'(z)]^2}C\frac{df}{dz}+C\frac{d^2f}{dz^2}\}+\frac{D}{1+[r'(z)]^2}\{\frac{1}{L}\frac{\partial L}{\partial z}\frac{\partial C}{\partial z}-\frac{r'(z)r''(z)}{1+[r'(z)]^2}\frac{\partial C}{\partial z}+\frac{\partial^2C}{\partial z^2}\} \quad 3$$

diffusion coefficient, and D_T is the translation diffusion coefficient.

The experimental results showed that the translation and diffusion of the macromolecules in the cell surface is nearly equal to zero during cytokinesis, that is, D_R and D_T are both zero. **Equation 13** can be considered as a linear formula.

The surface stress tensor related to the active stress, the distribution function C and the orientation N of the microfilament can be written as:

$$\gamma^{(\alpha\alpha)} = FLC\int\sqrt{a_{\alpha\alpha}}\,l^{\alpha}N(l)dl \qquad 14$$

where $\gamma^{(\alpha\alpha)}$ is the main stress tensor, and F and L are the filament contractile force and its length, respectively.

Based on assumption (7), the passive component relates to constitutive equations of the membrane. The Mooney-Rivilin material is selected to describe the membrane since it has been used to successfully simulate red blood cell deformation. The relationship between the main stress and the main stretch rate can be described as:

$$\gamma^{(11)} = 2h\hat{C}\left(\frac{\lambda_1}{\lambda_2} - \frac{1}{\lambda_1^3\lambda_2^3}\right)(1+\Gamma\lambda_2^2) \qquad 15a$$

$$\gamma^{(22)} = 2h\hat{C}\left(\frac{\lambda_2}{\lambda_1} - \frac{1}{\lambda_1^3\lambda_2^3}\right)(1+\Gamma\lambda_1^2) \qquad 15b$$

where 1 and 2 in subscript represent meridian direction and circulation direction, respectively; λ_α is the main stretch rate (α=1,2); h is the thickness of the membrane; $\hat{C}$ and Γ, the material constants [11], are both 0.1.

The relationships between the main stretch rate and the main strain as well as the main strain rate are:

$$\varepsilon_{\alpha\alpha} = \frac{1}{2}(\lambda_\alpha^2 - 1) \qquad 16$$

$$\dot{\varepsilon}_{\alpha\alpha} = \dot{\lambda}_\alpha\lambda_\alpha \qquad 17$$

Numerical calculation

The general cleavage process of eukaryotes is that of axial symmetry. The cylindrical coordinates (r, z, ϕ) are selected to describe the cell's 3-D deformation, and the coordinates (ϕ, z) are used as the surface coordinates. The equations are non-dimensionalized with γ_0, R, γ_0/μ for the scales of surface tension, length and velocity, respectively, and similarly with γ_0/R for the pressure scale. Here γ_0 denotes the uniform value of the initial surface tension. The initial concentration function of the microfilament is offered by the microfilament distribution after biochemical stimulus. It is assumed that the cell is in a state of no stress at the beginning of cytokinesis. **Equations 5–9** can be conveniently converted into the boundary integral equations that depend on the surface velocity and the surface tension. The microfilament distribution function C, orientation function N, strain ε and strain rate $\dot{\varepsilon}$ can be expressed with the surface velocity. As all the equations are nonlinear and the moving boundary problem needs to be solved, the method of boundary integral was selected to solve the equations. The procedures are as follows.

(1) During the mitosis telophase, the cell is a sphere and has a pair of normal mitotic apparatus. The distance between the aster centers of the two mitotic apparatus is one-third of the cell diameter. Using **Equations 1,2**, the microtubule density on the cell surface can be calculated. With the values k and D, the microfilament distribution function C can be calculated by **Equations 3,4** at the beginning of cytokinesis.

(2) The initial surface state is given. The surface force and the velocity distribution are calculated by the comprehension of function C.

(3) Solve the microfilament distribution function C and the orientation function N by **Equations 12,13**.

(4) Calculate the surface tension by **Equations 11,14–17**.

(5) Repeat the steps (2–4).

Results

This model mainly emphasizes the effect of the biochemical stimulus on cytokinesis. The microtubule density D of the asters at each point on the surface is plotted in **Fig. 1**. The row axis z of the coordinates represents the position of every point, and the column axis D indicates the microtubule density of the surface points. According to **Fig. 1**, the microtubule density is larger at the poles and smaller at the equator plane, and the density gradient of the microtubules in the membrane surface is formed. In accordance with assumption (2), the gradient of the biochemical stimulus is identical with that of the microtubule density, and the gradient is the driving force of the redistribution of the PE molecules.

The dynamic distribution of PE molecules is shown in **Fig. 2**. The axis t in the coordinates system represents the lasting time of biochemical stimulus, the axis P represents the position of the surface point, and the axis c illustrates the concentration of the PE molecules. **Fig. 2** shows that at the cell furrow, the PE molecules distribute from uniform to strong denseness, while at the polar zones the distribution of the PE molecules becomes very sparse from the original uniform distribution. Finally, a "mountain" peak is formed at the equator and a "mountain" valley around the equator is constructed. In terms of the mass conservation, the PE

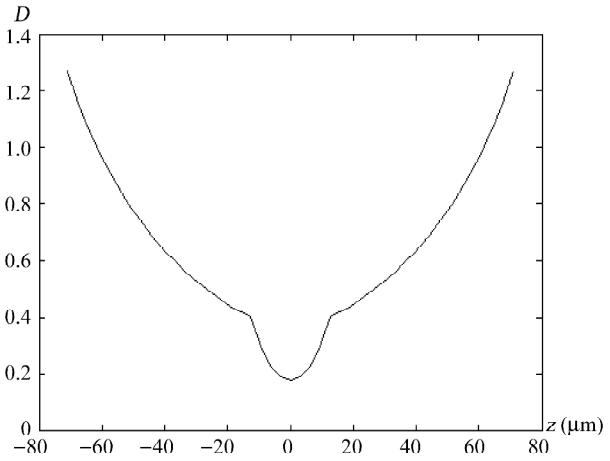

Fig. 1 Microtubule density of asters at each point on cell surface

The row axis z of the coordinates represents the position of every point, and the column axis D indicates the microtubule density of the surface points.

molecules move from the valley to the peak. Therefore, the result of **Fig. 2** explains the process of the microfilaments assembling at the equator plane and forming contractile ring.

Fig. 3 depicts the microfilament reorientation at t=2.8 min. In the coordinates system, the angle axis represents the angle between the microfilament axis and streamline, the axis p represents the position of the surface point, and axis N represents the number of microfilaments at a different point for a different orientation angle. It can be seen from **Fig. 3** that the surface velocity gradient of the cytoplasm induces a reorientation of the microfilaments, and that the microfilament axis parallels to meridian lines at the polar zones and parallels to the equator plane in the equator, which coincides with Hiramoto's experimental results.

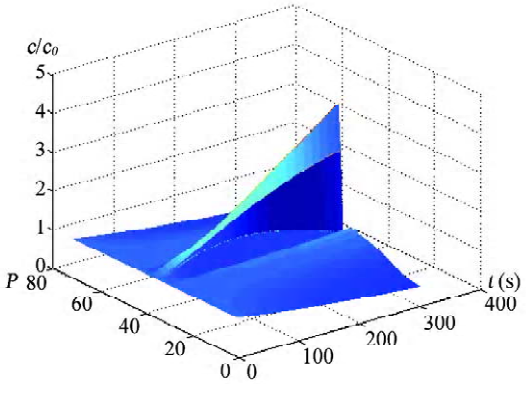

Fig. 2 Dynamic distribution of phosphatidylethanolamine (PE) molecules

The axis t in the coordinates system represents the lasting time of biochemical stimulus, the axis P represents the position of the surface point, and the axis c illustrates the concentration of the PE molecules. c_0 is the initial value of c.

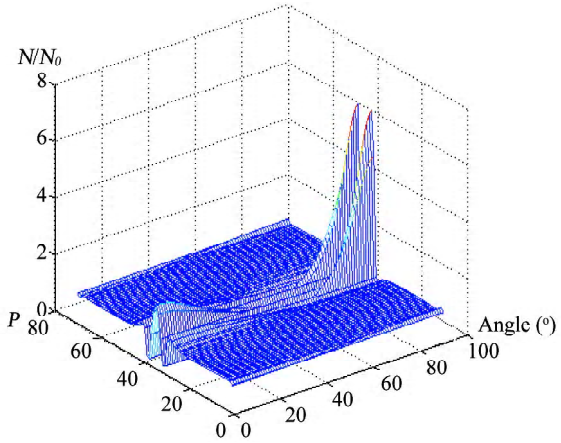

Fig. 3 Microfilament orientation at t of 2.8 min

The axis P represents the position of the surface point, and axis N represents the number of microfilaments at a different point for a different orientation angle. N_0 is the initial value of N.

The numerical results of our model, the Zinemanas-Nir model, and Hiramoto's experimental results are shown in **Fig. 4**. In the coordinates system, the row axis represents the cytokinesis process, and the column axis represents the nondimensional circular contractile force at the cell furrow. In spite of the similarity of the three curves in **Fig. 4**, the result of our model is closer to the experimental result than that of the Zinemanas-Nir model. The reason is that, in our model, the nonuniform distribution of microfilaments at the beginning of cytokinesis and the adjustment of the active contractile force are considered. The initial condition for the model is that the microfilament concentration at the cell furrow is more intense than that at the polar zones. During the cytokinesis process, because the connection of the microfilament and the membrane results in little change to the microfilament distribution at every point on the membrane surface relative to the initial distribution, the circular contractile force at the equator plane mainly relies on the reorientation of the microfilaments. However, in the Zinemanas-Nir model, the concentration of microfilaments enhances monotonously

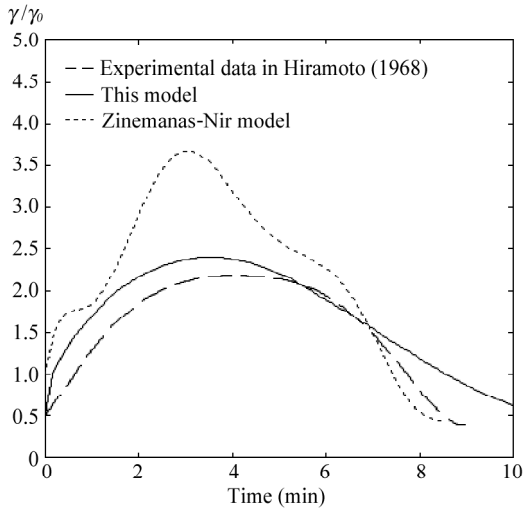

Fig. 4 Dynamic change in circular contractile force at cell furrow

In the coordinates system, the row axis represents the cytokinesis process, and the column axis represents the nondimensional circular contractile force at the cell furrow. γ_0 is the initial value of γ.

with the increase of time, and the circular contractile force depends not only on microfilament reorientation but also on its redistribution. The coupling of microfilament reorientation and its redistribution strengthens the circular contractile force.

Conclusion

Based on the Zinemanas-Nir model, we proposed a new model to simulate the entire process of membrane phospholipid redistribution in cytokinesis by coupling the biochemical stimulus with the mechanical actions. This model emphasizes the effects of phospholipid redistribution mainly on initial microfilament distribution and connection of the microfilament with the membrane. This model could explain some experimental phenomena during cytokinesis and predict cell deformation. The calculated results from this model demonstrated that the phospholipid redistribution and the connection of the microfilament with the membrane might play important roles in cytokinesis. However, the nature of the microfilament assembly and its active contraction is still unknown. In order to get an accurate cytokinesis model, more relative biochemistry groupware and experiments focusing on microfilament assembly are needed.

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Edited by
Ming-Hua XU