Cytokinesis: A problem in morphomechanics

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Abstract. The probable role of mechanics in understanding the cytokinesis in animal cell is discussed. A case is made for invoking mechanics to explain the phenomena of morphogenesis.

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1. Introduction

The subject of animate morphology, although an old discipline, has continually attracted the attention of investigators and has recently excited renewed interest. Questions as to how a cell divides, how the neural tube closes, how tissues get curved, and many more, have been intriguing biologists for a long time. They have been advancing various hypotheses, if only by way of analogies, to the effect that the same physical forces that are common to physics and mechanics are also responsible for animate morphogenesis.

Growth and morphogenesis are two essential features of living beings. The phenomena of morphogenesis are obscure to the extent that it is yet to be explained as to what causes morphogenetic movements as well as the changes in the form of an organism. Any movement of a particle having mass from one place to another in space that change of form implies, must be associated with some mechanical force or moment, unless Newtonian mechanics is to be denied. Any change in the form of matter, whether living or non-living, must be describable in terms of the action of a force or moment. Several physical phenomena such as surface tension, swelling, adhesion, crystallisation, diffusion, mechanical instability, differential growth and growth antagonism have been invoked as probable mechanisms to provide an insight into the phenomena of morphogenesis (Sahay 1981).

Cell division is one of the most basic characteristics of a living organism. People have long been curious about this physiological phenomenon. Cleavage is the process of cell division by which the egg divides into blastomers. The process of cell division involves several synchronous activities like furrow development, contractile ring formation and finally, the cell division. The cleavage is driven by a contractile ring in the shape of a furrow forming at the animal pole and then gradually extending around the cell equator and through it to the vegetal pole.

Phenomena like gastrulation, cell division, cerebral sulcation etc have long been considered by biologists as mechanistic in nature. But it is only recently that attempts are being made to examine such hypotheses in terms of rigorous mechanics; and the results are rather interesting. We intend to highlight, in this paper, the probable role of

mechanics in understanding the phenomena of morphogenesis, and to do so, a typical problem of morphogenesis namely the cytokinesis in animal cells is looked into and reviewed.

2. Cell division—As seen by biologists

It is interesting to know that the process of cleavage and cell division has been regarded as a problem in mechanics for more than one hundred years. Butschli in 1875 was apparently the first to propose that the variation in the surface tension of a cell was an important factor in cell division (Greenspan 1977).

Most of the experimental work on cleavage and cell division has been carried out on sea urchin eggs, mainly because they are easy to obtain and manipulate, besides being sufficiently transparent for individual cells to be followed by time lapse photography. The cleavage of a sea urchin egg can closely be modelled as a division of a sphere into spheres without any change in the volume. Certain common features have been summarised by Wolpert (1960) as quoted below:

- (i) The polar regions expand and the furrow regions contract.
- (ii) The 'stiffness' of the membrane increases just before cleavage and falls away during cleavage. This fall in stiffness occurs mainly at the poles, whereas the stiffness in the furrow region continues to rise.
- (iii) The cell rounds up before cleavage and during cleavage is weakest at the poles.
- (iv) There is an increase in birefringence at the surface before cleavage. During cleavage it decreases, this decrease starting at the poles and spreading to the furrow.

The fact that the division of a cell involves a considerable change in its shape has led to a general belief by several investigators that the process of cleavage should be explainable in terms of mechanics. This belief is the outcome of the general argument that a change in the shape of a body can only result from the action of forces on the body.

Wolpert (1960) has summarised and reviewed all such theories which attempt to explain cleavage in terms of forces and their mechanics, whether the force be of external or internal origin. He has classified the various postulates in the following categories depending upon the type and origin of the forces considered responsible for the cleavage. These are theories involving or based upon (i) the forces associated with the mitotic apparatus of the cells. (ii) diffusion forces, (iii) forces generated due to growth and (iv) the forces to be associated with the cell surface.

To be considered successful, any theory that describes cell division must explain first the mechanism by which a contractile ring or furrow is formed and then provide a mechanics to demonstrate how the forces in the contractile ring eventually lead to the division of the cell, besides being compatible with the known physiology of a cell during its division.

Cell division consists of nuclear division (mitosis), division of the cytoplasm and of the membrane (cytokinesis). We are concerned mainly with cytokinesis in this paper rather than questions like mitosis and of contractile ring formation. Nevertheless, it will be proper to mention here that both mitosis and contractile ring formation have mechanical implication and need to be examined from the viewpoint of mechanics.

Several theories have been advanced to shed some light on the mechanisms of cytokinesis and they can be summarised as below: (a) polar expansion, (b) polar

relaxation, (c) equatorial constriction, (d) cytoplasmic streaming, (e) formation of new membrane.

Rappaport (1971) has given a detailed discussion of these hypotheses. The currently accepted hypothesis is that of active muscle-like contraction of the furrow base itself which is similar to equatorial constriction as stated above.

The cell membrane has a thickness of 7 nm and it covers the cytoplasm which has a water concentration between 70 and 85 %. The Young's modulus of the cell membrane of sea-urchin has been found to lie between 0.91×10^3 and 2.08×10^3 Pa when determined by the suction method. But the compression method estimates the Young's modulus of the cell membrane as 0.12×10^3 Pa. Some investigators claim that the membrane is not even elastic. The very concept of a constant Young's modulus in the case of cell membrane is irrelevant.

There are certain issues regarding cell division that still continue to be debated. One group of investigators, for example, holds the view that the surface tension in cell membrane remains uniform as the cell deforms (as such the cell membrane is not elastic). The other group holds the opinion that the surface tension increases as cell deformation increases and hence the elastic membrane concept is relevant. The other issue under debate is whether the volume enclosed by the cell membrane during cell division remains constant or not. Yet another issue to be resolved is whether the cell membrane is initially stretched (*i.e.* inflated) or lies loose and wrinkled. It is generally accepted by the researchers that no new membrane is formed during cell division, but that the old cell membrane simply extends. Resolution of these issues is vital from the viewpoint of mechanics.

3. Mechanics of cytokinesis

It is our intention to highlight here the studies of only those who have investigated this problem in terms of rigorous mechanics, and have advanced such models to demonstrate that the cell division is explicable in mechanistic terms. Five such studies are relevant, and the researchers are Doerner (1976), Greenspan (1977), Pujara & Lardner (1979), and Akkas (1980 and 1981).

Prior knowledge of the constitutive equations and the relevant material constants of the cell membrane, cytoplasm and the contractile ring formed during cytokinesis is necessary for analysis. These difficulties have been circumvented by different researchers in different ways.

3.1 Doerner's model

Doerner's model to describe cell division assumes that the spherical shape of a cell changes into the shape of a short central cylinder capped at the ends with ellipsoid as cleavage begins. He then uses shell equations of Timoshenko and Woinowsky-Krieger to analyse his model. The analysis leads Doerner to conclude that the strain pattern produced in his model consisting of a short central cylinder with ellipsoidal ends guides the cell to divide either into two spherical cells or to take the shape of a long thin cell which may remain a single cell or divide into two thin cells.

Doerner's model and its analysis are, in brief, as follows. The initial shape of the cell is spherical in shape, which then changes into a differentially short cylinder with ellipsoidal ends.

Change in radius of the central cylinder according to Timoshenko and Woinowsky-Krieger, is

$$S_1 = \frac{Ta^2}{2Eh} (2 - \nu), \quad (1)$$

where T = prime mover for the system (Pa), a = radius of cylinder (cm), E = modulus of elasticity of membrane (Pa), h = thickness of membrane (cm) and ν = Poisson's ratio. The change of radius of the ellipsoids at the junction becomes

$$S_2 = \frac{Ta^2}{2Eh} \left(2 - \nu - \frac{a^2}{b^2} \right), \quad (2)$$

where b represents the semi minor-axis of the ellipsoid. The difference between S_1 and S_2 relates to the discontinuity stresses at the junction. If the membrane thickness of the cylindrical and ellipsoidal portions of the cell equals the same amount, the moment vanishes at the junction. The shearing force, Q_0 eliminates the interface discontinuity.

$$Q_0 = \frac{T(ah)^{1/2}}{8[3(1 - \nu)^2]^{1/4}} \frac{a^2}{b^2}. \quad (3)$$

The shearing force Q_0 acting at the furrowing region acts upon the cell to effect a change of shape. Doerner's model explains, as stated above, how a spherical cell may divide into two spherical cells, or produce a single new needle cell which may or may not progress to divide into two needle cells.

Doerner's model has many inherent problems—the most obvious being the fact that it is an elastic model based on the postulates of infinitesimal elasticity.

3.2 Greenspan's model

Greenspan (1977) has advanced a completely fluid model to describe cleavage and cell division and has explained the phenomena in terms of mechanics. It is a general observation that almost uniform tension exists on the surface of the cell before the onset of cytokinesis. Greenspan hypothesis states that the contraction of the local tension elements in a cell generates the surface tension which in turn leads to an effective surface tension within the boundary of the cell. The tension elements may be considered to be representative of the filaments of the cytoplasmic matrix or some similar surface modulating mechanism which contributes to the surface tension of a cell. The tension elements are assumed to be uniformly distributed before the onset of cleavage, thus maintaining a uniform surface tension which is counterbalanced by the internal pressure. This equilibrium can be disturbed by some internal biochemical activity, presumably by changing the surface concentration of the tension elements and can lead to a differential in equatorial and polar tension on the cell surface. Once such a differential is created, then, as has been demonstrated by Greenspan (1977) through the analysis of his fluid model based on the principles of mechanics, it results in a dynamic instability of the spherical fluid model which spontaneously leads towards cleavage. Greenspan's model thus characterises cleavage as a phenomenon in fluid motion induced by variable surface tension.

The initial state of the cell, according to Greenspan, is of static equilibrium, a quiescent fluid-filled sphere in which a constant pressure exactly balances the radially directed force of uniform surface tension. Equilibrium is disturbed when the surface

tension is altered by a change in the concentration of tension elements. The difference in tension draws the surface toward the equator and the resultant stress sets the protoplasm streaming. This motion acts to concentrate the tension elements, thereby increasing the contractile forces further, which in a self-perpetuating manner cause still greater motion, displacements and distortions. Greenspan's analysis assumes that the cell volume is ensured. He treats the cell as a very viscous (10–100 poise), homogenous fluid with uniformly distributed tension elements over the cell surface before the onset of cleavage. Greenspan's model is thus a completely fluid model.

The governing equations as formulated by Greenspan are as follows:

$$\nabla^2 p = 0, \quad (4)$$

$$\alpha \sigma \frac{\partial u}{\partial t} = -\frac{\partial p}{\partial r} + \frac{1}{r} \nabla^2 r u, \quad (5)$$

and the boundary conditions on $r = 1$

$$p - 2 \frac{\partial u}{\partial r} = - \left[\frac{\partial}{\partial \eta} (1 - \eta^2) - \frac{\partial \xi}{\partial \eta} + 2\xi \right] + 2c, \quad (6)$$

$$-\frac{\partial}{\partial r} \frac{1}{r^2} \frac{\partial}{\partial r} (r^2 u) + \frac{\partial}{\partial \eta} (1 - \eta^2) \frac{\partial u}{\partial \eta} = \frac{\partial}{\partial \eta} (1 - \eta^2) \frac{\partial c}{\partial \eta}, \quad (7)$$

$$u = \alpha \frac{\partial \xi}{\partial t}, \quad (8)$$

$$\frac{\partial c}{\partial t} - \frac{\partial u}{\partial r} = \chi \frac{\partial}{\partial \eta} (1 - \eta^2) \frac{\partial c}{\partial \eta}, \quad (9)$$

where ξ measures the amount of distortion and p , fluid pressure, u , v , radial and latitudinal velocity components, η , $\cos \theta$, α , σ , χ , are certain material parameters and c is concentration of tension elements.

The solution of this boundary value problem has been obtained in terms of Legendre polynomials. It so turns out in the solution that all modes are unstable and the growth rate increases with higher mode indices. Since the initial amplitude of the second mode must be very much larger than that of any other mode, the growth of a more unstable pattern can be offset for a period of time that possibly extends beyond the range of validity of the linear theory. Thus the characteristics of the instability and the initial form of the particular initial conditions combine to make the second mode predominate in the division process. Greenspan's cleavage mechanism can be sufficient only if the cell membrane has very fluid characteristics.

3.3 Pujara & Lardner's work

Pujara & Lardner (1979) have modelled a cell as a nonlinear elastic spherical membrane containing an incompressible fluid. They do not look into the question of initial formation of the contractile ring and also ignore the influence of the constricting furrow in their model analysis. They assume the internal fluid pressure in a cell and its surrounding external pressure to be equal before the onset of cleavage, thus giving a tension-free state of the undeformed cell membrane. Their analysis consists in determining the deformation of an initially spherical membrane containing an incompressible fluid for a prescribed deformation at the equator.

Pujara & Lardner model the sea urchin egg as a thin-walled spherical nonlinear elastic membrane enclosing an incompressible fluid. They assume that no tension exists in the membrane before the onset of cleavage. They also assume that the viscous stress at the fluid-membrane interface during deformation is negligible as compared to the pressure of the interior fluid. They then apply a prescribed displacement at the equator, subject to the condition that the enclosed volume is constant, and determine the deformation of the spherical cell membrane.

The following constitutive equations derived for an inflated spherical nonlinear membrane have been employed by Pujara & Lardner:

$$\lambda'_1 = \left(\frac{\delta \cos \Psi - \omega \sin \Psi}{\sin^2 \Psi} \right) (f_2/f_1) - \frac{\omega}{\delta} (f_3/f_1); \quad \delta' = \omega, \quad (10)$$

$$\omega' = \frac{\lambda'_1 \omega}{\lambda_1} + \frac{\lambda_1^2 - \omega^2}{\delta} (T_2/T_1) - \frac{\lambda_1 (\lambda_1^2 - \omega^2)^{1/2} P_n r_0}{T_1}, \quad (11)$$

where $f_1 = \frac{\partial T_1}{\partial \lambda_1}$; $f_2 = \frac{\partial T_1}{\partial \lambda_2}$; $f_3 = T_1 - T_2$,

and where primes indicate differentiation with respect to the angular position on the undeformed sphere: T_1, T_2 are the stress resultants, λ_1, λ_2 are the principal stretch ratios, $\delta = \lambda_2 \sin \Psi$, P_n is the normal external pressure and r_0 is the radius before inflation. The boundary conditions are:

$$\lambda_1 = \lambda_2 = \lambda_0; \quad \eta' = 0 \text{ at } \Psi = 0,$$

and $\lambda_2 = \hat{\lambda}_2$ at $\Psi = \frac{\pi}{2}$,

where $\hat{\lambda}_2$ is the specified value of the circumferential stretch ratio at the equator that determines the state of division.

The constant volume condition is written as

$$3 \int_0^{\pi/2} \delta^2 (\lambda_1^2 - \omega^2)^{1/2} d\Psi = 2. \quad (12)$$

To determine the values of λ_0 and P_n for a prescribed value of λ_2 constitutive equations are integrated iteratively until the constant volume condition is satisfied.

Pujara & Lardner have chosen the strain energy function as proposed by Mooney-Rivlin and Skalak *et al.* The two-dimensional constitutive equations for a Mooney-Rivlin material are

$$T_1 = 2hC_1 \left(\frac{\lambda_1}{\lambda_2} - \frac{1}{\lambda_1^3 \lambda_2^3} \right) (1 + \Gamma \lambda_2^2), \quad (13)$$

$$T_2 = 2hC_1 \left(\frac{\lambda_2}{\lambda_1} - \frac{1}{\lambda_1^3 \lambda_2^3} \right) (1 + \Gamma \lambda_1^2), \quad (14)$$

where C_1 and Γ are material constants and h is the initial thickness of the membrane.

For strc material the constitutive equations are

$$T_1 = \frac{C}{2} \frac{\lambda_1}{\lambda_2} [\Gamma (\lambda_1^2 - 1) + f(\Psi) \lambda_2^2 (\lambda_1^2 \lambda_2^2 - 1)], \quad (15)$$

$$T_2 = \frac{C}{2} \frac{\lambda_2}{\lambda_1} [\Gamma(\lambda_2^2 - 1) + f(\Psi) \lambda_1^2 (\lambda_1^2 \lambda_2^2 - 1)], \quad (16)$$

where C and Γ are material constants; and where the function

$$f(\Psi) = 1 - \frac{1}{\pi} \left[\tan^{-1} (\Psi - \Psi_0) + \frac{\pi}{2} \right] \quad (17)$$

takes care of the initial wrinkled region of the membrane. Ψ_0 in equation (17) corresponds to an angular position on the undeformed sphere.

Calculations as made by Pujara & Lardner agree well with the experimental results and thus provide additional support for the contractile ring theory of cell division. However, they have not taken into account the dynamics of the driving mechanism of active contraction of the contractile ring, and thus they call their model "passive".

3.4 Model of Akkas

The model proposed by Akkas (1980) is basically the same as that of Pujara & Lardner (1979), but Akkas, in contrast to the assumptions used by Pujara & Lardner (1970), considers the initial surface tension of the cell as observed by Hiramoto (1970). Pujara & Lardner (1979) have represented the cell membrane by the Mooney-Rivlin material and also by the Skalak, Tozeren, Zarda and Chien material (srzc material); whereas Akkas (1980) has used only the Mooney-Rivlin material for the model analysis. He also relaxed the condition of constancy of cell volume during its division, as found in the analysis presented by Pujara & Lardner (1979).

The set of equilibrium equations for the model proposed by Akkas are

$$\frac{d}{d\phi} (\bar{N}_\phi \bar{r} \sin \Psi) - \bar{p} \bar{r} \bar{F}_1 \lambda_\phi \lambda_\theta \cos \Psi = 0, \quad (18)$$

and

$$\frac{d}{d\phi} (\bar{N}_\phi \bar{r} \cos \Psi) - \bar{N}_\theta \bar{r}_1 + \bar{p} \bar{r} \bar{F}_1 \lambda_\phi \lambda_\theta \sin \Psi = 0, \quad (19)$$

where $\bar{N}_\phi$ and $\bar{N}_\theta$ are the membrane forces per unit length of the undeformed reference configuration, λ_ϕ and λ_θ are the stretch ratios in the meridional and circumferential direction and $\bar{r}, \bar{r}_1, \phi$ described the undeformed configuration.

The compatibility condition gives an additional relation among the variable

$$\frac{d\lambda_\theta}{d\phi} + \frac{\bar{r}_1}{r} \lambda_\theta \cos \phi - \frac{\bar{r}_1}{r} \lambda_\phi \cos \Psi = 0. \quad (20)$$

The constitutive relations for the Mooney-Rivlin material are

$$\bar{N}_\theta = (2hC_1/\lambda_\theta)(\lambda_\theta^2 - \lambda_\theta^{-2} \lambda_\phi^{-2})(1 + \alpha \lambda_\phi^2), \quad (21)$$

$$\bar{N}_\phi = (2hC_1/\lambda_\phi)(\lambda_\phi^2 - \lambda_\phi^{-2} \lambda_\theta^{-2})(1 + \alpha \lambda_\theta^2). \quad (22)$$

Akkas uses Runge-Kutta method to solve the equilibrium equation and compares his numerical solutions with some of the experimental evidence existing in literature. However, the investigator admits that various aspects of his proposed model need to be supported with experimental evidence. For instance, will the, say, 80 or 90% cleaved cell return to its initial configuration when its furrow ring is destroyed?

Akkas (1981) extended his earlier analysis by modifying his above mentioned hyperelastic model into a nonlinear viscoelastic model. In his viscoelastic model, Akkas (1981) has combined the features of Mooney-Rivlin model of nonlinear elasticity and the standard solid of linear viscoelasticity.

Akkas (1981) employs a simplified form of Winerman's (1972) stress-stretch ratio history expression as the constitutive equation given below

$$\bar{N}_\phi = 2\lambda_\phi^{-1}(\lambda_\phi^2 - \lambda_\theta^{-2}\lambda_\phi^{-2})\{1 - (1 - \gamma)(1 - \exp(-t/t_R))\}, \quad (23)$$

where $\bar{N}_\phi$, λ_ϕ and λ_θ have the same meaning as in (18). $\gamma = C_\infty/C_1$, where C_1 is the initial modulus of the Mooney-Rivlin material and C_∞ is the residual modulus. The constant t_R represents the relaxation time in the linearised theory. The simplified constitutive equation (23) assumes that (i) the relaxation time is not influenced by the amount of stretching and (ii) the material does not contain memory of the strain history at earlier times. The modified viscoelastic model, however, does not provide any extra information which is different from the previous elastic model (Akkas, 1981).

3.5 Discussion

Greenspan (1977), Pujara & Lardner (1979), and Akkas (1980 and 1981), all have modelled the cell as a spherical membrane and have analysed the phenomenon of cytokinesis in the cells as a problem in the instability of the hyperelastic or viscoelastic spherical membranes. Crisp & Hart-Smith (1971) have provided the general stability criteria for arbitrary membranes, not necessarily spherical. Besides, the phenomena of instability of multilobed inflated membranes as advanced by Crisp & Hart-Smith (1971) appear to be highly relevant in understanding the mechanics of cell division during the intermediate stages of cytokinesis when the shape is more like a two-lobed spherical membrane. The instability of any multilobed inflatable membraneous structure can be described as follows (see Crisp & Hart-Smith 1971): (i) a negative pressure-volume gradient is a critical condition for the instability to occur; and (ii) the second condition usually involves critical volume enclosed within the entire system.

The researchers have thus no doubt provided additional support to a general belief that cell division can be understood in terms of mechanics. It appears from the models and their analysis as advanced by the mechanists that it only requires some process just to trigger the onset of cleavage and the rest of the process of cell division is achieved according to the principles of mechanics.

4. Conclusion

The mechanics of cell division as examined and discussed in this paper leads to an important conclusion: that the morphogenesis of a growing organism may well be a consequence of the stresses induced in the organism as a result of its growth. The consequences of such a finding are far-reaching and profound; and it opens up a whole new field of Morphomechanics.

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