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A mechanism for the establishment of polar cell morphology based on the cytoskeleton-derived forces exerted on the cell boundary

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Abstract A mechanism for the establishment of polar cell morphology is presented, based on the internal forces that the cytoskeletal structures exert on the cell boundary. Cell shapes are determined by postulating that they correspond to the minimum of the total energy of the system, which is the sum of the bending energy of the cell boundary and the potential energies of the involved forces. Axisymmetrical cell shapes are considered, and it is assumed that the cytoskeletal structures exert an extensional axial force and are involved in controlling the area of the cell boundary. The dependence of cell shapes on the axial force is presented for different values of this area. The results show that, at increasing axial force, the cell undergoes a discontinuous transition from an oval shape, exhibiting an equatorial mirror symmetry into a polar shape. The proposed mechanism is related to previously documented specific effects of microtubule- and actin-modifying drugs on polar shapes of developing isolated retinal photoreceptor cells.

Discussion

Many unicellular organisms and isolated cells of multicellular organisms have polar morphology, i.e., their shapes exhibit a polar axis such that the curvature of the cell boundary, as it is resolved by an optical microscope, varies along the direction of this axis in an asymmetrical manner. Typical examples include the prokaryote *Caulobacter crescentus* (Shapiro 1993) and the zygote of brown alga (e.g. *Fucus*) (Brawley and Robinson 1985), which both exhibit the shape resembling a pear, the yeast *Saccharomyces cerevisiae*, with its budding stage consisting of two connected unequal spheres (Chant 1994), and cultured photoreceptor cells which are characterized by an extended shape composed essentially of two unequal oval widenings connected by a lengthy neck (Madreperla and Adler 1989). All these cells also exhibit polar distribution of their constituents and thus, as is commonly regarded, exhibit cell polarity (Drubin and Nelson 1996). There seems to be a correlation between polar cell morphology and polar distribution of cell constituents: before fertilization the fucoid egg has a spherical shape and the distribution of its cytoplasmic content is radially symmetrical; fertilization triggers a series of processes which lead to polar distribution of cell constituents as well as to polar morphology (Goodner and Quatrano 1993). Similarly, it was shown in cultured retinal photoreceptors that when they are round the distribution of investigated membrane constituents is uniform, whereas in the developmentally later stages where the morphology is polar, it is asymmetrical (Madreperla et al. 1989).

The polar cell morphology can be considered as an accompanying property of cell polarity. However, a hypothesis can also be put forward that it plays an essential role in the mechanism by which the cell polarity is established, in the sense that the polar distributions of cell constituents arise as the consequence of polar cell morphology. The basis for this hypothesis is an assertion that when a cell or a vesicle has a polar morphology and thus membrane curvature varies over its surface in a polar manner, any curvature-dependent interaction between membrane constituents causes their lateral density to vary correspondingly (Seifert 1993; Kralj-Iglič et al. 1996 and references therein). It is plausible to assume that membrane proteins could have acquired the structural characteristics needed for curvature-dependent interactions. For the sake of verifying the above hypothesis, it is then of interest to investigate any possible mechanism by which polar cell morphology can be established. One such mechanism may arise owing to a general property of vesicular membraneous objects such as phospholipid vesicles that some of their equilibrium shapes are polar, as was predicted theoretically (Deuling and Helfrich 1976; Svetina and Žekš 1989, 1990;

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Seifert et al. 1991) and proven experimentally (Berndl et al. 1990; Käs and Sackmann 1991; Farge and Devaux 1992; Käs et al. 1993; Döbereiner et al. 1997). Examples of predicted polar shapes include pear shapes, cup shapes, and shapes composed of two spheres of different radii connected by an infinitesimally small neck. Close similarity of these shapes to those observed in different cells with polar morphology suggests that similar principles which govern the shape behavior of vesicular membraneous objects may also be involved in governing the shapes of cells (Svetina and Žekš 1990) and possibly of other lamellar closed objects (Svetina and Žekš 1991). However, most cells involve cytoskeletal structures which exert forces on their boundary and thus affect their shape behavior. It is therefore of interest to extend the studies of the shape behavior of vesicular membraneous objects by including the effects of the cytoskeleton.

In this work we present an example of a simple mechanism by which polar cell morphology is established as a consequence of the cytoskeleton-derived forces exerted on the cell boundary. There are three motives for this study:

1. The strong effect of the cytoskeleton on shapes of vesicular membraneous objects is evidenced by the effects of encapsulated microtubules and microtubule bundles (Hotani and Miyamoto 1990; Kuchnir Fygenson et al. 1997) or thin actin shells (Häckl et al. 1998) on shapes of phospholipid vesicles.
2. The results of a recent systematic analysis of the effect of axial forces on the axisymmetrical shapes of closed lamellar membranes show that the occurrence of polar vesicle shapes in such systems is quite common (Heinrich et al. 1998).
3. Studies on cultured retinal photoreceptor cells showed that cell shapes, and in particular the development and maintenance of cell shape polarity, are governed by the cytoskeleton, in particular by the oppositely directed microtubule- and actin-dependent forces (Madreperla and Adler 1989).

In the following we shall first briefly outline the principles by which the shapes of flaccid closed membraneous objects were determined for phospholipid membranes and for cells without internal structures like erythrocytes, and the basic generalizations needed for taking into account some specific features of the cell boundaries. Then a simple model is introduced by which we can show the effect of a cytoskeleton-derived axial force on the symmetry of the cell morphology. Finally, the results are presented, in qualitative terms related to the shape behavior of cultured photoreceptor cells.

The shapes of vesicular membraneous structures such as phospholipid vesicles and erythrocytes are predicted theoretically by assuming that they correspond to the minimum of the elastic energy of the membrane (reviewed in Svetina and Žekš 1996; Seifert 1997). The minimization is performed under the constraint of constant vesicle or cell volume, and because the membranes in these systems can be considered to be smooth and unextendable, also at constant membrane area. Cellular plasma membranes are in general ruffled and folded, i.e., they exhibit invaginations or evaginations, take part in membrane protrusions such as microvilli, or have other reservoirs of membrane lipids. Therefore the membrane projected area, which we identify with the average contour of the cell boundary visible by an optical microscope, is smaller than would be the area of the smoothly extended plasma membrane. We approximate the energy state of a ruffled membrane by assuming that the membrane is distributed between two compartments, the first comprising the part of the membrane aligned along the projected area and exhibiting the corresponding bending energy, and the second comprising its other parts which are characterized by a different value of the membrane energy per unit area (Svetina et al. 1997). The difference between the membrane energies per unit area for the two compartments contributes to the membrane tension within the projected area. Furthermore, we assume that the projected area is determined by the area of the membrane cortex, i.e., a thin layer of cytoplasmic material particularly rich in actin and myosin microfilaments. We take into account that the cortex is laterally homogeneous. It has been demonstrated, for example, for blood granulocytes (Evans and Yeung 1989) and resting neutrophils (Needham and Hochmuth 1992; Tsai et al. 1994; Zhelev et al. 1995), that the membrane cortex exhibits specific viscoelastic properties such as lateral tension, area expansivity, elasticity, and bending energy, and that its mechanical state also depends on the cell activity. For the sake of demonstrating possible shape symmetries of cells with the described properties of the cell boundary, it is sufficient to determine the shapes for given values of the projected area because the energy contributions due to membrane and cortex tension, area expansivity, and possibly also the cell activity depend on the area and do not affect the symmetry of the shape at a given area. Thus, for the purposes of this work, it is not necessary to specify the mechanical properties of the cortex in detail. However, it has to be kept in mind that the area-dependent contributions to the energy of the cell boundary affect the shape trajectories in the shape/force phase diagram, and that, in general, the projected area is changing along these trajectories. We also assume that the cell volume (V) is well regulated and that it can therefore be assumed to be constant. The variable on which the shapes depend is then only the projected area, which can be conveniently measured in terms of the relative projected area, i.e., as $a = A/A_0$ where $A_0 = 4\pi R_0^2$ is the area of the sphere with the radius R_0 and the volume $V = 4\pi R_0^3/3$.

In order to demonstrate that the polar cell morphology can be the consequence of the effect of the cytoskeleton-derived forces acting on the cell boundary, we propose a simple model which can be analyzed by applying strict mathematical methods. Only axisymmetrical vesicular objects are considered. It is assumed that there is a cytoskeleton-derived axial force pushing apart the cell poles. The detailed mechanisms by which the cytoskeleton exerts this force are not essential to the model. The effect of the axial force is treated by the methods developed and comprehensively described previously (Božič et al. 1997). The ef-

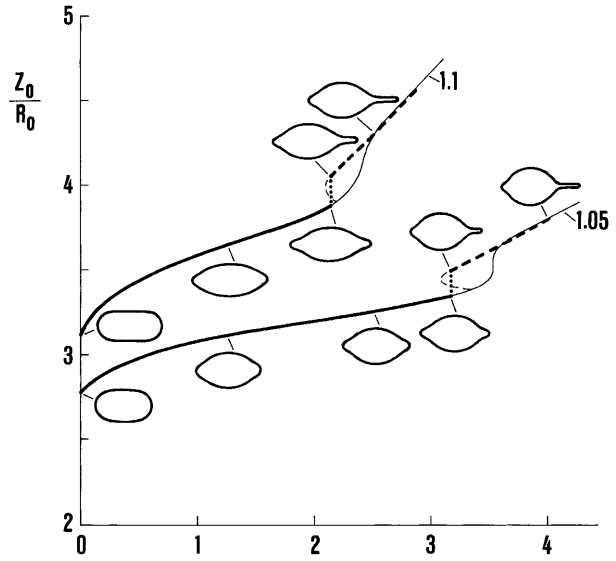

Fig. 1 The dependence of the axial length (Z_0) in units of R_0 on the axial force scaled in relative units as $f = FZ_0/8\pi k_c$ for two values of the relative projected area of the cell boundary (a = 1.05 and 1.10). *Wide sections* of the corresponding lines denote stable and *thin sections* unstable shapes. *Full lines* denote symmetrical shapes, i.e., shapes exhibiting equatorial mirror symmetry, and *dashed lines*, asymmetrical, i.e., polar shapes. The *dotted vertical lines* denote forces where the discontinuous shape transformations from symmetrical to asymmetrical shapes occur. Some examples of shapes are also presented

fects of the area difference elasticity (Svetina and Žekš 1996; Seifert 1997) are not taken into consideration because it can be concluded from the results of the previous analysis (Heinrich et al. 1998) that for reasonable values of the relevant elastic parameters they do not affect the conclusion obtained. For the same reason, the spontaneous curvature (Helfrich 1973) is also taken to be zero, and the assumption is made that the shear modulus of the cell boundary can be neglected. The shapes can then be determined by minimizing the energy functional which, in addition to the bending energy of the cell boundary (Canham 1970), $W_b = \frac{1}{2} k_c \int (c_1+c_2)^2 \, dA$, with k_c the bending modulus, c_1 and c_2 the two principal curvatures, and the integration performed over the whole area of the cell surface A, involves the potential energy of the axial force FZ_0, with F the force and Z_0 the cell axial length.

The predicted dependence of cell axial lengths on the force is presented in Fig. 1 for two values of the relative projected area a. Some representative examples of cell shapes are also shown. At a given area the increase of the force causes an increase of the axial length. It can be observed in both examples (a = 1.05 and a = 1.10) that in the treated region of relative areas there is a first-order transition exhibiting a discontinuous shape transformation, from the shape with the equatorial mirror symmetry to a polar shape. The polar shapes predicted have a characteristic lemon-like part, and on one side only, there is a tubular extension with an almost spherical ending of a radius which is slightly larger than the radius of the tube. It can be seen in Fig. 1 that the force at which the transition from mirror symmetry to polar shape occurs is larger at smaller areas. It can also be deduced from the results presented in Fig. 1 that there is a transition from the shape with the equatorial mirror symmetry to the polar shape when the area is increased at constant force. For instance, at the force f = 2.5 this transition occurs at the relative area which is larger than 1.05 and smaller than 1.10.

The shape behavior of the described model can be qualitatively compared to the shape behavior of cultured retinal photoreceptors (Madreperla and Adler 1989). Originally round cells, during in vitro development they undergo a sequence of morphogenetic transformations, including elongation and compartmentalization of the cell body. Madreperla and Adler (1989) showed that the complex developmental transformations leading to photoreceptor polarization are predominantly controlled by intracellular cytoskeletal forces. The cell-extending force could be attributed to microtubules. The evidence for this was that, when treated with the microtubule-depolymerizing agent nocodazole, the elongated photoreceptors became progressively shorter, eventually losing their compartmentalized structure and becoming round. Conversely, treatment of the actin-depolymerizing agent cytochalasin D caused the elongated photoreceptors to lengthen even further. The described gross behavior of photoreceptor cells can be compared to the behavior of the presented model as depicted in Fig. 1, if it is assumed that the axial force is due to microtubule-derived forces and that actin-derived forces control the area of the cell surface in the sense that an enhanced actin activity causes the area to decrease. Let us consider that our model cell is in the region of polar shapes in Fig. 1. Then, by applying nocodazole which disrupts the microtubules, the axial force would diminish, which would drive the system into the region of oval shapes involving an equatorial mirror symmetry. By applying cytochalasin D which disrupts actin filaments, the relative area at these conditions would increase, causing an increase of the cell axial length.

The main conclusion of this work is that the mechanism for the establishment of polar cell morphology can be based on the macroscopic mechanical properties of cell boundaries, and in turn on the cytoskeleton-derived forces exerted on the latter. In the particular model analyzed, the mechanism involved a cytoskeletal structure which exerts an axial extensional force. A possible applicability of the above conclusion is supported by the qualitative similarity between the behavior of the proposed model and the behavior of cultured retinal photoreceptor cells (Madreperla and Adler 1989), in which the cytoskeletal forces acting on the cell boundary were demonstrated experimentally. A particularly relevant property revealed by the treated model is that, depending on the strength of the cytoskeletal axial force, the shape makes a transformation from a higher to a lower, polar symmetry. The structural parameters on which this transformation depends are, in general, the elastic properties of the cell boundary and of the cell constituents exerting forces on it. For instance, the important macroscopic parameter in the presented model is the ratio between the axial force and the bending modulus of

the cell boundary. In general, the analysis of the system's behavior at the macroscopic level as presented here reveals the relevant macroscopic parameters and also gives the conditions under which the polar cell shapes can be established and maintained. It must be understood that the same macroscopic properties may be realized by different chemical and structural means, and that this may add to the variety of the molecular bases causing the arising of polar cell morphologies. It still has to be proven whether polar cell morphology also represents a sufficient basis for the establishment of cell polarity. The suggested hypothesis for the establishment of cell polarity implies that in unraveling its molecular and biochemical basis it might be worthwhile to pay attention to the curvature-dependent properties of those involved cell constituents which also interact with the cell boundary.

References

Berndl K, Käs J, Lipowsky R, Sackmann E, Seifert U (1990) Shape transformations of giant vesicles: extreme sensitivity to bilayer asymmetry. Europhys Lett 13: 659–664

Božič B, Svetina S, Žekš B (1997) Theoretical analysis of the formation of membrane microtubes on axially strained vesicles. Phys Rev E 55: 5834–5842

Brawley SH, Robinson KR (1985) Cytochalasin treatment disrupts the endogeneous currents associated with cell polarization in fucoid zygotes: studies of the role of F-actin in embryogenesis. J Cell Biol 100: 1173–1184

Canham PB (1970) The minimum energy of bending as a possible explanation of the biconcave shape of the human red blood cell. J Theor Biol 26: 61–81

Chant J (1994) Cell polarity in yeast. Trends Genet 10: 328–333

Deuling HL, Helfrich W (1976) The curvature elasticity of fluid membranes: a catalogue of vesicle shapes. J Phys (Paris) 37: 1335–1345

Döbereiner H-G, Evans E, Kraus M, Seifert U, Wortis M (1997) Mapping vesicle shapes into the phase diagram: a comparison of experiment and theory. Phys Rev E 55: 4458–4474

Drubin DG, Nelson WJ (1996) Origins of cell polarity. Cell 84: 335–344

Evans E, Yeung A (1989) Apparent viscosity and cortical tension of blood granulocytes determined by micropipet aspiration. Biophys J 56: 151–160

Farge E, Devaux PF (1992) Shape changes of giant liposomes induced by an asymmetric transmembrane distribution of phospholipids. Biophys J 61: 347–357

Goodner B, Quatrano RS (1993) *Fucus* embryogenesis: a model to study the establishment of polarity. Plant Cell 5: 1471–1481

Häckl W, Bärmann M, Sackmann E (1998) Shape changes of self-assembled actin bilayer composite membranes. Phys Rev Lett 80: 1786–1789

Heinrich V, Božič B, Svetina S, Žekš B (1998) Vesicle deformation by an external load: from axially elongated shapes to tethered vesicles. Biophys J (in press)

Helfrich W (1973) Elastic properties of lipid bilayers: theory and possible experiments. Z Naturforsch 28c: 693–703

Hotani H, Miyamoto H (1990) Dynamic features of microtubules as visualized by dark-field microscopy. Adv Biophys 26: 135–156

Käs J, Sackmann E (1991) Shape transitions and shape stability of giant phospholipid vesicles in pure water induced by area-to-volume changes. Biophys J 60: 825–844

Käs J, Sackmann E, Podgornik R, Svetina S, Žekš B (1993) Thermally induced budding of phospholipid vesicles – a discontinuous process. J Phys II 3: 631–645

Kralj-Iglič V, Svetina S, Žekš B (1996) Shapes of bilayer vesicles with membrane embedded molecules. Eur Biophys J 24: 311–321

Kuchnir Fygenson D, Marko JF, Libchaber A (1997) Mechanisms of microtubule-based membrane extension. Phys Rev Lett 79: 4497–4500

Madreperla SA, Adler R (1989) Opposing microtubule- and actin-dependent forces in the development and maintenance of structural polarity in retinal photoreceptors. Dev Biol 131: 149–160

Madreperla SA, Edidin M, Adler R (1989) Na^+,K^+-adenosine triphosphate polarity in retinal photoreceptors: a role for cytoskeletal attachments. J Cell Biol 109: 1483–1493

Needham D, Hochmuth RM (1992) A sensitive measure of surface stress in the resting neutrophil. Biophys J 61: 1664–1670

Seifert U (1993) Curvature induced lateral phase segregation in two component vesicles. Phys Rev Lett 70: 1335–1338

Seifert U (1997) Configurations of fluid membranes and vesicles. Adv Phys 46: 13–137

Seifert U, Berndl K, Lipowsky R (1991) Shape transformations of vesicles: phase diagram for spontaneous curvature and bilayer coupling models. Phys Rev A 44: 1182–1202

Shapiro L (1993) Protein localization and asymmetry in the bacterial cell. Cell 73: 841–855

Svetina S, Žekš B (1989) Membrane bending energy and shape determination of phospholipid vesicles and red blood cells. Eur Biophys J 17: 101–111

Svetina S, Žekš B (1990) The mechanical behaviour of cell membranes as a possible physical origin of cell polarity. J Theor Biol 146: 116–122

Svetina S, Žekš B (1991) Mechanical behavior of closed lamellar membranes as a possible common mechanism for the establishment of developmental shapes. Int J Dev Biol 35: 359–365

Svetina S, Žekš B (1996) Elastic properties of closed bilayer membranes and the shapes of giant phospholipid vesicles. In: Lasic DD, Barenholz Y (eds) Handbook of nonmedical applications of liposomes, vol 1. CRC Press, Boca Raton, pp 13–42

Svetina S, Božič B, Žekš B (1997) Shape behavior of closed layered membranes and cytokinesis. In: Alt W, Deutsch A, Dunn G (eds) Dynamics of cell and tissue motion. Birkhäuser, Basel, pp 67–71

Tsai MA, Frank RS, Waugh RE (1994) Passive mechanical behavior of human neutrophils: effect of cytochalasin B. Biophys J 66: 2166–2172

Zhelev DV, Needham D, Hochmuth RM (1995) Role of the membrane cortex in neutrophil deformation in small pipets. Biophys J 67: 696–705