Radiol Oncol 1998; 32(1): 119–124

On mechanisms of cell plasma membrane vesiculation

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Vesiculation of the cell membrane is studied. It is proposed that the shape of the membrane segment free of the cytoskeleton is driven towards the shape of its maximal possible difference between the two membrane layer areas by the rearrangement of the laterally mobile membrane constituents. It is shown that the shapes corresponding to the extrema of the area difference can be spherical, planar and cylindrical, depending on the enforced constraints. Correspondingly, the spherical vesicles and the cylindrical protrusions observed in vesiculating MCF7 cancer (human breast adenocarcinoma) cells are shown. The proposed mechanism of vesiculation also provides an explanation for different relative content of some substances in the membrane of the released vesicles than in the membrane of the residual cells.

Key words: budding; membrane bilayer; vesiculation

Introduction

Membranes of some cells can form during the budding process small protrusions which are eventually released from the membrane as vesicles. The amount of the involved membrane varies from relatively large portions with or without enclosed elements of the cytoplasmic material to very small fragments filled only with the cytosol. It is a common feature that the disruption of the cytoskeleton or its detachment from the membrane bilayer occurs prior to vesiculation. It was also observed that the membrane of the released vesicles differs from the membrane of the residual cell in the relative content of some membrane constituents, indicating that the rearrangement of the membrane constituents occurs during the budding. The vesiculation is therefore a mechanism through which the cell membrane looses some substances. In cancer cells the budding and vesiculation process occurs spontaneously thereby causing a continuous loss of some important substances from the cell membrane and leading to the alteration of the cell function.[1,2,3]

A system that is due to its simplicity convenient to study the general features of the membrane budding and vesiculation is the

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mammalian erythrocyte. In erythrocytes, it was found that a wide variety of treatments and conditions such as incubation with dimyristoyl phosphatidyl choline vesicles,[4,5] incubation with various amphiphiles,[6,7] ATP depletion[8] and extreme pH in the suspension[9,10] may influence the vesiculation process. As the features observed in vesiculation of the erythrocytes are relevant also in general[11], there is a possibility to manipulate the cancer cells in such a way as to stabilize the membrane and prevent the loss of the important membrane constituents from the membrane. It is therefore of interest to understand the mechanisms taking place in membrane budding and vesiculation. In this work we focus on the features involving the membrane segments that are already detached from the cytoskeleton.

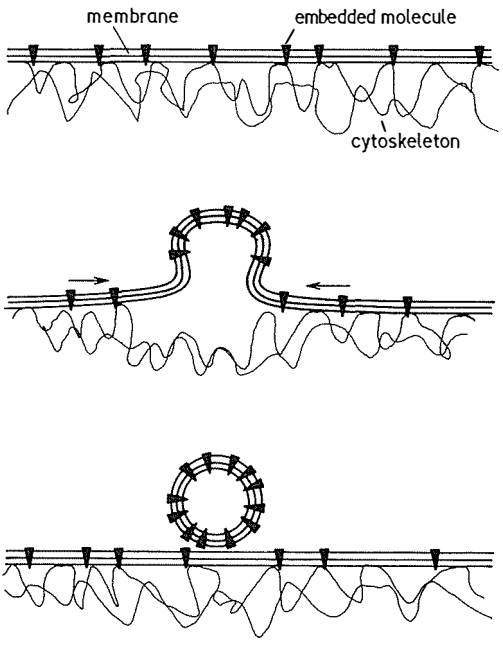

Figure 1. A scheme of the proposed mechanism of the budding process. The membrane with embedded molecules that favour large membrane curvature is shown. First, the membrane becomes locally detached from the cytoskeleton thereby increasing the pool of laterally mobile embedded molecules. The membrane segment free of the cytoskeleton then forms a protrusion as this is energetically favourable. The laterally mobile membrane embedded molecules accumulate in the region of favourable curvature so that the area density of the number of the embedded molecules is higher in the membrane of the vesicles than in the membrane of the residual cell.

Material and methods

The proposed mechanism of budding and vesiculation

The proposed mechanism is schematically represented in Figure 1.

In the description of the membrane segment the membrane is taken to be a two dimensional liquid composed of phospholipid molecules, in which various other molecules such as the membrane proteins are embedded. The embedded molecules are more or less free to move laterally over the membrane surface. The connections of the membrane with the intracellular and the extracellular matrix are of importance, since they may impose limits and obstacles for the lateral motion of the membrane constituents. The disruption or detachment of the cytoskeleton from the membrane thus increases the pool of the laterally mobile molecules.

It is proposed that following the detachment of the cytoskeleton, the development of the bud can be described as a local event involving the cytoskeleton free membrane segment. Further, it is proposed that the exchange of the laterally mobile membrane constituents between the membrane segment and the membrane of the residual cell provides the driving mechanism for the bud development, ending with the formation of the vesicle.

As the cytoskeleton free membrane segment is very small comparing to the membrane of the residual cell, the membrane of the residual cell can be treated as a reservoir for the laterally mobile membrane con-

stituents. The energy of the embedded molecule at a given site in the membrane depends on the local membrane curvature,[12] so that the laterally mobile molecules tend to accumulate in the regions at which the membrane curvature is energetically more favourable while the membrane segment attains the shape with larger regions of the favourable curvature.[12] As a result, a protrusion of the membrane exhibiting higher curvature than the residual cell is formed and the laterally mobile membrane constituents that favour large curvature accumulate in the protrusion.

In turn, the molecule that favours large curvature may be expected to occupy larger portion of the outer membrane layer than the inner one. A presence of such a molecule in the membrane segment increases the area of the outer layer of the segment with respect to the area of the inner one. If the cytoskeleton free membrane segment forms a bud, the laterally mobile molecules that favour large membrane curvature flow to the bud from the membrane of the residual cell and cause the area of the outer layer of the segment to increase relative to the inner one. The process may proceed up to a limit imposed by the geometrical constraints where the shape of the maximal possible area difference is reached.

Theory

In order to obtain the shapes of the bilayer segment of an extreme area difference ΔA at a given area of the bilayer neutral surface A a variational problem is stated by constructing a functional

$$G = \Delta A - \Lambda_A \left(\int dA - A\right), \qquad (1)$$

where for thin bilayers

$$\Delta A = h \int (C_1 + C_2)\, dA, \qquad (2)$$

Λ_A is the Lagrange multiplier, C_1 and C_2 are the principal membrane curvatures, ΔA is the area element and h is the distance between the neutral surfaces of the two bilayer monolayers in the direction perpendicular to the membrane surface. The analysis is restricted to axisymmetric shapes. It is chosen that the symmetry axis of the body coincides with the x axis, so that the shape is given by the rotation of the function $y(x)$ around the x axis. In this case the principal curvatures are expressed by $y(x)$ and its derivatives with respect to x; $y'=\partial y/\partial x$ and $y''=\partial^2 y/\partial x^2$, as $C_1=1/y(1+y'^2)^{1/2}$ and $C_2 = -y''/(1+y'^2)^{3/2}$ while the area element is $\Delta A=2\pi\,(1+y'^2)^{1/2}\, y\, dx$. The sign of the principal curvatures is taken to be positive for a sphere. Inserting the above expressions for C_1, C_2 and ΔA into (1) and rearranging, the functional normalized with respect to $2\pi h$ becomes

$$G = \int g\left(x, y, y', y''\right) dx \qquad (3)$$

where

$$g(x,y,y',y'') = 1 - yy''/(1+y'^2) - \lambda_A\, y\, (1+y'^2)^{1/2}, \qquad (4)$$

$\lambda_A=\Lambda_A/h$. The variation $\delta G = 0$ is performed by solving the Poisson – Euler equation

$$\frac{\partial g}{\partial y} - \frac{d}{dx}\left(\frac{\partial g}{\partial y'}\right) + \frac{d^2}{dx^2}\left(\frac{\partial g}{\partial y''}\right) = 0\ . \qquad (5)$$

Obtaining the necessary differentiations of (4), the Poisson – Euler equation is expressed as

$$2y''/(1+y'^2)^2 + \lambda_A\left((1+y'^2)^{-1/2} - yy''(1+y'^2)^{-3/2}\right) = 0. \qquad (6)$$

If the area of the segment is fixed ($\lambda_A \neq 0$), there is an analytical solution of (6), given by a circle of the radius r_{cir} : $y = (r_{cir}^2 - x^2)^{1/2}$. This solution represents spheres of a radius $1/r_{cir}=\lambda_A$, and a segment of a plane $1/r_{cir}=0$.
If the area A is not fixed i.e if $\lambda_A = 0$ the possi-

ble analytical solution of the equation (6) is a constant $y = const$, representing a cylinder.

Experiment

Cells: The cells MCF7 (human breast adenocarcinoma) were grown in Eagle MEM, supplemented with 1 percent nonessential amino acids, 10 percent fetal calf serum (FCS), penicillin (100 U/ml) and streptomycin (100 μg/ml) at 37°C in a CO_2 incubator.

Induction of membrane vesiculation: Exponentially growing MCF7 cells were detached by 0.25 percent trypsin solution. The cells were resuspended in Eagle MEM without FCS and put on ice (4°C). After one hour the cell suspension was placed on 37°C for two hours.[13] The cells were then observed by the phase contrast microscope (Obj. Ph 3, 100X, NA 1.2).

Results and discussion

It was shown above that the shapes of the bilayer segment corresponding to the extreme difference between the two monolayer areas are spherical, planar and cylindrical. The spherical shape and a planar circular segment are characterized by one parameter, respectively. The respective parameter can be determined from the constraint requiring a fixed area. Therefore the sphere and the planar segment can be established as the shapes of the extreme area difference.[14] If the sphere is involved, the extremum is a maximum, as calculated by the minimization of the membrane bending energy for a sequence of shapes describing the formation of a spherical vesicle.[15] In order to establish a cylindrical shape as a shape of the extreme area difference, a boundary condition should be stated, such as a requirement for a fixed radius of the cylinder. It can be concluded that the spherical and the planar shapes of the maximal area difference are connected to the fixed area of the membrane segment while the cylindrical shape is unconstrained, but confined at its radius.

The theoretical predictions are compared to the phenomena observed in the experiment. In a vesiculating cancer cell, spherical vesicles as well as cylindrical protrusions can be observed (Figs. 2A and 2B, respectively). The vesiculation was promoted as described in the Material and methods ensuring that the integrity of the cytoskeleton was destroyed.[13] The observed shapes of the vesicles and protrusions correspond well to the theoretically predicted ones.

It was indicated by experiments that the properties of the membrane constituents strongly influence the nature of the protrusions and vesicles. In erythrocyte suspension, incubated with the exogenously added amphiphiles the budding and vesiculation of the erythrocyte membrane was observed.[6,7]

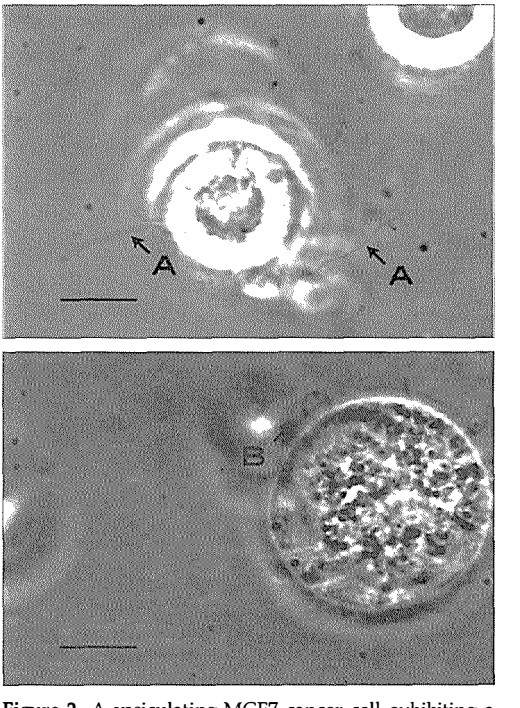

Figure 2. A vesiculating MCF7 cancer cell exhibiting a cylindrical protrusion (A) and a spherical vesicle (B), (bar=10μm).

The released vesicles were spherical and cylindrical, depending on the species of the added amphiphiles. Besides the properties of the membrane constituents, the manner of the disruption or the detachment of the skeleton may also influence the character of the vesicles by determining the amount of the membrane segment available to the bud, and also by determining the amount of the laterally mobile membrane embedded molecules. In deciding whether the protrusion will lead to a spherical vesicle or to a cylindrical protrusion, it should therefore be established which of the two processes is possible. If both of them are possible it should be distinguished, which of them would be energetically more favourable. In this case, the free energy of the segment under consideration should be minimized, taking into account the local composition of the segment. This is however beyond the scope of this work.

The laterally mobile molecules that favour large membrane curvature are accumulated in the buds which develop into vesicles and are released from the membrane. Thereby the relative content of these molecules in the residual cell membrane is diminished which may be unfavourable regarding the cell function. In this context, adding to the cell membrane the substances that decrease the area difference ΔA would tend to inhibit the budding and vesiculation. Indeed, chlorpromazine which was proved to intercalate into the inner membrane layer of erythrocytes[16,17] was also shown to have therapeutic effects in cancer treatment.[18] These promissing results encourage further studies of the mechanisms taking place in the membrane during budding and vesiculation.

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