Mechanobiology of Endocytosis and the Physics of Vesicle Formation

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1 Modeling the Mechanbiology of Endocytosis in Yeast

1.1 Introduction

Yeast endocytosis is a complex process that involves both physical as well as chemical changes in the local environment. It has been studied extensively using biochemical and genetic techniques which have provided valuable insight into the proteins involved in this process.[1] Moreover, development of biophysical techniques and advances in microscopy have also helped visualize the physical changes that occur during vesicle formation.[2]

The data that has been acquired from these diverse techniques is being used to construct mathematical as well as physical models for vesicle formation. A number of different models have been created that can recreate vesicle movement and formation.[3,4] The 2D necklace model has been used to simulate the movement of a vesicle under forces.[5] Continuous 3D models are being used to simulate vesicle formation using energy minimization, bending elasticity and curvature models.[6] A recent attempt at unifying the current knowledge to provide a holistic picture has been very successful.[7] This model successfully simulates the changing quantities of proteins during the process as well as the mutant phenotypes. However, it only models the changes in protein concentrations and describes the physical effect, without going into the physical equations. We will discuss these models, their assumptions and how well they fit the actual process. Moreover, we will also attempt to integrate the knowledge from the holistic model with the 2D and 3D models.

Yeast has been used as a model organism for many studies due to its simple genome and high similarity with the human genome. The endocytic process in yeast is similar to the process in mammalian cells. Both involve clathrin, adaptor proteins and cytoskeletal proteins. However, there are key differences in the processes as well. Yeast endocytosis forms a tubular rather than a spherical

invagination and lacks the protein dynamin. It also requires actin assembly while the mammalian process is aided by, but does not require actin assembly.[6] In this paper we will focus entirely on yeast endocytosis.

1.2 Mechanism

The process of endocytosis involves two major steps: the invagination of the membrane and the pinching off of the bud. Both processes have a large energy penalty because the cell cortex is resistant to deformation. Therefore, mechanical forces need to be applied for vesicle formation. The pulling force for membrane invagination is obtained from the actin myosin interaction. The pinching force is more elusive in yeast because in mammals a protein called dynamin is known to provide this force. However, it has been shown that yeast endocytosis does not involve dynamin so there has to be another source for this force. It has been hypothesized that an interfacial force is created due to the phase separation of the lipid bilayer, which causes the pinching [6].

1.3 Components of the endocytic machinery [2]

The molecules involved in endocytosis can be broadly divided into 4 categories or modules. It is useful to look at categories rather than specific proteins as it helps to generalize the process for all organisms.

The first module is of phosphoinositides (such as PIP2). They are created by lipid kinases and hydrolyzed by phosphatases. The balance of the action of these two enzymes determines their local concentration. Their function is to cover the endocytic membrane and recruit endocytic proteins.

The second module is of the coat proteins such as clathrin and Sla1. These proteins accumulate on the vesicle via PIP2 and PIP2 associated adaptor proteins. They anchor actin filaments and impart curvature to the bud region.

The third module is BAR domain proteins (BDPs), which have both membrane deforming as well as membrane curvature sensing ability. They accumulate in the tubule region and help block the hydrolysis of PIP2, creating the lipid phase boundary.

The fourth module is of the actin and actin associated proteins such as myosin, Cap 1/2 and Arp2/3. They anchor to the bud via the coat proteins and generate a pulling force on the bud. This pulling force helps create BDP binding sites and recruit BDP.

The fifth module consists of proteins that hydrolyze PIP2, such as synaptojanin and Sjl2p which accumulate later in the process.[6] They help create the

lipid phase separation. It has been shown that local membrane curvature influences the rate of hydrolysis i.e the greater the curvature, the more lipid heads are exposed and the more hydrolysis occurs.

The proteins involved, their role and time of accumulation is illustrated in Figure 1.

1.4 Timeline

From time 0-20s, coat proteins accumulate at the endocytic site. A bud begins to form that has a width of about 50 nm and a height of less than 50 nm. PIP2 is uniformly accumulating at this time. Between 20-25s the actin myosin pulling force comes into action and creates an invagination. BDP accumulates along the tubule region very rapidly. It reaches its peak from an initial 10 percent value in only 3s. The fast accumulation is the result of a positive feedback loop between membrane curvature and BDP accumulation (Figure 2A).

At about 21s, the PIP2 phase separation begins to form and an interfacial force starts to develop. The phase separation is a result of different hydrolysis rates of PIP2 at the tubule and bud regions due to lipid protein interactions. The rate of hydrolysis is decreased in the tubule region due to the presence of BDP proteins that provide a protective covering (Figure 2C). The phase separation produces a force due to two possible effects. Firstly, the number of hydrogen bonds are decreased which decreases the hydrogen shielding of hydrophobic hydrocarbons. Their interaction with water is energetically unfavourable so a line tension (Figure 2C) is produced that constricts the neck. Secondly, less hydrogen bonds lower the surface tension, making the bud expand. Since the bud cannot expand in the radial direction due to the constraint put by osmotic pressure, it will expand tangentially and produce a pinching force (Figure 2D).

Between 21-27 s, the force grows sharply due to another positive feedback loop, this time between the membrane curvature and PIP2 hydrolysis (Figure 2B). There is a sharp dip in PIP2 levels at the interface between the phases.

There is an optimal membrane radius of curvature for BDP binding (about 30nm). As the pinching forces curves the membrane further, it deviates from this optimal value. This acts as a disassembly signal for BDP. Phosphatase activity causes disassembly of the coat and actin attachments to the bud as well as tubule weaken. PIP2 hydrolysis can occur all over again. This decreases the pinching force but it is still enough to drive scission. At about 29s, the interfacial force narrows the neck to less than 5nm. At this distance, the opposite bilayers can fuse spontaneously. Timing is very important in this process in vivo. There is a very short time window in which successful endocytosis can take place.

2 Physics of Vesicle formation

2.1 Introduction

We started to model vesicle formation as a 2D necklace model of circular particles connected by springs (Figure 4), but soon realised that the model made many assumptions and because of no third dimension we lost many important aspects of vesicle formation. Moreover we could only model bending of a membrane from it and it was impossible to model the pinching and the formation of the actual vesicle without introducing unrealistic assumptions.

So we introduce a continuous and 3 dimensional membrane model which is a much better model and gives us more insights about the physics behind vesicle formation. I start with deriving the spontaneous curvature model of Helfrich and study in detail the most basic model by making assumptions along the way. In the end I give an overview of how we can further introduce sophistication and use our model to study other interesting cases.

2.2 Derivation of spontaneous-curvature model

So we take a 2D membrane(no kinks or discontinuities) and embed it in 3D space. See figure 3A. We can have two radii of curvature R_1 and R_2 to characterize this 2D surface. These radii determine the Gaussian Curvature K and the mean curvature H.

$$H \equiv (1/R_1 + 1/R_2)/2 \tag{1}$$
$$K \equiv 1/(R_1 R_2) \tag{2}$$

The curvature model for vesicles is based on the assumption that the local energy, f_1, associated with bending the membrane can be expanded in H and K to some order.

$$f_1 \equiv (\kappa/2)(2H - C_o)^2 + \kappa_G K \tag{3}$$

The parameter κ is the bending rigidity, κ_G is the Gaussian bending rigidity, C_o is a spontaneous curvature term that reflects a possible asymmetry of the membrane. This term would be useful if we incorporate bilayer structure to our model.

We can also include at least one additional variable which is reflective of the fluidity of the membrane. A fluid can be characterized by its local density ϕ. Small deviations of this density from the equilibrium density ϕ_o lead to an elastic energy. (As a side note this is the term we would have gotten if we had assumed a necklace model of particles connected with a string approximated to

a continuous model).

$$f_2 \equiv k(\phi/\phi_o - 1)^2 \tag{4}$$

Adding these two energies f_1 and f_2 and integrating them over the closed vesicle surface yields the total energy. For now we will consider a very simplified model and ignore the f_2 elastic term and put a constraint of fixed total area of the membrane.
What we get is the curvature energy F_{SC}, it has to be minimized at constant total area and constant enclosed volume. This constitutes the *spontaneous curvature model* as introduced by Helfrich.

$$F_{SC} = (\kappa/2) \oint dA(2H - C_o)^2 + \kappa_G \oint dAK \tag{5}$$

Three mathematical properties of this energy should be noted. First, by virtue of the GaussBonnet theorem the integral over the Gaussian curvature K is a topological invariant and depends on genus. So as long as the topology does not change, the second term can thus be ignored. Secondly, for $C_o = 0$, the curvature energy is scale-invariant, i.e. the energy of a vesicle does not depend on its size since $\oint dAH^2$ is a dimensionless quantity.

2.3 Domain induced budding and vesicle formation

Phase separation within the lipid bilayer can lead to domain-induced budding. We would look at vesicle formation through domain-induced budding for a flat membrane matrix.

We denote the two coexisting phases of the lipid mixture by α and β as shown in figure 3B. We would not discuss the biological players who cause the spontaneous curvature or the factors that cause the nucleated growth of the domain (figure 5). We would only discuss the physics for now and integrate it later with the yeast model discussed in the first section, at the end of this paper.

The edge of the inter-membrane domain has an energy which is proportional to the edge length. Therefore it has a tendency to attain a circular shape in order to minimize its edge energy. We define a quantity line tension, Σ_e, which is equal to the edge energy per unit length. A flat domain will form a circular disk in order to attain a state with minimal edge length. For a circular domain with radius L, the edge energy, F_e, is given by

$$F_e = 2\pi L \Sigma_e \tag{6}$$

However, as far as the edge energy is concerned, a flat circular disk does not represent the state of lowest energy since the length of the edge can be further reduced if the domain forms a bud: the domain edge now forms the neck of the bud, and this neck narrows down during the budding process, see fig 3B. Budding involves an increase in the curvature and thus in the bending energy of the domain. Therefore, the budding process of fluid membranes is governed by the competition between the bending rigidity κ of the domain and the line tension F_e of the domain edge. This competition leads to the characteristic invagination length, ξ.

$$\xi = \kappa/\Sigma_e \tag{7}$$

To understand this competition between the edge and bending energies we will look at our simple model, we assume that the matrix is flat and the membrane domain forms a spherical cap with radius R. If the domain has surface area $A = \pi L^2$, mean curvature $C = 1/R$ and spontaneous curvature $C_{sp} = C_o/2$, its total energy is $F = F_{SC} + F_e$ and has the following form which we can get using equation 5:

$$F = 2\pi\kappa \left(LC - LC_{sp}\right)^2 + (L/\xi)\sqrt{1 - (LC/2)^2} \tag{8}$$

The energy F has several minima and maxima, there are two boundary minima at $LC = \pm 2$ which correspond to spheres which have the lowest energy and we call them complete bud.

Now consider a domain where you fix the spontaneous curvature, invagination length. The domain size L however changes with time and so plays a role of a control parameter for the budding process. So for different values of L we can have incomplete bud growth, and as L grows there would be a value for which the energy is minimized and we would get a complete bud. We can find this critical value L_o in terms of the parameters by rearranging the equation above. When the domain has grown upto $L = L_o$, it becomes unstable and it has to undergo the complete budding process.

$$L_o = 8\xi \left[1 + \left(4\xi C_{sp}\right)^{2/3}\right]^{3/2} \tag{9}$$

2.4 Further Models

We just talked about domain induced budding (vesicle formation) from a flat matrix membrane. We can further look at domain-induced budding of vesicles, in which smaller vesicles can be formed from larger spherical vesicle which is composed of two types of domains α and β. Another interesting model is domain-induced budding in an elongated nucleated domain but we won't discuss these models here.

Now we have all the tools and can keep on adding further sophistication to our models, we can introduce bilayer for our membranes in which the inner and outer membranes have separate bending rigidities and so we don't ignore the local fluid density ϕ and so don't take the equation 4 equal to zero. Also we can model for vesicles with asymmetrical geometry like the torus and other geometries with higher genus. For that case we would have to use the Gaussian curvature that we ignored in equation 5.

3 Integration of Physical and Biological model

Although the model is based on domain induced budding without the presence of external forces, we can gather some insight from it. The phase segregation α and β can be mapped on to the PIP2 rich and PIP2 poor regions respectively. So, the model shows theoretically that phase segregation can induce budding in membranes.

Secondly, the invagination length being limited to a particular value which is the ratio of bending rigidity k to the line tension gives an intuition about the competition between two forces: the increasing bending energy required to invaginate the membrane and the minimization of the line tension. This results in a characteristic invagination length which comes out to be 10nm for lipid bilayers. Additional forces will need to be applied for longer tubular invaginations such as those in yeast (200nm). This could be one of the reasons why actin assembly is essential for yeast endocytosis as it provides the necessary energy for longer invaginations.

Another interesting result obtained during the calculations is that the bending energy is scale invariant if we consider a single layer membrane.

The next steps would be to use the nucleated domain model, which is an extension of this model, to look at the effect of actin assembly in detail. Moreover, the model of endocytosis can also be improved by adding more detail such as the mechanism of recruitment of PIP2 phosphatases and also by confirming that the bud becomes PIP2 poor. Although less plausible, but it is possible that the opposite happens since that case would also result in phase segregation.

4 Bibliography

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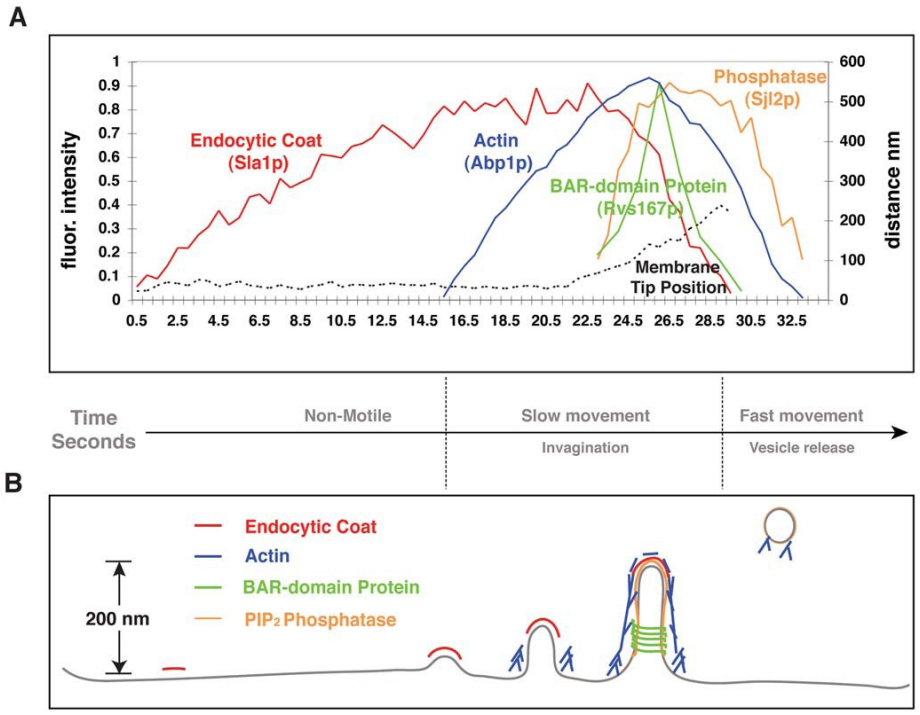

Figure 1: A) Multicolor fluorescence microscopy analysis is used to determine the timeline for endocytic protein recruitment. The results show that the coat protein is recruited at the earliest times followed by actin and then BAR domain proteins. The phosphatases are recruited at the latest stage. B) Spatio-temporal profile of the proteins involved in endoctyosis. The membrane tip position increases with time as illustrated in part A.

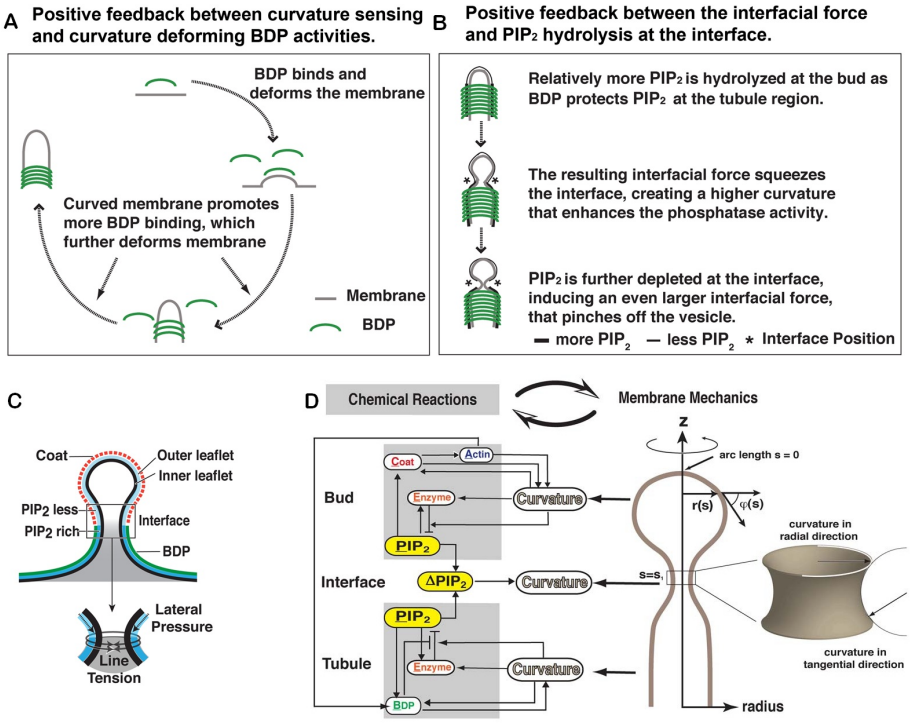

Figure 2: A), B) Diagrammatic representation of the positive feedback loops between BDP and membrane curvature in the tubule region, and the interfacial force and PIP2 hydrolysis at the phase segregation interface. C)Difference in PIP2 content between the bud and tubule and the resulting line tension that constricts the neck. D) The chemical cycle involved in causing phase segregation and forces it generates.

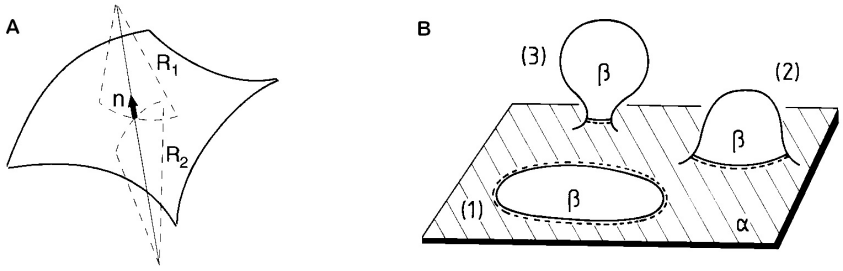

Figure 3: Budding of the membrane domain β embedded in the membrane matrix α. The domain edge is the full-broken line. The length of this edge decreases during the budding process (1 to 3).

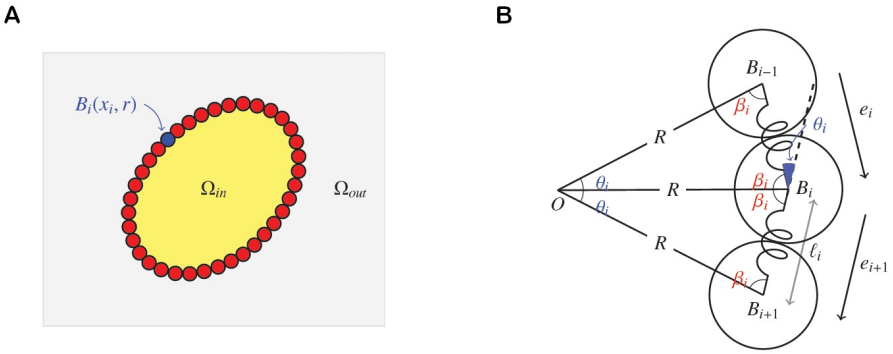

Figure 4: A) 2D necklace model of a vesicle, where Bi stands for each particle, and $\Omega_i n$ is the internal fluid and $\Omega_o ut$ is the external suspending medium. B) The beads are held together by spring forces. Each successive particle experiences a stretching energy through an elastic force which keeps them stuck. In addition, a triplet of particles will experience an angular force that would tend to align them. e is the unit vector between successive particles.

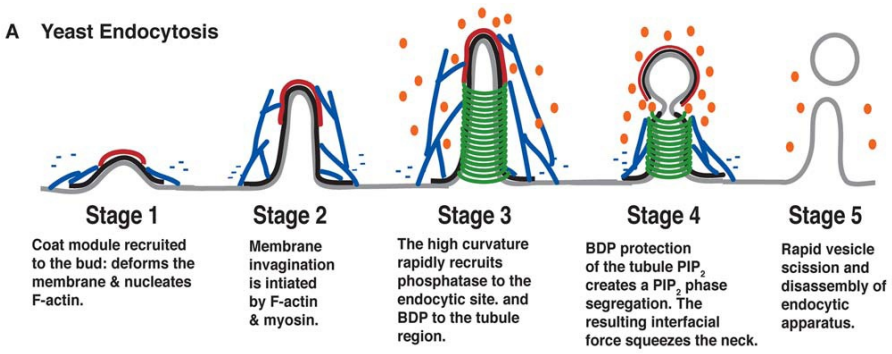

Figure 5: The biological model of yeast endocytosis