Domain-Mediated Endocytic Budding in a Raft Model Membrane

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It is important to understand the physicochemical mechanisms that govern the morphological changes in cell membrane structure in response to various internal and external stimuli because of the possible implications in membrane trafficking and endosomal systems. Recently, microdomains within a bilayer membrane, such as lipid rafts, have attracted considerable attention as one of the major mechanisms which mediate membrane internalization. A curved-out raft domain in a fluid membrane is predicted theoretically; line tension at the phase boundary drives a spherical cap for each domain connected by a sharp cusp to reduce the interface length [1]. Here, we studied the dynamic response of a raft-exhibiting giant liposome to external stimuli, such as the addition of Triton X-100 or osmotic stress. Giant liposomes were formed from a ternary mixture of saturated and unsaturated phospholipids and cholesterol. This ternary system is characterized by phase separation between liquid-ordered (Lo) and liquid-disordered (Ld) phases, where each phase corresponds to rafts and a surrounding fluid bilayer, respectively.

Figure 1 shows fluorescent images of the raft domains in two-phase liposomes upon osmotic stress, using a fluorescent dye (CtxB-488) that preferentially partitions into the raft phase. The heterogeneous membrane surface was covered by several raft domains (Fig. 1A). The floating rafts show negative curvature, i.e., the domains are curved toward the center of the liposome. Figure 2B shows enlarged snapshots of the budding process of a raft domain (the raft on the left is budding). The negative curvature of a spherical cap gradually increased, and then the edge closed to form an endocytic vesicle. When Triton X-100 was applied, the vesicles underwent essentially the same budding phenomena.

Next, we focused on much larger raft domains which covered approximately a third of the vesicular surface. We know from our experiments that the domains become larger through collision and fusion during thermal motion to decrease phase boundaries, and finally the entire membrane surface is covered with two different phase regions [2]. Thus, small and large sizes on the raft domain correspond to the early and late kinetic stages of domain growth. Figure 2 illustrates the process of endocytic vesicle formation in two-phase liposomes with one large raft domain under treatment with Triton X-100. The phase boundary was excited to form waves, and the top of the wave interface spontaneously progressed to a flask-shaped bud, which produced endocytic vesicles. Notably, the generated endocytic vesicles were typically within the same size range. In addition, we observed a wavy interface that produces endocytic vesicles under osmotic stimuli.

We found that multi-component liposomes show two different types of internalization process depending on the size of the raft domains. If several small rafts exist in a membrane, simple budding occurs due to the invagination of a whole raft domain area (Fig. 1). In contrast, large raft domains tended to produce monodisperse daughter vesicles from a wavy boundary (Fig. 2). The mechanism of this difference in raft domain internalization processes will be discussed by considering the effects of the line energy of domain boundaries and the bending energy of bilayer membranes [3].

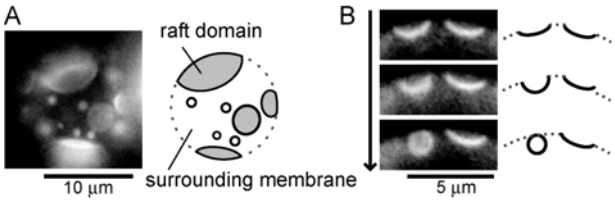

Fig. 1 Budding through invagination. (A) Fluorescent images (left) of raft domains in a two-phase liposome surface with schematic illustrations (right). (B) Time series of the budding process of a raft domain. The elapsed time between snapshots is 66 ms [3].

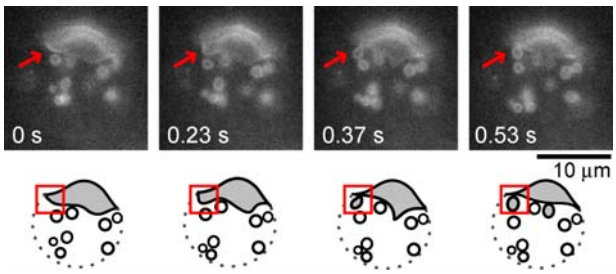

Fig. 2 Wavy budding: endocytic process of a larger raft domain in two-phase liposomes. Fluorescent image sequence (upper) of the production of satellite vesicles from a wave-excited domain interface, with schematic illustrations (lower) [3].

References

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