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Spontaneous Formation and Behavior of Lipid Nanotubes

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Abstract

Lipid nanotubes are unique membrane structures that have been used as nanofluidic constructs and models for determining membrane properties. Most recently, they have been observed as cytoplasmic bridges spanning between live cells suggesting a newly discovered form of intercellular communication. It is not understood, however, how these transient structures form between cells and how they enable the transport of cellular components. We have been exploring how nanotube structures form spontaneously using protein-membrane interactions to induce membrane curvature and the role that membrane rigidity plays in the assembly process. We have found that simple protein interactions, such as with streptavidin, induce the formation of nanotubes and that a threshold exists for membrane rigidity that allows nanotube formation to occur.

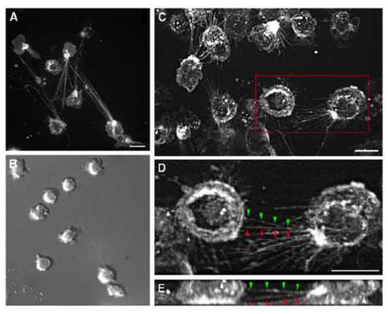

Figure 1: (A-E) Confocal images of THP-1 cells (a monocytic cell line that can acquire macrophage function following LPS or PMA treatment), Nanotubes observed running between cells over distances over 100 µm. Similar results found for dendritic cells. Calcium fluxes and cell communication very rapid between connected cells - possible pathway for rapid immune response. (Watkins, S. C. and Salter, R. D., *Immunity* **2005**, *23*, 309.)

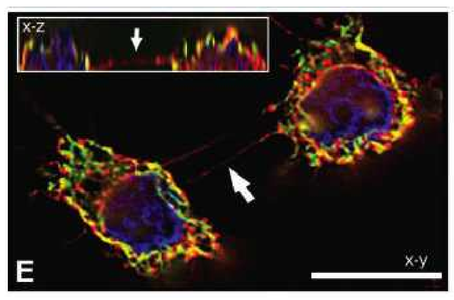

Figure 2: Rat pheochromocytoma PC12 cells showing nanotube connections. (E) staining of cells with fluorescent labeled antibodies, (*Science* **2004**, *303*, 1007).

Introduction

The lipid nanotube structures under investigation can be formed through a combination of lipid geometry, mechanical extension, or interaction with boomerang-shaped proteins (i.e. amphiphysin). As materials these structures have been used as nanofluidic constructs and models for membrane tension studies. More recent studies, however, have shown that lipid nanotubes also serve as cytoplasmic bridges between live cells with directed transport of ions, proteins, and even organelles observed. This process was monitored in a diverse range of cells, suggesting a totally new and potentially important form of cellular communication. It has also been recently discovered in our lab that the simple protein-membrane interaction between streptavidin and giant lipid vesicles can induce the formation of lipid nanotubes. We will show that this nanotube formation is dependent upon the rigidity of the lipid membrane (κ) and mechanical stress placed upon the system. Understanding the interfacial interactions and membrane structures that enable the formation of nanotubular architectures is essential in elucidating biological processes and pathways towards novel self-organized assemblies.

Methods

Giant unilamellar vesicles (GUVs) were prepared according to the procedures outlined by Moscho. In short, a solution of 5% 1,2-dioleoy-sn-glycero-3-phosphoethanolamine-N-biotin (DOPE-biotin) with a phosphocholine lipid (see Table 1) and 0.3% Di-A (fluorescent label) in chloroform was mixed with a small quantity of methanol and a quantity of MOPS (0.02M morpholino-N-propylsulfonic acid, 0.1M NaCl, pH 7.4) buffer solution. Removal of the organic solvent under reduced pressure was effected by rotary evaporation at slow rotation speed. The resultant giant vesicles had a visible size range of 1 - 20 microns. Streptavidin was then added to the vesicle solution and following incubation at room temperature for more than 30 minutes a sample was placed between a slide and coverslip for microscopic observation. Images of each type of vesicles and the resulting nanotubes were gathered and scrutinized for observable patterns.

Figure 3: Diagram of the suggested formation mechanism for GUVs. Initially there is an ordered monolayer of phospholipids at the interface between an aqueous and an organic phase (A). During evaporation, bubbles form (B) that rupture the phospholipids film into fragments (C). The resulting phospholipids monolayer fragments fuse to bilayers (E), which spontaneously vesiculate (F). An alternative way for the formation of the bilayered phospholipids fragments (D) involves micellar structures having entrapped liquid organic solvent. (A. Moscho, O. Orwar, D. T. Chiu, B. P. Modi, R. N. Zare, Proc. Natl. Acad. Sci. USA 1996, 93, 1143)

Results

Spontaneous nanotube formation was observed with DOPC, DLPC, and POPC, indicating that κ values of 3.9 and lower are sufficiently low for nanotubes to form. Spontaneous formation lacked, however, with egg PC, DMPC, DSPC, and DPPC, showing that κ values of 8.0 and higher are indicative of a lipid membrane too rigid to form nanotubules in the presence of streptavidin. This blatant barrier within the data indicates that there exists a rigidity threshold controlling nanotube formation. In addition, this threshold suggests that protein-membrane interactions that induce curvature, like those resulting of amphiphysin, may be dependent on the membrane's resistance to curvature. Although streptavidin is not known to induce curvature, this may be a possible explanation for the nanotube formation. Also, mechanical stretching of the membrane as two vesicles collide and separate can produce nanotubes. Streptavidin-membrane interaction may induce high membrane tension that produces the observed taught nanotube structures.

Table 1: Lipid Membrane Rigidity Constants

Lipid	κ value ($\times 10^{-20}$ J)	Nanotube Formation
DOPC	1.9	yes
DLPC	3.37	yes
POPC	3.9	yes
egg PC	8.0	no
DMPC	14.7	no
DSPC	18	no
DPPC	20	no

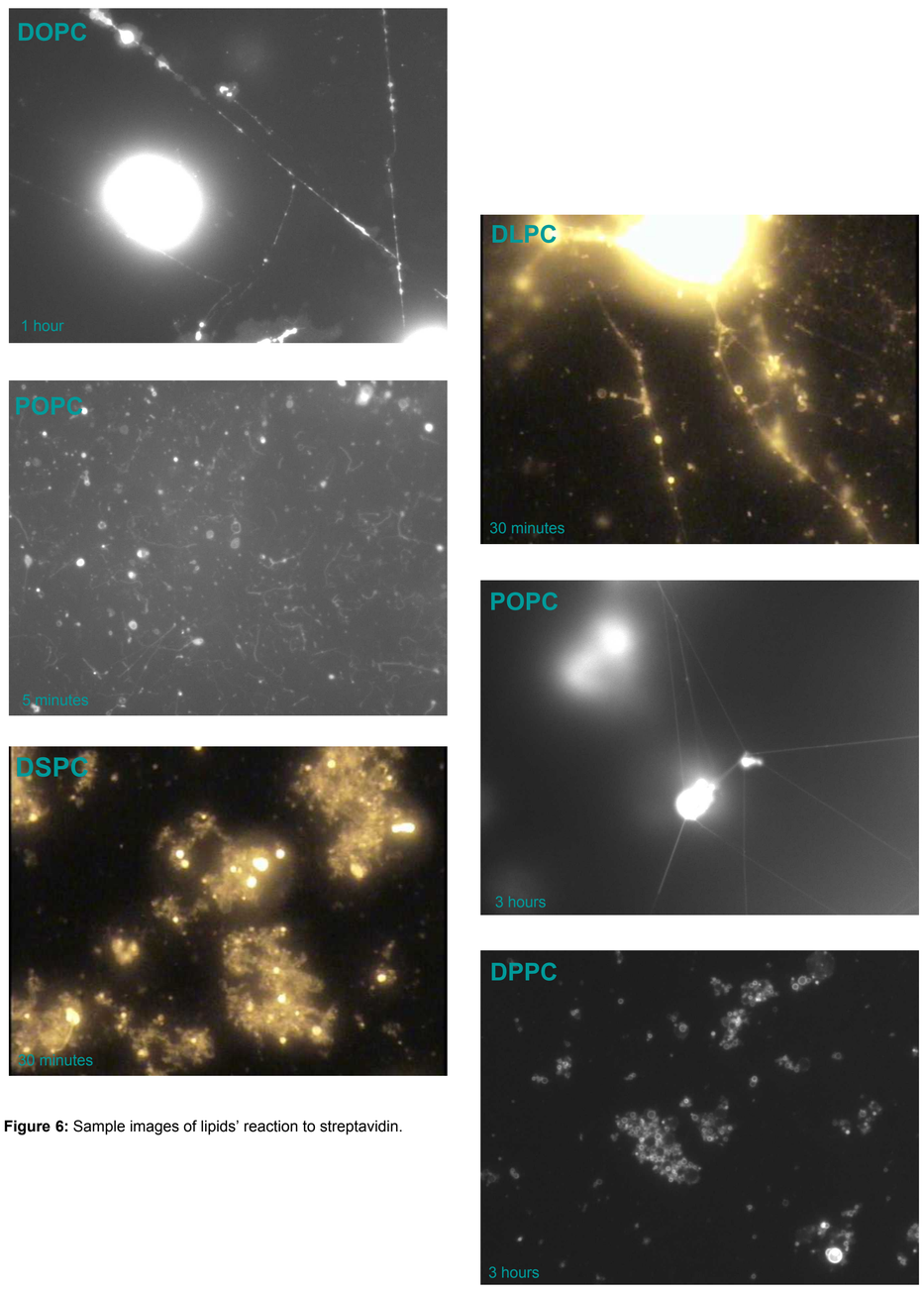

Figure 6: Sample images of lipids' reaction to streptavidin.

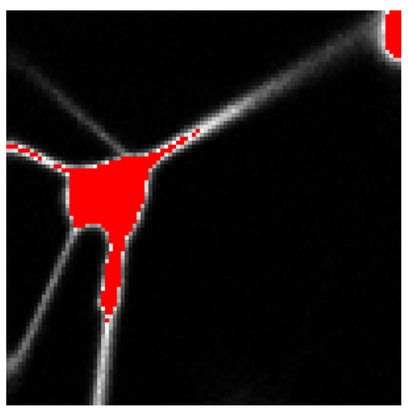

Figure 5: Confocal microscope image of POPC GUVs with TMR labeled streptavidin. The red labeling is indicative of the protein's attachment to the membrane surface.

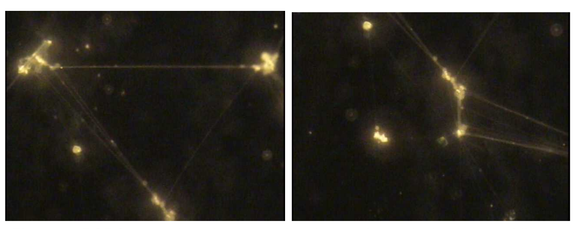

Figure 4: POPC nanotubes demonstrating the tension typical to streptavidin induced constructs

Conclusions

- Simple protein-membrane interaction can induce nanotube formation
- Nanotube formation is dependent upon membrane bending rigidity
- Streptavidin either induces membrane curvature, mechanically stretches the membrane, and/or increases membrane tension, enabling the exclusive formation of taught nanotubes

Future work will include investigations into the effects of the strength and coordination of the protein-membrane complex on nanotube formation.