Lipid localization in bacterial membranes

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Boulder Summer School 2007

Thanks to: Kerwyn Casey Huang and Ranjan Mukhopadhyay

Support: NIH, HHWF(KCH)
Outline

• Introduction
  - Protein localization at bacterial poles
  - Cardiolipin localization
• Biophysics of lipid-cluster formation
• Curvature-induced polar localization of lipid clusters
• Future directions
Protein localization at bacterial poles

- Protein-protein interactions
- Curvature (?)
- Turing oscillations
- Protein-lipid interactions (?)

Tsrr micellar assemblies

Weis et al. (2004)

DivIVA-GFP in *B. subtilis* outgrown from spores

Harry & Lewis (2003)
Bacterial phospholipids

Cardiolipin (CL)

Phosphatidylglycerol (PG)

Phosphatidylethanolamine (PE)
Evidence for lipid localization

- Cardiolipin (CL) can be stained with NAO (10-N-nonyl acridine-orange)
- CL levels enhanced at the poles and in minicells of *E. coli*

Mileykovskaya *et al.* (2000)

Koppelman *et al.* (2001)
More cardiolipin localization...

- CL also located at poles and septa in *B. subtilis*

Exponential growth  Sporulation

Kawaiii (2004)
And cardiolipin delocalization

- CL delocalized in round *rodA*− *E. coli* cells

(live cells w/ 100nM NAO)
Can a single lipid find the poles based on a preference for a highly curved geometry?

Curvature $C_{lipid} < (1 \text{ nm})^{-1}$
Bending rigidity modulus $\kappa = 100k_B T$

$$C_{cyl} = (500 \text{ nm})^{-1}$$

$$C_{pole} = 2 C_{cyl}$$

$$\Delta E_{pole} \sim \kappa C_{cyl} C_{lipid} \sim (100k_B T) \times \left( \frac{1}{500 \text{ nm}} \right) \times \left( \frac{1}{1 \text{ nm}} \right) \times (\text{nm}^2) = 0.2k_B T$$

Only $\approx 20\%$ enrichment for single lipids at poles
Lipid phase separation in vesicles

Baumgart et al. (2003)
Domains and budding in lipid mixtures

Budding of lipid domains is due to:

i. spontaneous curvature of lipids

ii. line tension at domain boundaries

Laradjji and Kumar (2005)

Lipid domains in vesicles from particle-dynamics simulations

Question: What is the effect of the cell wall on lipid domains?
Energy model for membrane

- Short-range attraction between like lipids can lead to phase separation:

\[ E_{\text{coupling}} = \sum_{i} \sum_{\text{n.n. } j} \frac{\varepsilon}{2} \left[ \sigma_i (1 - \sigma_j) + \sigma_j (1 - \sigma_i) \right], \quad \sigma_i = 0,1 \]
Energy model for membrane, continued

• Elastic energy of membrane

\[ E_{\text{elastic}} = \int \left[ \frac{\kappa}{2} (\nabla^2 h - C)^2 + \frac{\lambda}{2} h^2 \right] dA \]

Lipid cluster size is limited by energy penalty for curving membrane away from cell wall...
Elastic energy of membrane mediates long-range lipid-lipid repulsion

- Minimize energy with respect to membrane height $h$
- Leads to effective repulsion between lipid A molecules
  given by $V(\vec{r} - \vec{r}')$

$V(|\vec{r} - \vec{r}'|) / (k_B T)$
Short-range attraction vs long-range repulsion

- Can lead to stable clusters
- Cluster size controlled by lipid-lipid attraction, spontaneous curvature of lipids, and pinning of membrane by cell wall

“Cardiolipin” clusters (from Monte Carlo simulations)
Effect of changing $\gamma$

Decreasing $\gamma$ has the same effect as increasing $\varepsilon$ (cluster size $\sim \varepsilon / \gamma^2$)

- $\varepsilon = 2.5k_B T$
- $\varepsilon = 3k_B T$
- $\varepsilon = 4k_B T$

$\gamma = 0.2/\text{nm}$  $\gamma = 0.4/\text{nm}$  $\gamma = 0.6/\text{nm}$
Effect of curved poles

\[ \epsilon = 2.5k_B T \]

\[ \lambda = 0.25k_B T/\text{nm} \]

\[ \gamma = 0.4/\text{nm} \]

\[ \gamma_0 = 0.04/\text{nm} \]

\[ \Delta E_{\text{pole}} \sim 5kT \text{ per cluster} \]

Clustering is crucial for polar localization
Effect of increased concentration

fraction of lipid A: $\phi = 0.15$

$k = 25k_B T$
$\lambda = 0.25k_B T/\text{nm}^2$
$\gamma = 0.4/\text{nm}$
$\gamma_0 = 0.04/\text{nm}$

fraction of lipid A: $\phi = 0.3$

Domain size not a function of $\phi$...
Conclusions

• Lipids can form clusters due to a competition between phase separation and pinning by the cell wall.

• Clusters of high-curvature lipids can localize to both poles of the cell.

• Cardiolipin may serve as a target for polar localization of proteins.
Future directions

• Biophysics experiments (w/Rob Phillips@Caltech)
  – Does cardiolipin go to highly curved regions of lipid vesicles?
  – Will cardiolipin mediate localization of proteins (e.g. DivIVA)?

• Biology experiments
  – Co-imaging of cardiolipin and DivIVA, etc., particularly in cells that branch (e.g. Streptomyces).
  – Cardiolipin “knockouts” (hard to kill).
Lateral compartmentalization of lipids, cholesterol, and protein molecules, violates the Fluid Mosaic Model (Singer-Nicholson, Science ’72)

Rafts found on the outer leaflet of the plasma membrane of animal cells and is rich in sphingolipids, cholesterol and anchored proteins (Simons and Ikonen, Nature ’97)

Recent evidence of lipid localization in plasma membranes of bacteria.