3.7: Lipid Phase Coexistence

Lipid phase coexistence occurs when a membrane is composed of a heterogeneous mix of lipid physical states organized into lateral domains that can range from gel (solid phase) to liquid disordered and to liquid ordered phases.

Lipid phase differences within the plane of the membrane give rise to heterogeneity in the overall function and structure of the membrane. The structural heterogeneity is caused by the presence of multiple types of lipids with varying physical properties such as: saturation state, presence of double bonds, length of the hydrophobic acyl chains as well as, some suggest, the size of the head group. Phase coexistence is characterized by diverse lipid types and their interactions between each other within the same membrane. Generally, any physical component of the lipid which leads to less or more packing affinity between other lipids will affect the phase state at certain environmental conditions. Phase coexistence is caused by heterogeneity of lipid phases in the same environmental conditions.

Multiple Lipid Phases

The ordered state of lipids can be thought of generally as the "packedness" of the lipids which is specifically determined by the orientation of the hydrophobic acyl tails--straight and extended (ordered) or free to bend and relatively unpacked (disordered). There are liquid ordered and liquid disordered phases in which the acyl tails are more or less ordered together in a uniform arrangement, respectively. Lipids of different physical properties will exist in various phases (gel or liquid) simultaneously even in the same temperature.

Gel phase lipids and liquid phase lipids coexist in the plane of the membrane as distinct lateral domains. This frequently occurs when low melting point lipids mix with high melting point lipids--in which case the low melting point (low Tm) lipids will preferentially remain in the liquid phase at a set of temperatures where the high melting point lipids would be in the gel phase. The physical structure of these gel phase lipids alters the overall membrane structure by changing the height of the membrane over those domains and causing tension along the boundary between the different domains.

The physical property differences between ordered and disordered lipids also alter the propensity of the membrane to curve or flex in certain directions. This curvature between liquid and gel phase domains give rise to tension within the lipid layers--which can be seen in electron microscopy images as tilting of the lipids in response to the tension induced by the physical differences of the lipid phase domains. Furthermore, membrane flexability and curvature is important for proper membrane function which involves vesicular budding, membrane tubulation, and other cellular trafficking processes.

Methods of Observation

Various methods of detecting lipid phase coexistence in membranes and testing related hypotheses are used including utilizing unique fluorescent probes with phase sensitive spectra, measuring the infrared spectra of lipids in a membrane, and examining the surface of the membrane. While these methods are somewhat diverse--from observing the topology to using fluorophores and infrared light excitation and emission to detect lipid heterogeneity--each is very useful in elucidating the nature and organization of lipid phase coexistence in membranes.

- **Fourier Transform Infrared Spectroscopy (FTIR):** A common technique in detecting phase differences in membranes is by shining infrared light on the sample and measuring the spectroscopic properties of the sample following excitation with various wavelengths. Differences in the spectra equate to differences in membrane composition or, more specifically, different lipid phases. FTIR has a wide variety of uses but is particularly useful in membrane and lipid studies due to its ability to capture spectra for nonhomogenous lipid samples at a very high resolution.
- **Atomic Force Microscopy (AFM):** Phase coexistence of lipid membranes can be readily observed in real time, as the lipids undergo phase transitions, using AFM while adjusting the temperature to induce phase changes. Generally, by using a probe attached to the end of a lever (upon which laser is directed) to scan over the top of a sample, the lever will move up and down according to the topology of the sample surface, causing the laser deflection to change--which can then be interpreted as the sample surface. Since lipids in different phases exhibit different height characteristics when the tails straighten or are relaxed AFM allows you to visualize those height differences by scanning topology of the membrane surface.
- **Laurdan (6-dodecanoyl-2-dimethylaminonaphthalene):** Laurdan is a fluorescent molecular dye that inserts into the membrane bilayer at the boundary between the hydrophobic and hydrophilic portions of the lipids. The usefulness of this molecule is the wide emission spectra shift it undergoes upon entering different polar environments. As the lipids surrounding the Laurdan molecule change phase and become either less or more ordered in response to stimuli (temperature for example)

the polarity environment also shifts. Laurdan molecules are sensitive to this polarity change in the membrane in that the emission wavelength shifts significantly. This emission difference, modulated by the lipid phase environment, allows researchers to easily distinguish between lipids at different phases and following transitions. Laurdan dye can be used to study phase coexistence in that it pinpoints areas of differing phases in the membrane.

Biological significance

Heterogeneity at the lipid phase level is important for proper membrane function in cells, especially given the diverse range of functions and structures in which the membrane is involved. Most notably, lipid phase differences within the bilayer membrane allow for (and even encourage) a wide variety of shape changes that are necessary for fundamental cellular processes such as vesicular trafficking (through the production of vesicles) as well as cell signaling and membrane associated-protein function.

Membrane curvature

While proteins are known to mediate membrane curvature, lipid composition and the phase state also plays a role in curvature and bending of the bilayer into the diverse array of membrane shapes we see in the cell. Specifically, lipids of different phase can encourage membrane curvature at the boundary between the two phases. Gel and liquid phase lipid domains, for example, creates line tension along the border of the phase domains. The difference in the bending properties between the two lipids at different phases encourages out-of-plane bending of the membrane, which could then lead to vesicle budding or fission. By changing the temperature you can alter the lipid phases and therefore the membrane curvature as it shifts due to the different physical organization of the lipids.

Intracellular trafficking

Membrane curvature is fundamental to cellular processes like endomembrane trafficking (e.g,g, endocytosis) in which vesicles bud and fuse to various membranes like the plasma membrane, the Golgi apparatus stacks, endoplasmic reticulum tubules, and numerous other organelles. Therefore, lipid phase coexistence and membrane heterogeneity is important in maintaining lipid and membrane composition between the organelles/subcellular locations (by encouraging membrane budding and therefore membrane recycling/flow throughout the cell) but also plays a role in trafficking of physiologically important material in the cell.

Phase coexistence and membrane proteins

Lipid phase coexistence could also affect membrane associated-protein function by altering the structure and mechanical properties of the membrane in which the protein is embedded. Also, some proteins preferentially reside in one phase over others; alpha-helix proteins are more commonly found in liquid phase lipid domains. Conversely, membrane proteins can influence the phase state of the surrounding lipids by altering the local organization of lipids in the bilayer and the interactions between lipid chains.

Summary

Model membranes are often composed of homogenous lipids in order to maintain uniformity in experiments. However, in reality membranes can be quite heterogeneous in their lipid composition and the phase state of those lipids. These lipids are organized into lateral domains in the membrane and contribute to many fundamental processes within the cell such as endocytosis and secretion. The mechanical membrane properties dictated by lipid phase is modulated by the coexistence of different lipid types and phases under various conditions. Membrane shape and curvature is also directly affected by the phase of the lipids of which it is made. Further work is focused on deciphering the details of lipid phase coexistence and its relation to biological processes.

References

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