

Tutorials, while not mandatory, will allow you to improve your final grade in this course. Thank you for your attendance to date. These notes are not a substitute for the discussions that we will have class.

This week's topic will be:

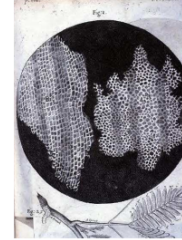
Evidence for the Fluid Mosaic Model.

Developing theories, testing hypotheses and techniques for visualizing cells

Cell Theory: 17th Century, Robert Hooke

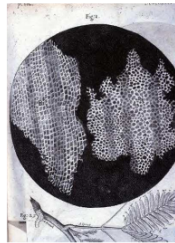
This is what Robert Hooke wrote when he looked at a piece of cork through his microscope.

"I...perceived it to be perforated and porous much like a honeycomb but that the pores were not regular.
... the walls of the pores were not as thin as those of a honeycomb which enclose hexagonal cells.
...these pores or cells consisted of a great many boxes.
...I found there were about threescore of these small Cells in an eighteenth part of an inch, (less than 2 millimetres) about 12 hundred million in a cubic inch. (about 80 million per cubic centimetre)"



<http://www.mhs.ox.ac.uk/wp-content/uploads/the-world-through-a-microscope.pdf>

Why does Hooke compare the cork to a honeycomb?



This was the first use of the word "cell" to describe parts of an organism. What does "cell" mean to you?

How is Hooke's use of the word "cell" different to your own?

19th century:

A semi-permeable barrier must exist around a cell. WHY?

What is this barrier?

- 1) Behaviour of oils in water
- 2) Anesthetic molecules led to the theory that this barrier might be made of some sort of fat.

Criticisms?

Only lipid-soluble, hydrophobic molecules can get past - no mechanism for energy-dependent selective transport.

Lipid bilayer: Gorter and Grendel, 1925

- Prepared a solvent extract of lipids from a red blood cell and spread them into a monolayer.
- How can this be used to tell us if the lipid layer of the cell is a monolayer or bilayer or other structure?

Compare the area of the monolayer to the surface area of the cells, they found a ratio of two to one.

Despite several problems with this experiment the fundamental conclusion - that the cell membrane is a lipid bilayer - was correct.

Possible models incorporating proteins into the bilipid membrane theory:

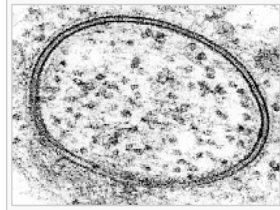
Davson and Danielli, 1935

The lipid bilayer is coated on either side with a layer of globular proteins.

Sjöstrand et al., 1954

A single molecular layer of protein lies in between the two lipid layers.

Electron microscopy:
Sjöstrand et al., 1954.



After staining with heavy metal labels two thin dark bands separated by a light region were visible. Interpreted as a single layer of protein.

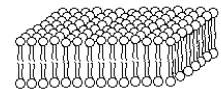
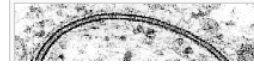
Electron microscopy:

J. David Robertson, 1955.

Reinterpretation of same data.

The dark electron-dense bands were the headgroups and associated proteins of two apposed lipid monolayers.

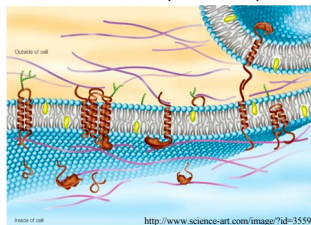
The bilayer structure was assigned to all cell membranes and organelle membranes.



Structure of cell wall bilipid layer

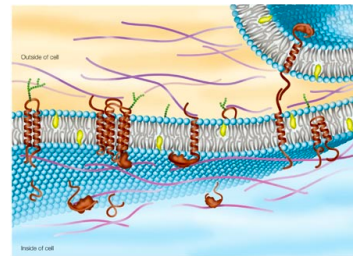
Fluid mosaic model: Singer and Nicolson, 1972

A biological membrane is composed of a "lipid bilayer" that is essentially a two-dimensional solution composed of lipids and proteins.



The lipid bilayer functions as a solvent for integral proteins and a permeability barrier.

Evidence for the fluid mosaic model?



What are at least two characteristics of membranes that you can assay that would provide support for the fluid mosaic model?

1) Hypothesis 1: Membrane proteins are randomly distributed in membrane EM, fluorescence

2) Hypothesis 2: Proteins can redistribute from one cell to another in a fusion

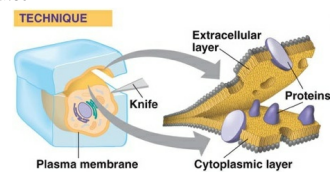
1) Are proteins randomly distributed throughout the plane of the membrane due to their mobility (lateral diffusion)?

Freeze fracture and electron microscopy

Technique:

1. Cells are frozen in liquid nitrogen.
2. Frozen cells are fractured using a knife.

The fracture occurs on lines of weakness like between the lipid bilayer of the plasma membrane. This exposes proteins embedded in the surface.



http://en.wikibooks.org/wiki/Structural_Biochemistry/Lipids/Membrane_Fluidity

3. Freeze Etching uses a vacuum to remove surface ice.

4. The first part of making a replica is shadowing with platinum vapor at a 45-degree angle to the surface.

5. The next part of making a replica is evaporating a very thin layer of Carbon onto the surface at a 90-degree angle.

6. The final replica is revealed by degrading the organic cell material away with an acid or base.

7. The Carbon-Platinum replica is then studied under an electron microscope, and the pattern of membrane proteins is shown by the shadowed craters and bumps.

http://en.wikibooks.org/wiki/Structural_Biochemistry/Lipids/Membrane_Fluidity

Results:



Inside of extracellular layer

e face :
the outer lamella of the plasma membrane viewed as if from within the cell



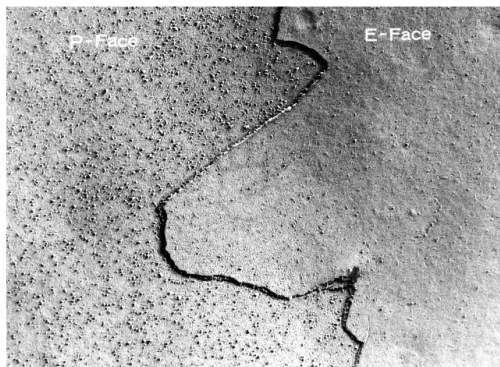
Inside of cytoplasmic layer

p face :
the inner lamella viewed from outside the cell.

1) membrane proteins are randomly dispersed throughout the phospholipid bilayer

2) integral transmembrane proteins that span the entire membrane.

http://en.wikibooks.org/wiki/Structural_Biochemistry/Lipids/Membrane_Fluidity



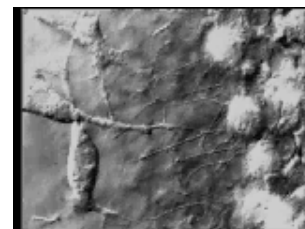
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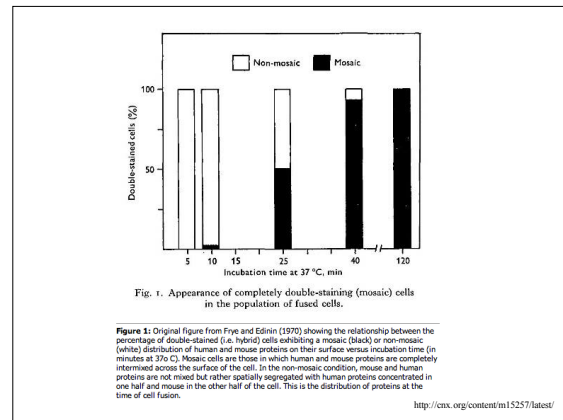
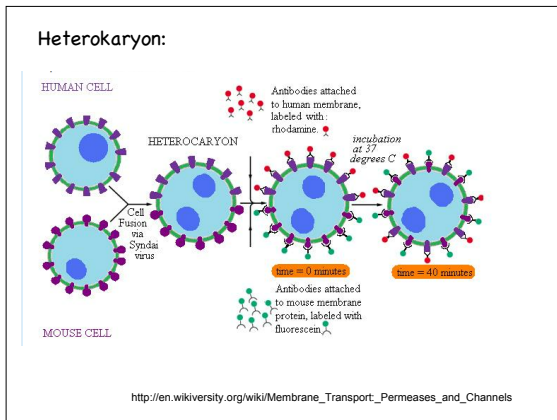
2) Do proteins and lipids undergo lateral diffusion within the membrane?

Heterokaryon: cell fusion

Frye and Eddidin, 1970

"The surface of membranes of animal cells rapidly change shape as the cells move, form pseudopods, or ingest materials from their environment. These rapid changes in shape suggest that the plasma membrane itself is fluid, rather than rigid in character, and that at least some of its component macromolecules are free to move relative to one another within the fluid. We have attempted to demonstrate such freedom of movement using specific antigen markers of 2 unlike unlike cell surfaces." (Frye and Eddidin, 1970, p.320)





1. Carefully examine the figure legend, axes labels and data. Summarize the results of this experiment as depicted in the figure above in a sentence or two. Be sure to describe only what the figure shows.

2. What do their results suggest about the position of proteins within the cell membrane over time? Why? Please explain.

Singer and Nicolson (1972) cite this study as evidence that the membrane behaves as a fluid through which proteins can diffuse.

Review the results above, do you think they can be unequivocally interpreted as evidence for a fluid cell membrane or could these same results be caused by alternative cellular mechanisms?

What are your alternative explanations?

Other possible explanations:

- 1) new proteins were rapidly synthesized and inserted into the membrane over the course of the experiment.
- 2) proteins were being removed from the surface in one location and reinserted in another.
- 3) proteins synthesized in both the mouse and human cell prior to cell fusion were being inserted into the membrane after fusion.

The researchers treated the cells with chemicals that inhibited protein or ATP synthesis either before fusion (to test model 3) or after fusion (to test models 1 and 2). None of these treatments impeded protein redistribution.

Temperature at which the cells were incubated did, however, affect rates of mosaic formation (Frye and Edidin, 1970). Was this outcome consistent with the explanation that protein redistribution results from diffusion through a fluid membrane?

Consider the process of diffusion. How would you expect temperature to affect the rate at which molecules diffuse?

Sketch a graph that shows how you would expect temperature to affect the number of mosaic cells formed after 40 minutes of incubation if protein movement was diffusion driven.

Imagine incubating a set of newly formed heterokaryons at a constant, preselected temperature and counting the number of mosaics after 40 minutes and then repeating this procedure for a series of predetermined temperatures between 0 and 37 degrees Celsius.

What types of results (relationships between temperature and mosaic formation) would not support the fluid mosaic model? Please explain.

Sketch the results that would contradict the fluid mosaic model.

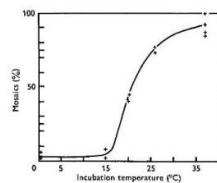


Fig. 2. Effect of temperature on the appearance of mosaic cells within 40 min of cell fusion.

Figure 2: Original figure from Frye and Edidin (1970) describing the relationship between the percentage cells exhibiting mosaicism after a 40 minute incubation at temperatures (degrees Celsius) indicated by + symbols. Curve fit to data.

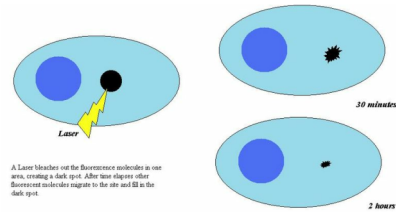
Examine the figure legend, axes labels and data. Summarize the results of this experiment in a sentence or two. Be sure to describe only what the figure shows.

Do these results support a fluid model of the cell membrane in which proteins move by diffusion? Please explain.

2) Do proteins and lipids undergo lateral diffusion within the membrane?

FRAP: Fluorescence Recovery After Photobleaching

FRAP follows the recovery of the bleached region or redistribution of fluorescent molecules into the bleached region.



http://en.wikiversity.org/wiki/Membrane_Transport_Permeases_and_Channels

Animations for FRAP:

1)

<http://www.dnatube.com/video/67/Flourescence-recovery-after-photobleaching>

2)

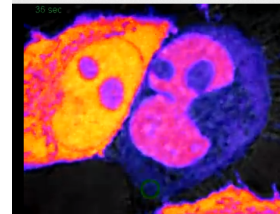
<http://www.youtube.com/watch?v=ipyGVh7JKvw>

FLIP: Fluorescence Loss in Photobleaching

FLIP follows the redistribution of the bleached fluorophores.

Animation:

<http://www.youtube.com/watch?v=Av0xdKJkO0s>



Shortcomings and simplifications in the original theory

- Lipid rafts can limit diffusion.

- Free diffusion on the cell surface is often limited to areas a few tens of nanometers across due to cytoskeleton anchors and aggregated protein structures.

- Little of the plasma membrane is “bare” lipid ; much of the cell surface is likely protein-associated.

In despite this, the fluid mosaic model remains a popular and most often referenced theory for the structure of biological membranes.