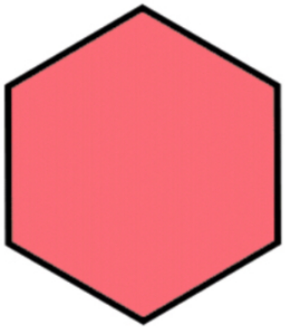


Cytology

Doctor 2019 | Medicine | JU

◉ Sheet ○ Slides

Done By

Nada Odeh

Contributed in the Scientific Correction

Contributed in the Grammatical Correction

Doctor

Dr.Ziad Shraideh

Sheet(1) / part 2/ concept 8.7

The dynamic nature of the plasma membrane

1/ The mobility of individual lipid molecules within the bilayer of the plasma membrane can be directly observed under the microscope by linking the polar heads of the lipids to gold particles or fluorescent compounds

2/ Types of lipids' movement

a. Flip- flop (transverse diffusion)

While the diffusion of phospholipids from one end of a bacterium to the other end in a second or two , it takes a phospholipid molecule a matter of hours to days to move across to the other leaflet

Why this form of motion is the most restricted ?

** because for flip-flop to occur, the hydrophilic head group of the lipid must pass through the internal hydrophobic sheet of the membrane, which is thermodynamically unfavorable (hydrophilic & hydrophobic)

Note: Lipids lacking polar groups, such as cholesterol, can move across the bilayer quite rapidly.

** the existence of flippases (enzymes that actively move certain phospholipids from one leaflet to the other) makes this motion possible and plays an important role in the lipid asymmetry as explained previously in 8.2

b. Lateral shift (flex) : easier / more frequent

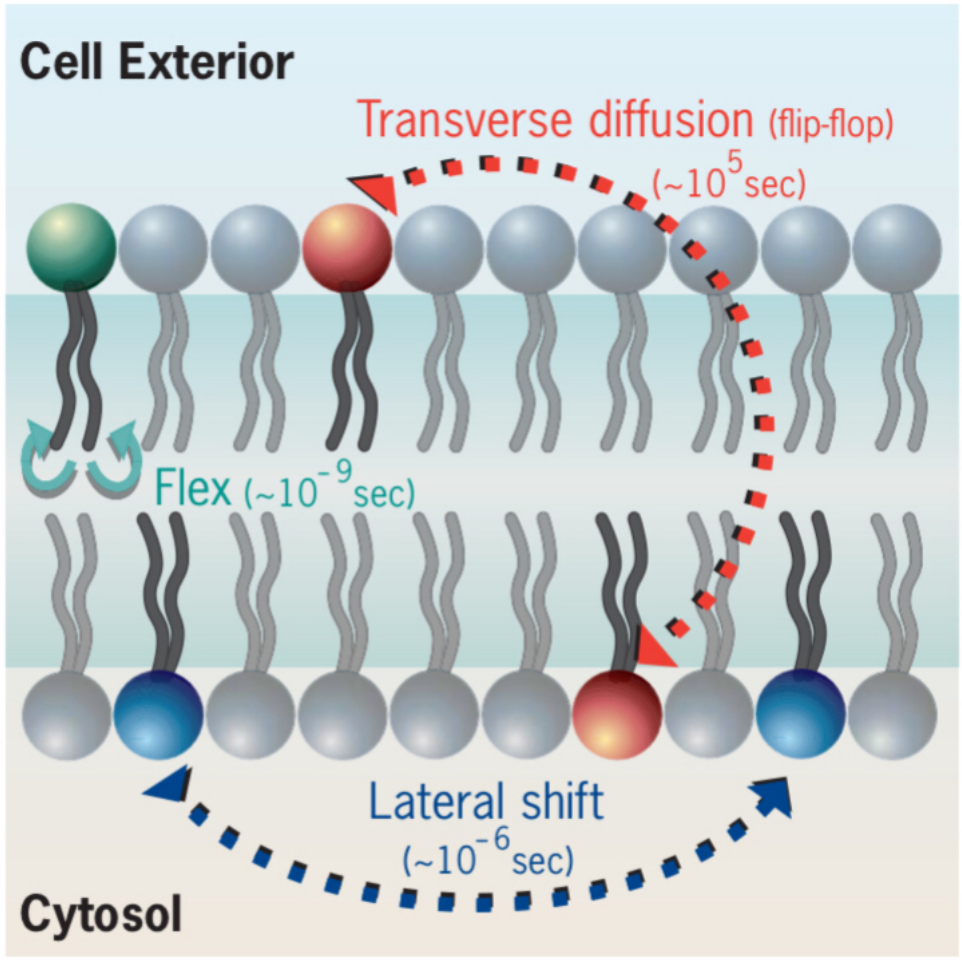


FIGURE 8.25 The possible movements of phospholipids in a membrane.

The diffusion of membrane proteins after cell fusion

1/ the physical state of the lipid is an important determinant of the mobility of integral proteins

2/ **Cell fusion** :
A technique whereby two different types of cells, or cells from two different species, can be fused to produce one cell with a common cytoplasm and a **single**, **continuous** plasma membrane and is used to prepare specific antibodies.

3/ Cells are induced to fuse with one another by making the outer surface of the cells "sticky" and this can be achieved by one of two different ways :
[1] addition of certain inactivated viruses that attach to the surface membrane, by adding the compound polyethylene glycol
[2] mild electric shock

4/ Experiment:
Scientists: Frye & Edidin
Technique: Cell fusion
Microscope: fluorescence light microscope
Process:

a. Mouse and human cells were fused (according to what was mentioned earlier above)

b. follow the distribution of integral proteins , antibodies against one or the other type of protein were prepared and covalently linked to fluorescent dyes (antibodies against the mouse proteins fluoresces green & antibodies against human proteins fluoresces red.) . When the antibodies were added to fused cells, they bound to the human or mouse proteins.

c. At the time of fusion, the plasma membrane appeared half human and half mouse; that is, the two protein types remained segregated in their own hemisphere

d. By about 40 minutes, each species' proteins were uniformly distributed around the entire hybrid cell membrane

تم دمج الخليتين معًا و لتتبع حركة البروتينات تم تحضير أجسام مضادة مرتبطة بمادة مشعة برابطة تساهمية و لهذه الأجسام القدرة على الارتباط بالبروتينات المختلفة لتحديد مواقعها
في البداية فقد بين المجهر أن بروتينات كل كائن حي ما زالت تتركز في الجزء الخاص به من الغشاء البلازمي الجديد إلا أنه و بعد مرور ٤٠ دقيقة توزعت بانتظام على كافة أجزاء الغشاء البلازمي الجديد الناتج عن الاندماج

Note: at lower temperature the membrane's viscosity increased and the proteins mobility decreased

**** this experiment concluded that integral proteins have unrestricted movement but as we will see later this is not completely correct

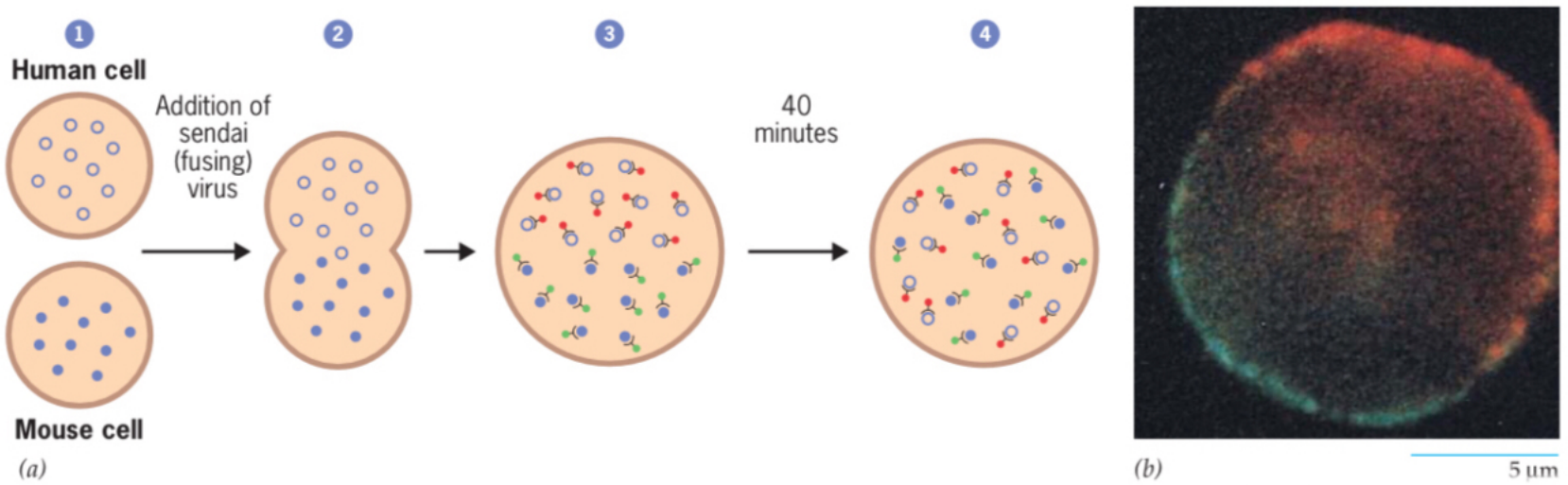


FIGURE 8.26 The use of cell fusion to reveal mobility of membrane proteins.

Restrictions on Protein and Lipid Mobility

Two techniques:

[1] FRAP (fluorescence recovery after photobleaching)

integral membrane components in cultured cells are first labeled by linkage to a fluorescent dye(particular ones can be labeled by certain probe such as fluorescent antibodies) and then cells are placed under the microscope and irradiated by a sharply focused laser beam that bleaches the fluorescent molecules in its path, leaving a circular spot on the surface of the cell that is largely devoid of fluorescence.

The recovery of fluorescence in the bleached region is followed over time (the random movements of the labeled molecules should produce a gradual reappearance of fluorescence in the irradiated circle) which indicates their mobility

Note: The rate of fluorescence recovery provides a direct measure of the rate of diffusion(proportional to it)

Results :

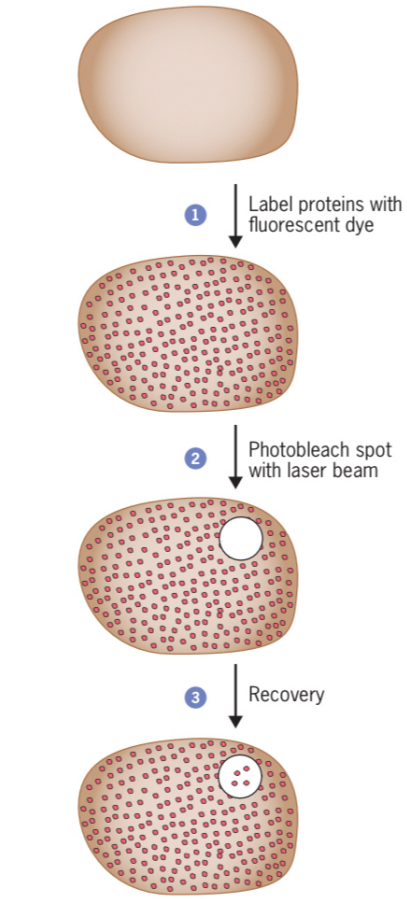
#1# membrane proteins moved much more slowly in the plasma membrane of living cells than they would in a pure lipid bilayer

#2# a significant fraction of membrane proteins (30 to 70 percent) were not free to diffuse back into the irradiated circle.

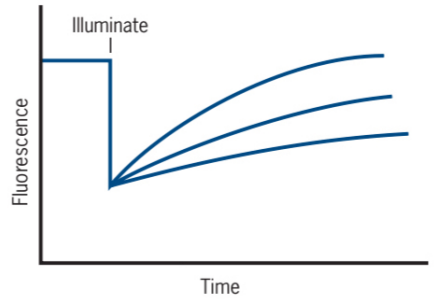
Drawbacks :

#1# FRAP can only follow the average movement of a relatively large number of labeled molecules as they diffuse over a relatively large distance

#2# Using FRAP cannot distinguish between proteins that are truly immobile and ones that can only diffuse over a limited distance in the time allowed.



(a)



(b)

FIGURE 8.27 Measuring the diffusion rates of membrane proteins by fluorescence recovery after photobleaching (FRAP).

An alternate technique is needed >>
[2] single-particle tracking (SPT)
individual membrane protein molecules are labeled . The movements of the labeled molecules are then followed by a type of microscopy known as TIRF (Total Internal Reflection Fluorescence) that is specialized for imaging fluorescent molecules at the surface of cells. The results of these studies often **depend on the particular protein being investigated**.

E.g :

a. Some membrane proteins move randomly throughout the membrane at rates considerably less than would be measured in an artificial lipid bilayer if protein mobility were based strictly on physical parameters such as lipid viscosity and protein size

b. Some membrane proteins fail to move and are considered to be immobilized

c. In some cases, a protein is found to move in a highly directed manner toward one part of the cell or another

d. In most studies, the largest fraction of protein species exhibit random (Brownian) movement within the membrane at rates consistent with free diffusion

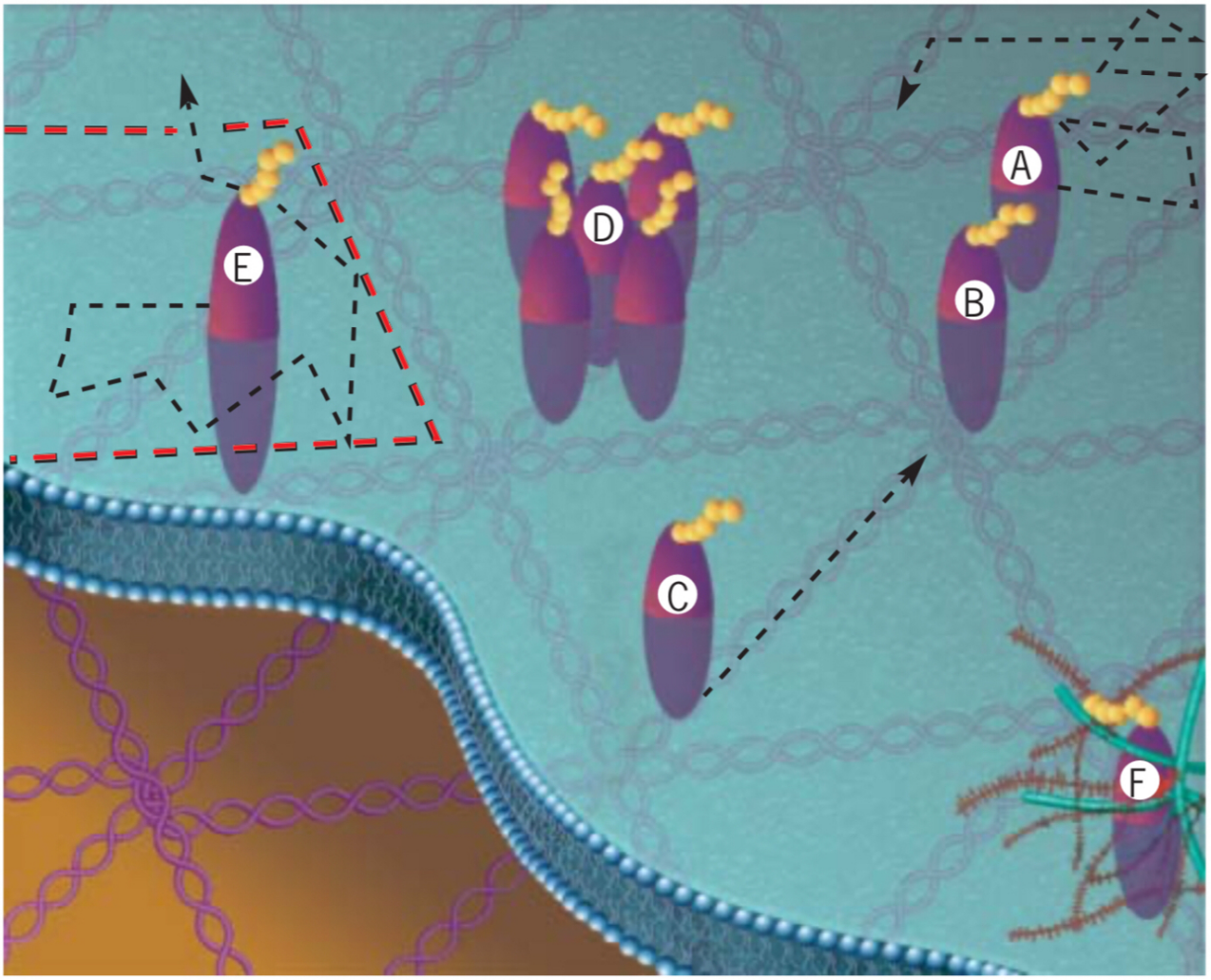


FIGURE 8.28 Patterns of movement of integral membrane proteins.

Control of membrane protein mobility is excluded

Membrane Lipid mobility

The diffusion of phospholipids as many other molecules is restricted

a. When individual phospholipid molecules of a plasma membrane are tagged and followed under the microscope using ultra-high-speed cameras .

b. Computer analysis indicates that the phospho- lipid diffuses freely within one compartment (shaded in purple) before it jumps the “fence” into a neighboring compartment (shaded in blue) and then over another fence into an adjacent compartment (shaded in green), and so forth (hop from one confined area to another.)

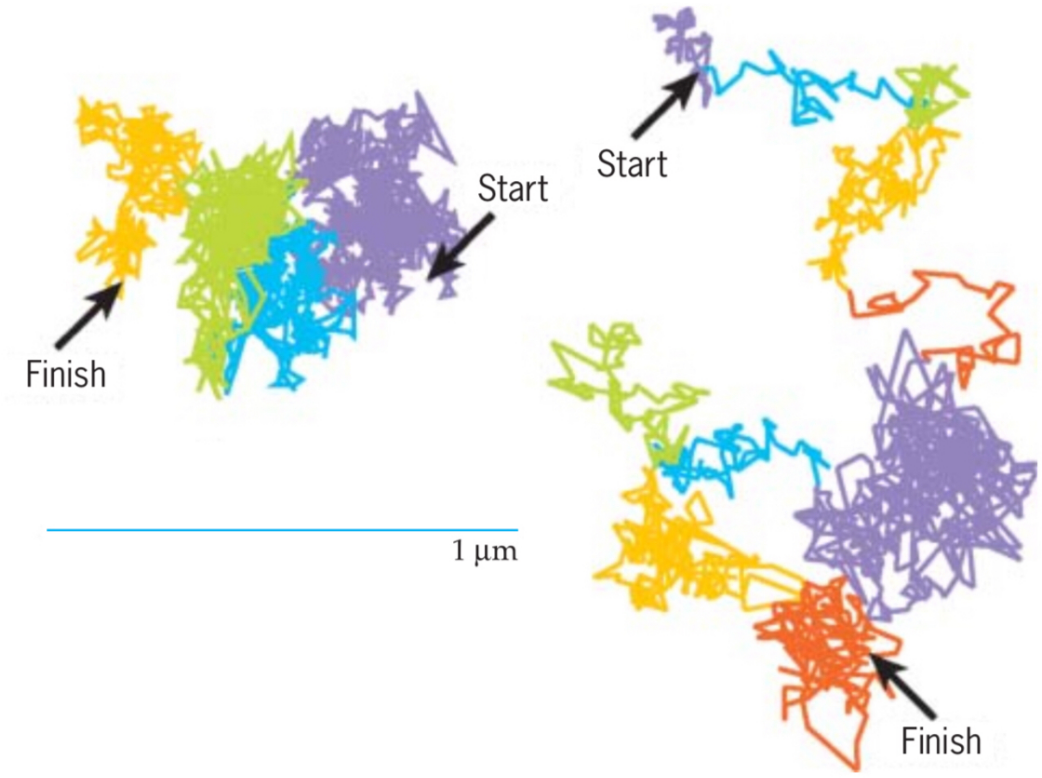


FIGURE 8.29 **Experimental demonstration that diffusion of phospholipids within the plasma membrane is confined.**

It was discovered that those fences were constructed by an underlying membrane skeleton but if the membrane skeleton lies beneath the lipid bilayer, how can it interfere with phospholipid movement?

**** The authors of these studies speculate that the fences are constructed of rows of integral membrane proteins whose cytoplasmic domains are attached to the membrane skeleton and integral proteins are embedded in the lipid bilayer

Membrane domains and cell polarity

***Most membranes, however, exhibit dis- tinct variations in protein composition and mobility, especially in cells whose various surfaces display distinct functions.
E.g :
a. epithelial cells which are highly polarized cells whose different surfaces carry out different functions
Apical surface : absorption from the lumen and secretion to it
Lateral : interaction with adjacent cells
Basal : attachment to ECM
& different membrane proteins and enzymes

b. In other examples, the receptors for neurotransmitter substances are concentrated into regions of the plasma membrane located within synapses , and receptors for low- density lipoproteins are concentrated into patches of the plasma membrane specialized to facilitate their internalization

في أمثلة أخرى ، تتركز مستقبلات مواد النقل العصبي في مناطق من غشاء البلازما الموجود داخل المشابك العصبية ، وتتركز مستقبلات البروتينات الدهنية منخفضة الكثافة في بقع غشاء البلازما المتخصصة لتسهيل استيعابها

c. sperm may have the most highly differentiated structure as a mature sperm can be divided into head, midpiece, and tail

Note: Although divided into a number of distinct parts, a sperm is covered by a continuous plasma membrane

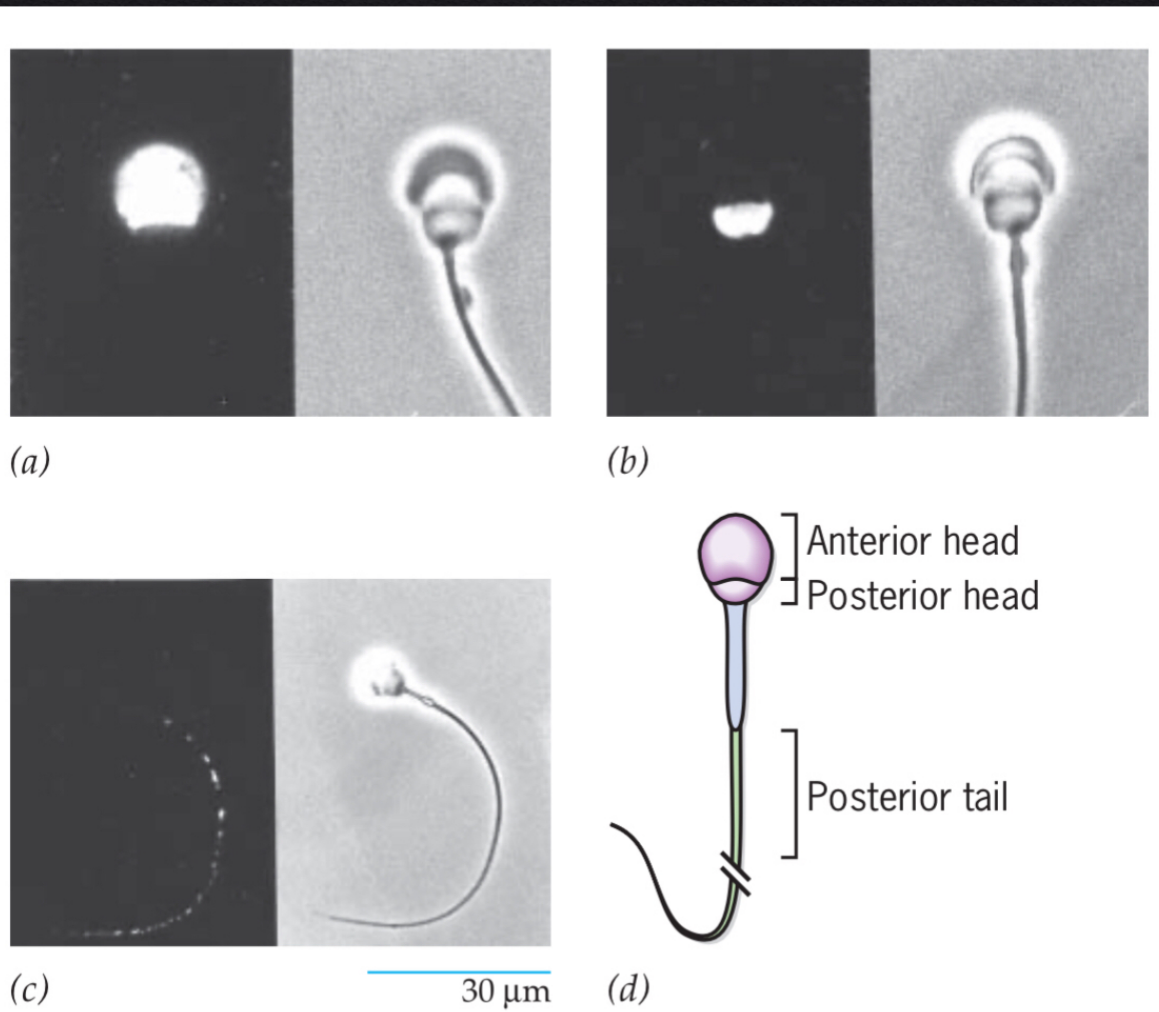


FIGURE 8.31 **Differentiation of the mammalian sperm plasma membrane as revealed by fluorescent antibodies.**

The end

Wish you all the best