Chapter 12 - Membranes & Transport

- Major consituents are lipids & proteins
- Membrane structure best described by "Fluid Mosaic" model
- Membrane proteins are associated with the lipid bilayer in many ways
- Membrane fluidity is critical for many processes
- Movement of molecules across membranes is restricted
- Transport can be "passive" or "active" (energy-requiring)

1

Membranes

- Define external boundaries
- Regulate "traffic" across boundary
- Segregate metabolic processes
- Bar movement of polar compounds

2

Membrane features

- Flexible
- Tough
- Self-sealing
- Selectively permeable
- Contain an array of proteins

3

Molecular consituents of Membranes

table 12–1

Major Components of Plasma Membranes in Various Organisms

	Components (% by weight)				
	Protein	Phospholipid	Sterol	Sterol type	Other lipids
Human myelin sheath	30	30	19	Cholesterol	Galactolipids, plasmalogens
Mouse liver	45	27	25	Cholesterol	—
Maize leaf	47	26	7	Sitosterol	Galactolipids
Yeast	52	7	4	Ergosterol	Triacylglycerols, steryl esters
Paramecium (ciliated protist)	56	40	4	Stigmasterol	—
E. coli	75	25	0	—	—

Varies according to species, tissue and membrane "face"

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Membrane Structure - The Fluid Mosaic Model

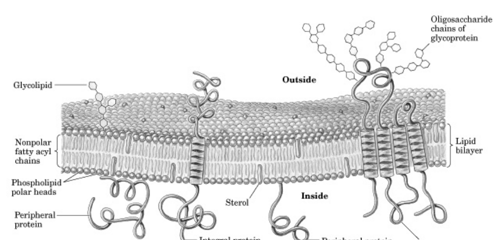

Fig 12.3

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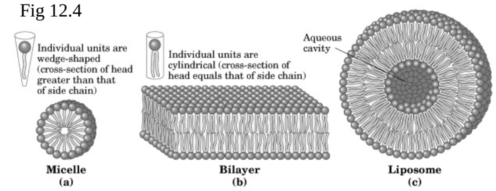
The Lipid bilayer

Fig 12.4



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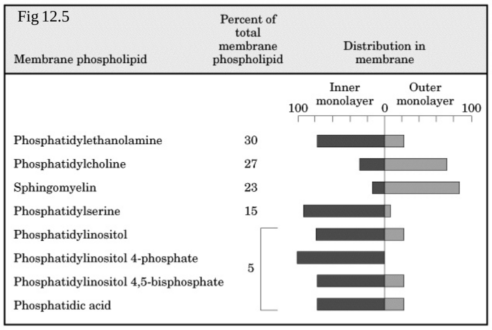
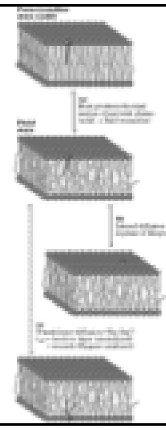
Membrane lipids are asymmetrically distributed



Membrane lipids are in constant motion



Cells regulate lipid composition

table 12–2

Fatty Acid Composition of *E. coli* Cells Cultured at Different Temperatures

	Percentage of total fatty acids*			
	10 °C	20 °C	30 °C	40 °C
Myristic acid (14:0)	4	4	4	8
Palmitic acid (16:0)	18	25	29	48
Palmitoleic acid (16:1)	26	24	23	9
Oleic acid (18:1)	38	34	30	12
Hydroxymyristic acid	13	10	10	8
Ratio of unsaturated to saturated†	2.9	2.0	1.6	0.38

Source: Data from Marr, A.G. & Ingraham, J.L. (1962) Effect of temperature on the composition of fatty acids in *Escherichia coli*. *J. Bacteriol.* **84,** 1260.

*The exact fatty acid composition depends not only on growth temperature but on growth stage and growth medium composition.

†Calculated as the total percentage of 16:1 plus 18:1 divided by the total percentage of 14:0 plus 16:0. Hydroxymyristic acid was omitted from this calculation.

Membrane proteins diffuse laterally in the bilayer

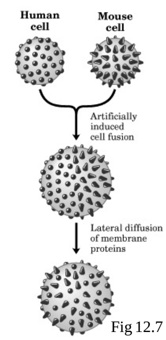
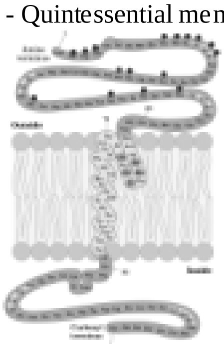


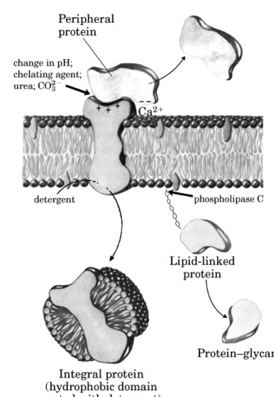
Fig 12.7

Glycophorin - Quintessential membrane protein

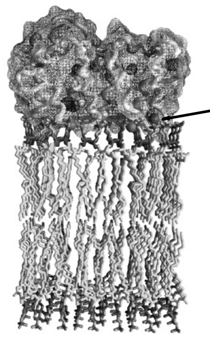


Outer surface, inner surface & transmembrane domains

Peripheral & integral membrane proteins



Electrostatic interactions - 1 biological role for Ca^{2+}



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Covalently attached lipids anchor some peripheral proteins

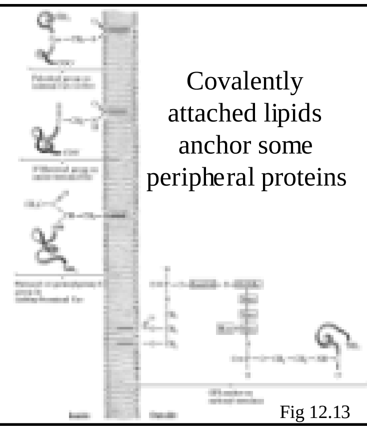

Fig 12.13

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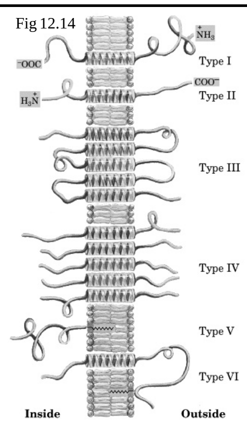
Integral membrane proteins

Membrane-spanning α-helices (and β-sheets)

6 categories

Fig 12.14



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β-sheet-containing integral membrane proteins have "β-barrel" motifs

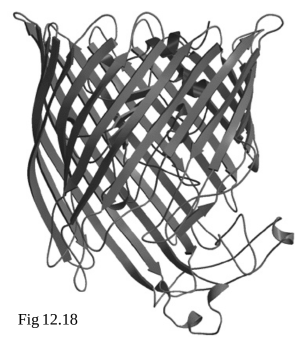

Fig 12.18

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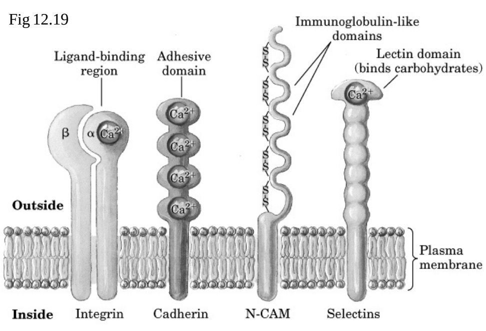
Integral membrane proteins mediate cell-cell interactions

Fig 12.19



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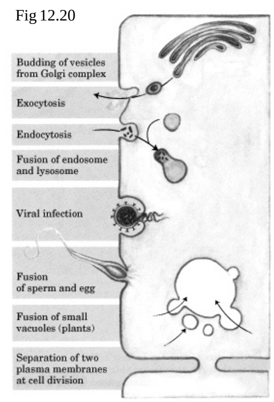
Membrane fusion central to many biological processes

Fig 12.20



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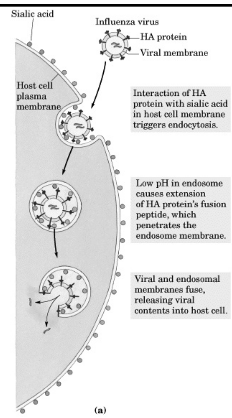
Influenza virus infection



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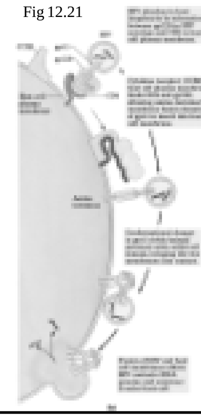
HIV infection (AIDS)

Fig 12.21



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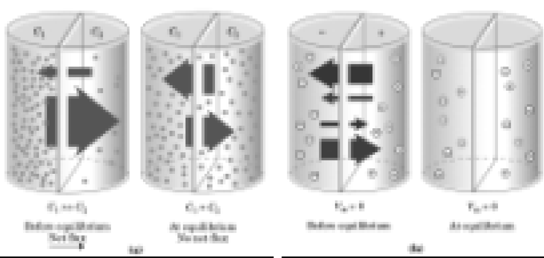
Solute transport across membranes



Concentration gradient | Electrochemical gradient

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Opposition to solute movement across membranes

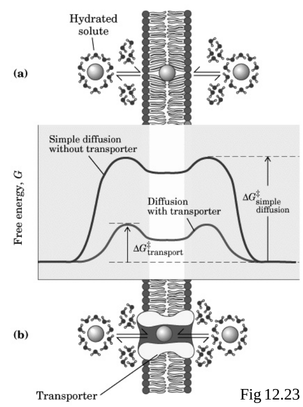

Fig 12.23

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Transmembrane passage of polar compounds

- Integral membrane proteins (transporters)
- "Passive" or "active" transport
- Action analogous to enzymes
  - Bind substrates via multiple weak interactions
  - Negative free energy of binding counterbalances positive free energy of H_2O removal
  - Span of lipid bilayer by transporter provides alternative path for substrate movement

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3 examples of Passive transport (RBC's)

- Aquaporins (H_2O)
- Glucose Transporter
- Chloride-bicarbonate exchanger

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Passive transport - Hallmarks

- High rates of diffusion <u>down</u> concentration or electrochemical gradients (high → low)
- Saturability of transporter
- Specificity of transporter

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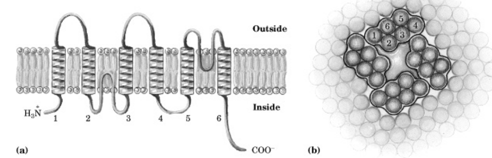
Aquaporins

Fig 12.24



Channel for <u>rapid</u> ($5x10^8$ s^{-1}) movement of water

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Glucose transporter (GluT1)

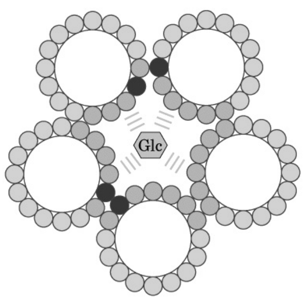

Fig 12.25 (c)

50,000 x more glucose transported

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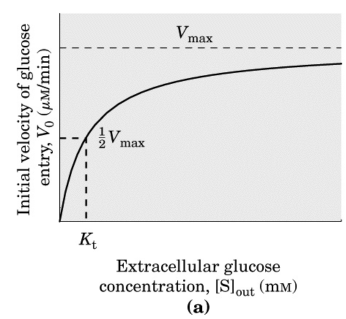
Transport analogous to enzymatic reaction



Substrate = $Glucose_{outside}$

Product = $Glucose_{inside}$

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Blood [glucose] = 5 mM

GluT1 K_T = 1.5 mM

GluT1 saturated - operates at V_{max}

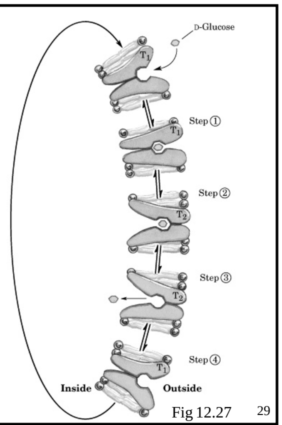

Fig 12.27

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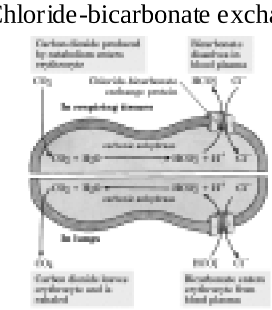
Chloride-bicarbonate exchanger



- <u>Cotransport</u> system: simultaneous & <u>opposite</u> movement of solutes
- Essential for CO_2 transport; ↑ permeability by 10^6

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Cotransport systems

- Antiport (Chloride-bicarbonate exchanger): 2 substrates in opposite direction
- Symport: 2 substrates in same direction
- Uniport (Glucose transporter): one substrate

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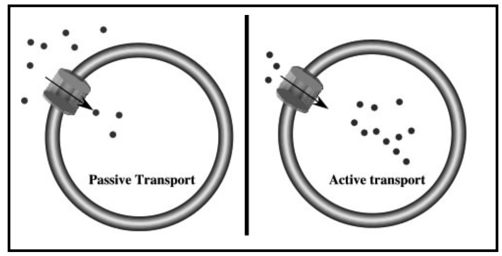
Active transport moves solutes against their concentration or electrochemical gradient



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Primary & Secondary Active Transport

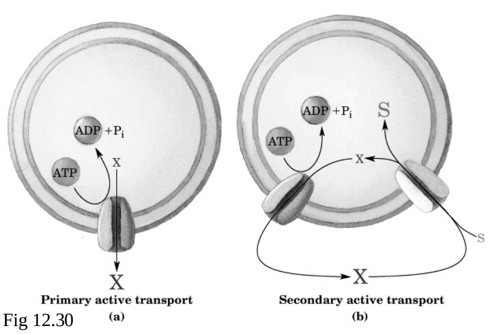

Fig 12.30

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Active Transport - ATPase

table 12–4

Four Classes of Transport ATPases

	Organism or tissue	Type of membrane	Role of ATPase
P-type ATPases			
Na^+K^+	Animal tissues	Plasma	Maintains low [Na^+], high [K^+] inside cell; creates transmembrane electrical potential
H^+K^+	Acid-secreting (parietal) cells of mammals	Plasma	Acidifies contents of stomach
H^+	Fungi (*Neurospora*)	Plasma	Create H^+ gradient to drive secondary transport of extracellular solutes into cell
H^+	Higher plants	Plasma	
Ca^{2+}	Animal tissues	Plasma	Maintains low [Ca^{2+}] in cytosol
Ca^{2+}	Myocytes of animals	Sarcoplasmic reticulum (endoplasmic reticulum)	Sequesters intracellular Ca^{2+}, keeping cytosolic [Ca^{2+}] low
Cd^{2+}, Hg^{2+}, Cu^{2+}	Bacteria	Plasma	Pumps heavy metal ions out of cell
V-type ATPases			
H^+	Animals	Lysosomal, endosomal, secretory vesicles	Create low pH in compartment, activating proteases and other hydrolytic enzymes
H^+	Higher plants	Vacuolar	
H^+	Fungi	Vacuolar	
F-type ATPases			
H^+	Eukaryotes	Inner mitochondrial	Catalyze formation of ATP from ADP + P_i
H^+	Higher plants	Thylakoid	
H^+	Prokaryotes	Plasma	
Multidrug transporter			
	Animal tumor cells	Plasma	Removes a wide variety of hydrophobic natural products and synthetic drugs from cytosol, including vinblastine, doxorubicin, actinomycin D, mitomycin, taxol, colchicine, and puromycin

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P-type ATPases

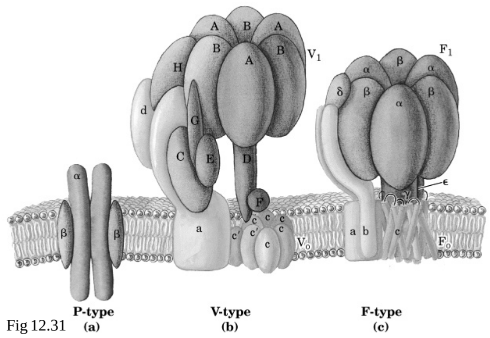

Fig 12.31

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P-type ATPase: The Na^+K^+ATPase

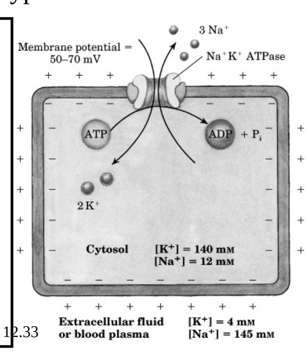

Fig 12.33

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Each ATP hydrolysis:

$3\ Na^+$ out of cell

$2\ K^+$ into cell

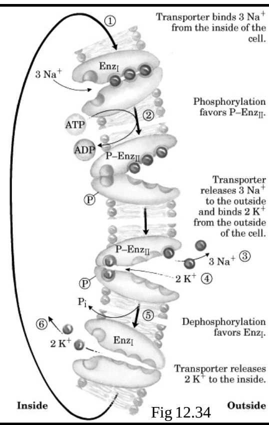

Fig 12.34

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V-type ATPases

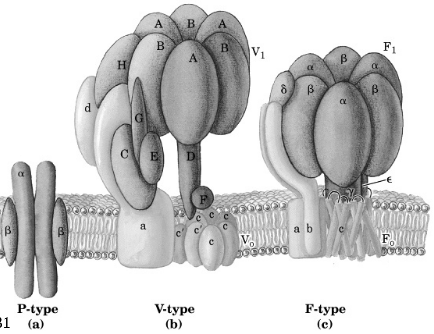

Fig 12.31

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F-type ATPases

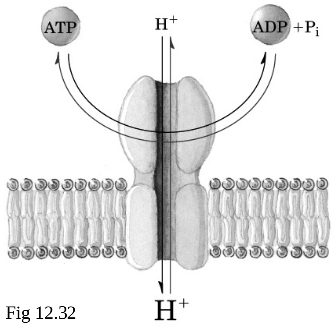

Fig 12.32

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"Bad ATPase" - The multidrug transporter

- Found in plasma membrane of some tumor cells
- ATP transporter
- Pumps many hydrophobic drugs <u>out</u> of tumor cells

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ATP-driven Ca^{2+} pumps (P-type)

- Ca^{2+} far lower inside vs. outside cells
- Ca^{2+} forms insoluble compounds with phosphate
- Ca^{2+} pumped out of cells by specific pumps

- Other ATPases (SERCA's) move Ca^{2+} into the ER and sarcoplasmic reticulum (SR) of myocites
- Similar to Na^+K^+ ATPase -cycling between phosphorylated & dephosphorylated forms

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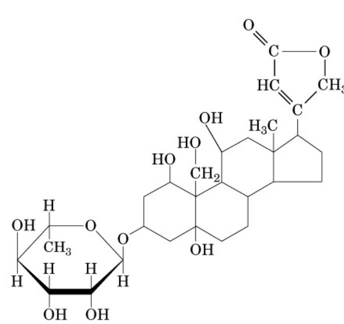
ATPases are targets of many poisons & medicines



Ouabain

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Ion gradients provide the energy for 2° active transport

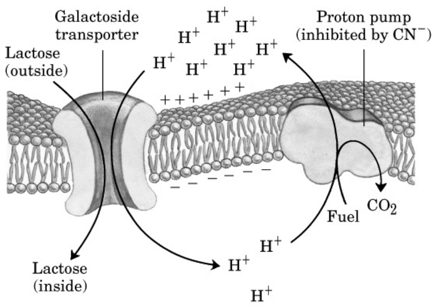

Fig 12.35

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Glucose transport in intestinal epithelial cells

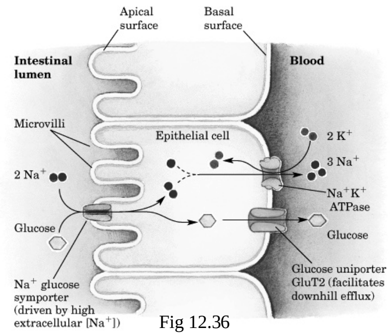

Fig 12.36

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Ionophores "short circuit" ion gradients

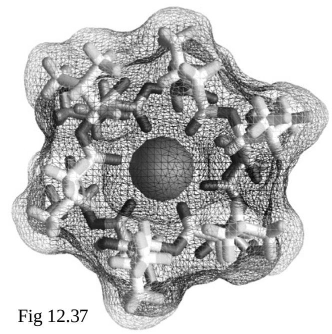

Fig 12.37

Valinomycin - potent antibiotic

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Ion channels allow rapid movement of ions across membranes

Ion channels distinguished from transporters

- Flux much higher than channels ($\uparrow 10^8$ ions·s^{-1})
- Ion <u>channels</u> are <u>not</u> saturable
  - No V_{max}, linear increase with concentration
- Ion channels are <u>gated</u>
  - Ligand-gated channels
  - Voltage-gated channels

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3 examples

- Bacterial K^+ channel
- Acetylcholine receptor ion channel
- Voltage-dependent Na^+ channel (neurons)

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Bacterial K^+ channel

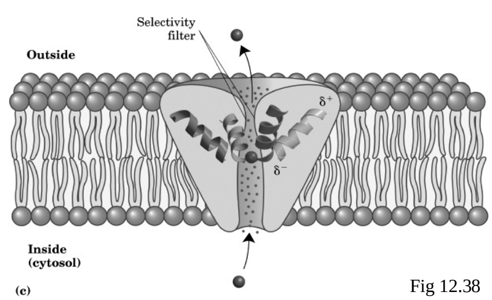

Fig 12.38

Selectivity filter screens out alternative ions

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Acetylcholine receptor

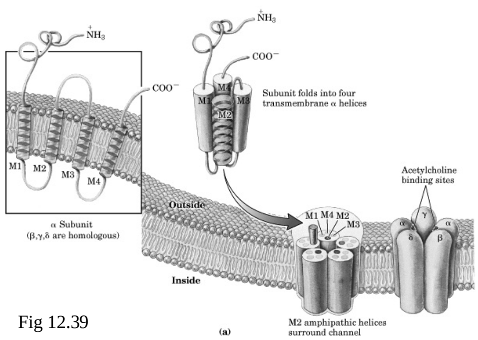
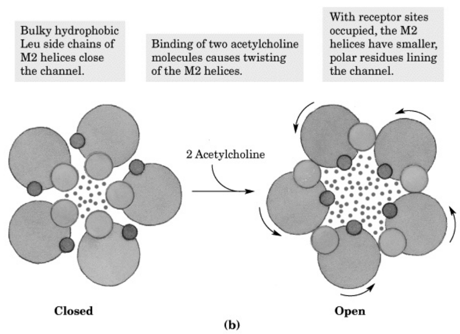


Fig 12.39

A ligand-gated ion channel

Acetylcholine binding → conformational change



Clears channel for ion passage

The neuronal Na^+ channel

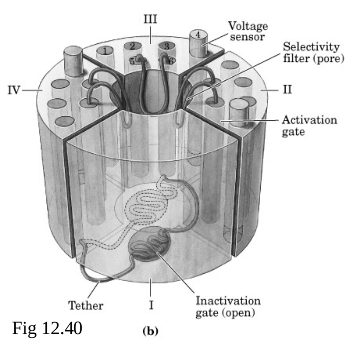


Fig 12.40

A voltage-gated ion channel

Movement of helix 4 in response to change in transmembrane potential triggers opening

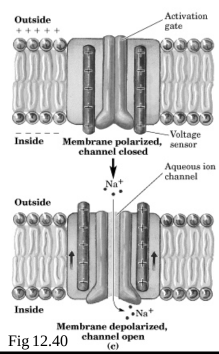


Fig 12.40

Inactivation "gate" closes opening

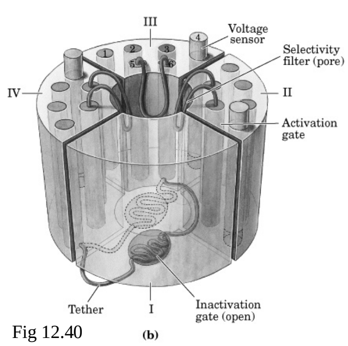


Fig 12.40

Length of time depends on length of protein tether

Naturally occuring toxins target ion channels

Puffer fish

Curare - arrow poison

Porins are channels for small molecules

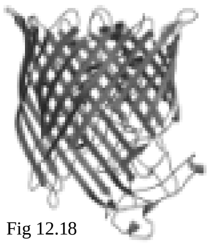


Fig 12.18

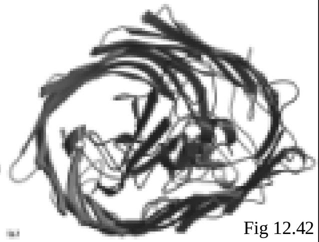


Fig 12.42

Allow passage of molecules much larger than ions, but in manner more similar to ion channels

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Summary of transport systems

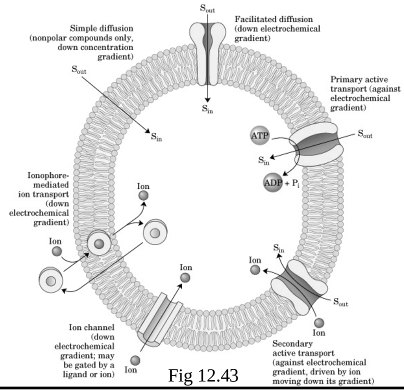

Fig 12.43

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Chapter 12 - Summary

- Biological membranes
  - Define cellular boundaries
  - Divide cells into discrete compartments
  - Organize reaction sequences (pathways, etc.)
  - Roles in signal reception, transduction
- Membrane composition
  - Lipids (phospholipids, sphingolipids, glycolipids)
  - Protein (peripheral & integral)
  - Varies in composition according to species, cell type, organelle & even “face” of membrane

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- Fluid Mosaic Model
  - Lipid bilayer
  - Fatty acyl chains of phospholipids & sterols oriented towards interior
  - Lipids & proteins free to diffuse laterally
  - Fluidity affected by temperature, fatty acid composition (saturation) & sterol content
- Cells regulate their lipid composition to reflect changes in their environment

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Membrane proteins

- Peripheral
  - Loose association with membrane
  - Electrostatic interactions, H-bonds or covalent attachment to lipids
- Integral
  - Firm association with membrane
  - Hydrophobic interactions with lipid bilayer
  - Both α-helical and β-sheet-type structures

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Membranes are <u>structurally</u> & <u>functionally</u>

Asymmetric

- Two sides differ in lipid/protein composition
- Different functional roles (adhesion, signal reception vs. cytoskeleton attachment, exocytosis, etc.)
- Variety of proteins mediate protein fusion (SNARE’s)

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Lipid bilayer is impermeable to polar substances
- Exception = water (concentration v. high)
- Other polar molecules cross via transporters or ion channels

Transporters
- Similar to enzymes - show saturation & substrate specificity
- Passive or Active transport
  - Passive - down an electrochemical gradient
  - Active - against an electrochemical gradient, requires energy (ATP , oxidation, light, 2º transport)

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Transport
- Uniport: single species transport
- Symport: simultaneous transport, same direction
- Antiport: simultaneous transport,opposite direction

Differences in $[Na^+]$ & $[K^+]$ between inside/outside of Cell maintained by active transport via Na^+K^+ ATPase
- Resulting gradient used as energy source for 2º transport

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4 general types of ATPases
- P-type
  - Reversible phosphorylation
  - Vanadate inhibition (P_i analog)
  - Ex's: Na^+K^+ ATPase, SERCA (Ca^{2+} transporter)
- V-type
  - Produce proton gradients across intracellular membranes
- F-type
  - Produce proton gradients
  - Reverse reaction (Proton flow) drives ATP synthesis
  - Mitochondria & chloroplasts

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- Multidrug transporter
  - Tumor cells
  - Energy of ATP to export hydrophobic drugs
- Ionophores
  - Lipid soluble
  - Bind & transport ions across membranes
  - Short-circuit electrochemical gradients

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Ion channels
- Provide hydrophilic pores for movement of specific ions
- Movement down electrochemical gradient
- Differ from transporters
  - Unsaturable
  - V. high flux rate
- Gated by ligand binding or voltage potentials
- K^+ channel prototype
  - Ion movement restricted by “selectivity filter”
  - Protein domain “gate” obstructs channel when not activated

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Porins
- Integral membrane β-barrel proteins
- Substrate binding opens pore, allowing movement of molecule to interior

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