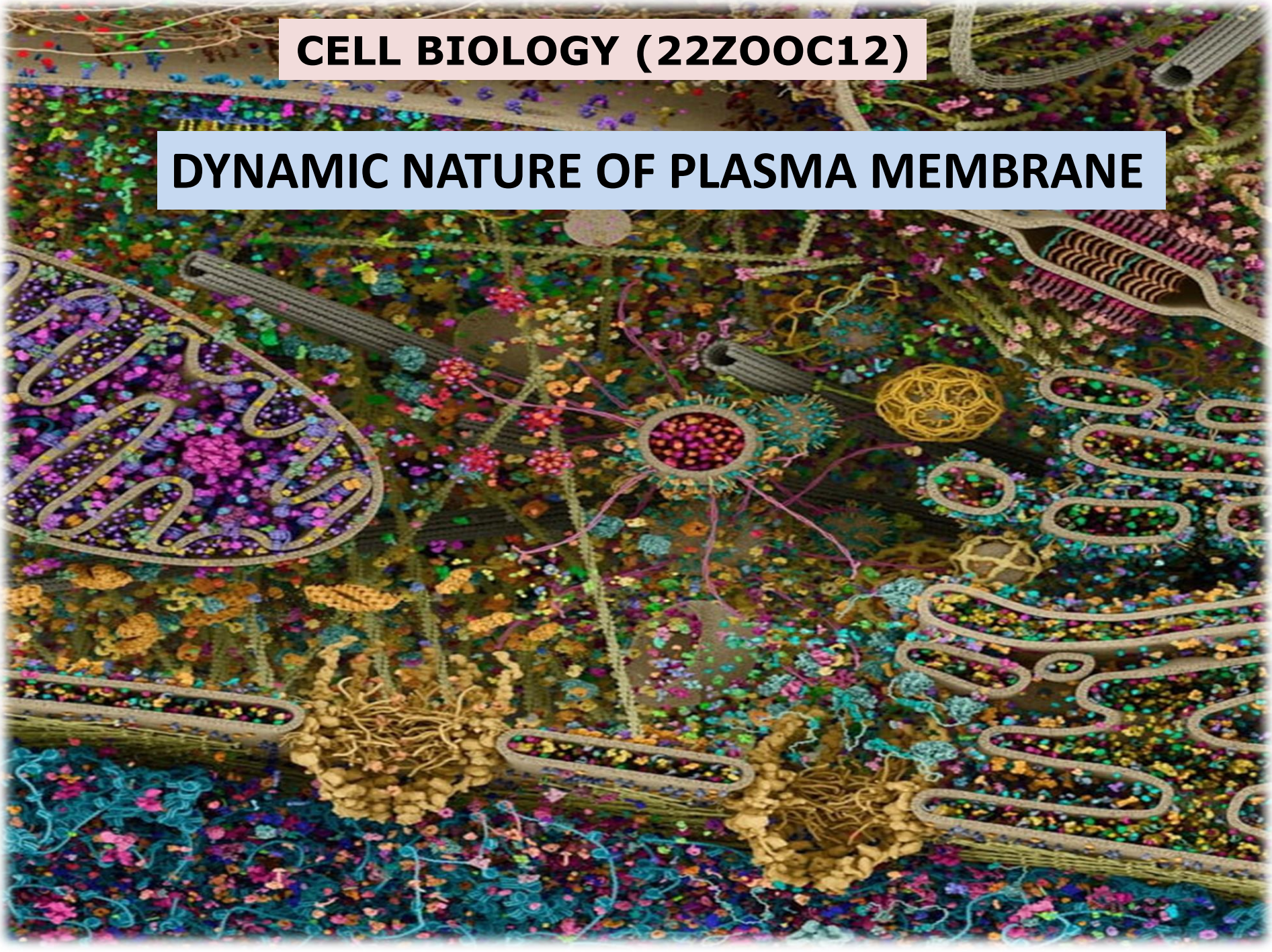
CELL BIOLOGY (22ZOOC12)

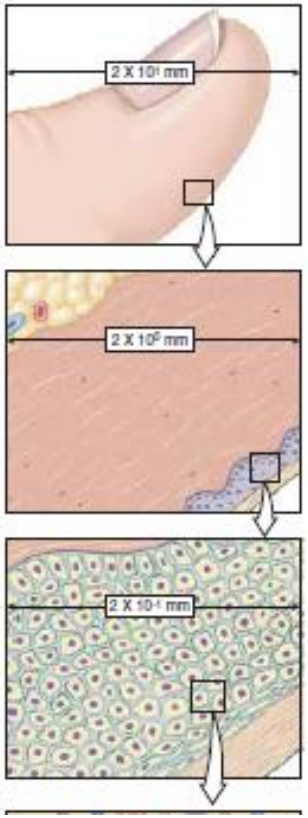


DYNAMIC NATURE OF PLASMA MEMBRANE

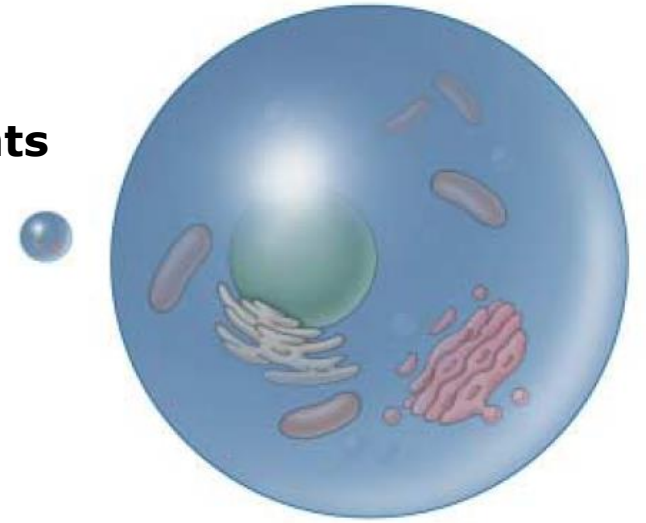
Why Aren't Cells Larger?

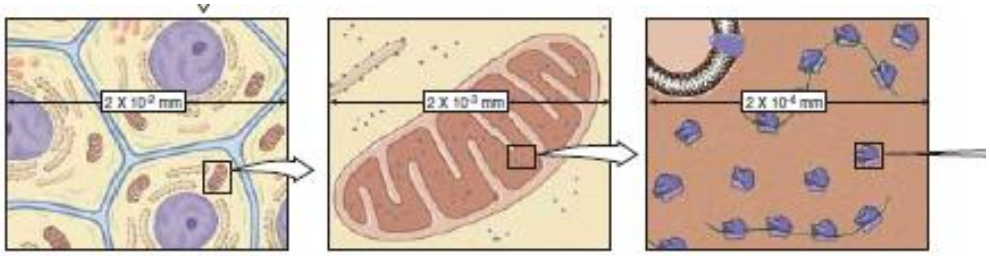
Multicellular organisms usually consist of many small cells rather than a few large ones because **small cells function more efficiently**.

They have a **greater relative surface area**, enabling more rapid communication between the center of the cell and the environment.



The size of cells and their contents





Cell radius (r)	1 cm	10 cm
Surface area ($4\pi r^2$)	12.57 cm^2	1257 cm^2
Volume ($\frac{4}{3}\pi r^3$)	4.189 cm^3	4189 cm^3

Transport across plasma membrane

1. **Internal composition of cell is maintained** because of the pm (Selectively permeable) structure.

2. It is a **barrier** for free exchange of molecules.

3. In a **protein free** phospholipid bilayer (or Synthetic lipid bilayer)

   Hydrophobic molecules (O_2, CO_2, N_2, Benzene)
   Small uncharged polar molecules (H_2O, Urea, Glycerol)
   Large uncharged polar molecules (Glucose, Sucrose)

BILAYER IS "**PERMEABLE**"

- **Rate of Diffusion :**

FICK'S LAW OF DIFFUSION

$$J = - D \left[\frac{\triangle C}{\triangle X} \right]$$

J = Flux / unit area

D = Diffusion coefficient (cm^2/ sec)

$\triangle$C = Concentration difference between two regions

$\triangle$ x = distance between two regions (membrane thickness)

Gases (O2, CO2, N2)
Hydrophobic molecules (Benzene)
Small uncharged polar molecules (H2O, Urea, Glycerol)
Large uncharged polar molecules (Glucose, Sucrose)

PASSIVE TRANSPORT

* Occurs along concentration gradient.
* No usage of metabolic energy.

Types:

a. SIMPLE DIFFUSION: (Non selective process)

- Molecule simply **dissolves** in plb and **diffuses** across it.
- **No** membrane proteins are involved.
- Direction of transport is by **concentration** – inside and outside of the cell.
- **Relative Diffusion rate ∞ Concentration gradient + Hydrophobicity + Size**

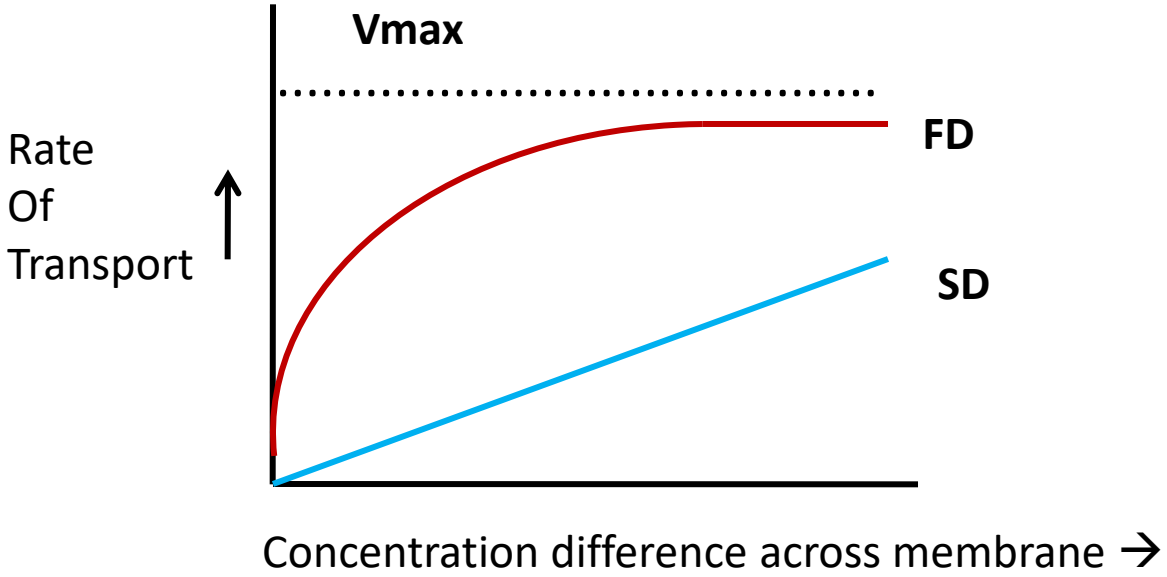
- Hydrophobicity of a substance is measured by its **Partition coefficient.**

"Equilibrium constant for partition of the molecule between oil & water".

- Movement of solutes by diffusion is always **from [higher] to a [lower].**

b. FACILITATED DIFFUSION:
(Non selective ; Transport protein process)

- Movement of molecules by their relative concentration.
- By transport proteins - **Permease** (it is **Selective**).
- Rate of transport is **greater.**
- It **allows polar & charged molecules** (Carbohydrates, AA, nucleosides, ions).



Plasma membrane : It allows polar molecules (Ions, amino acids, etc).

- Presence of special transport proteins (CARRIER / TRANSPORT) are responsible.

- **TRANSPORT:**
  - LIPID BILAYER
  - TRANSPORT PROTEINS

Thus transport across pm is of two types:

1. **PASSIVE TRANSPORT**

   *** Simple &**

   *** Facilitated Diffusion** (Proteins – Channel; Carrier and Ionophores)

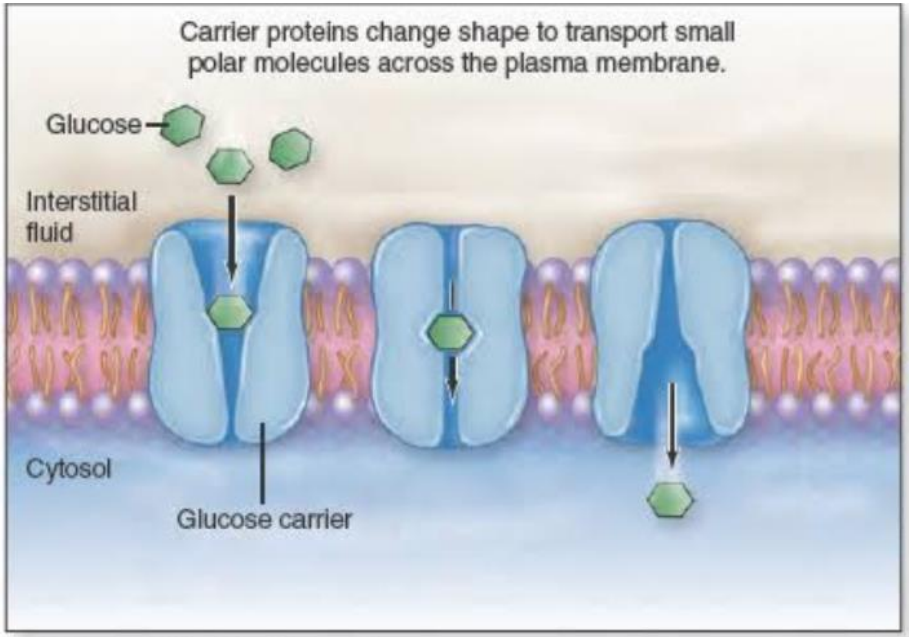
2. **ACTIVE TRANSPORT**

   *** Primary (direct) type**

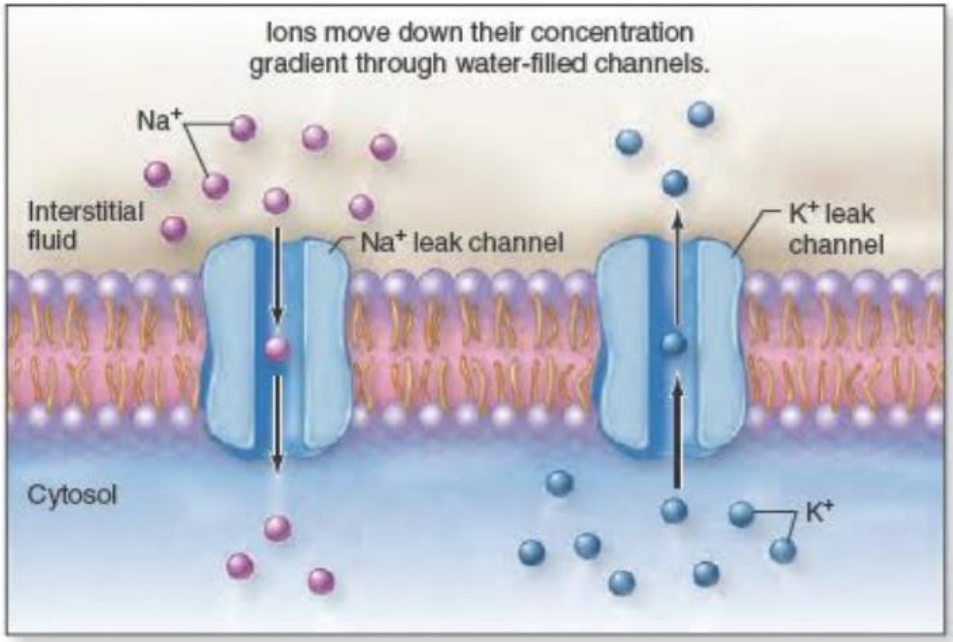
   *** Secondary (indirect) active transport**

Classes of proteins mediating facilitated diffusion:

1. Carrier mediated proteins (Transporters / Permeases)
2. Channel mediated proteins



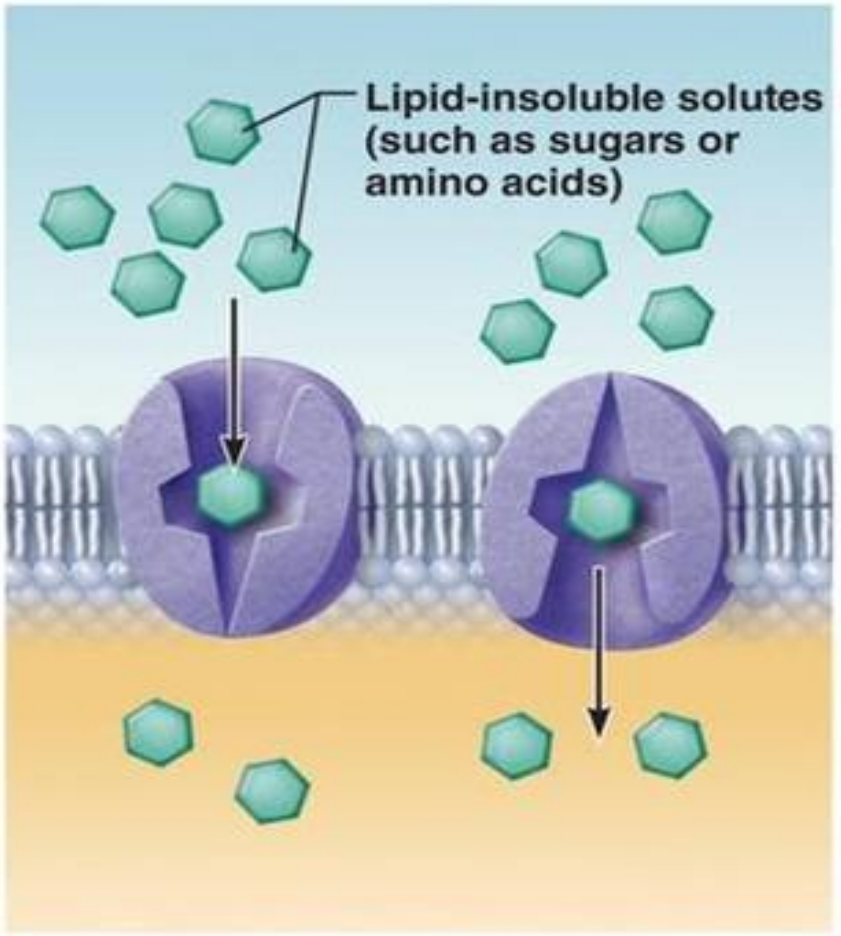
(b) Carrier-mediated diffusion



(a) Channel-mediated diffusion

1. Carrier proteins mediated (Transporters / Permeases)

- **Binds covalently** to specific molecules for transporting on one side of membrane.
- Then undergo **conformational changes** → molecules to release on other side.



(b) Carrier-mediated facilitated diffusion
via protein carrier specific for one chemical; binding of substrate causes transport protein to change shape

GLUT (GLUcose Transporters) – Movement of glucose mediated by carrier protein found in plasma membrane

GLUT family – Isomers:

~ 500 AA; 12 transmembrane segments.

GLUT- 1 (RBC)
GLUT- 2 (Liver)
GLUT- 3 (Brain)
GLUT- 5 (small intestine)

} at Extracellular surface

GLUT – 4 (Predominant) → Intracellular vesicles
(Skeletal muscle ; Adipose tissue)

Eg: GLUT 4 (Insulin sensing)

GLUT 2 (Glucose sensing)

RATE OF TRANSPORT (V) described by the equation:

$$V = V_{max} / (1 + K_m / C)$$

V max = Maximal transport velocity

Km = Substrate (glucose) concentration at which half maximal transport is attained

C = Concentration of substrate

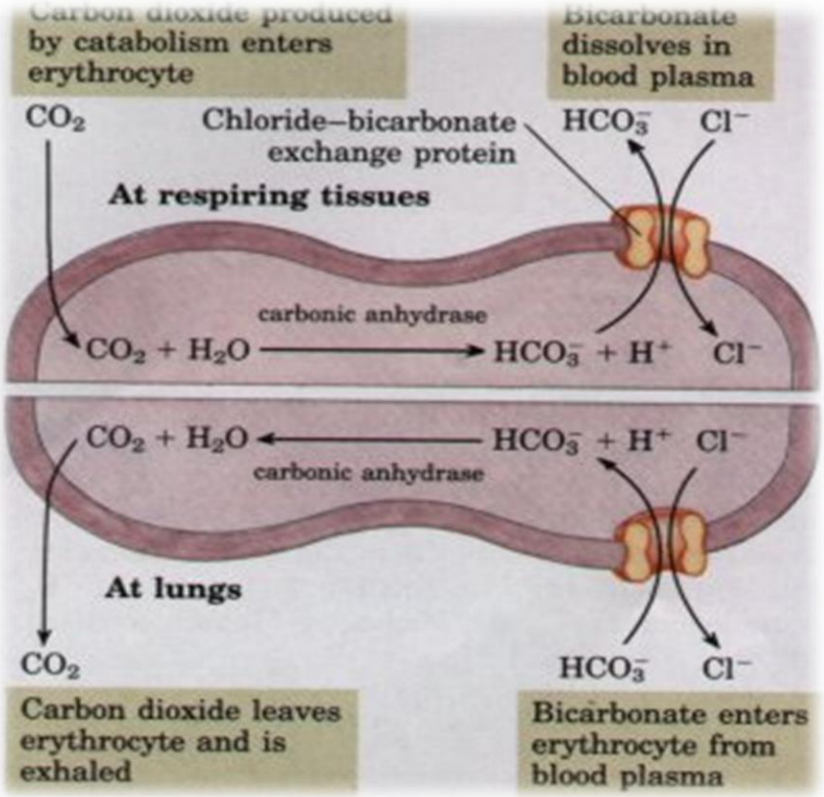
This relationship is formally equivalent with **Michaelis-Menten equation**

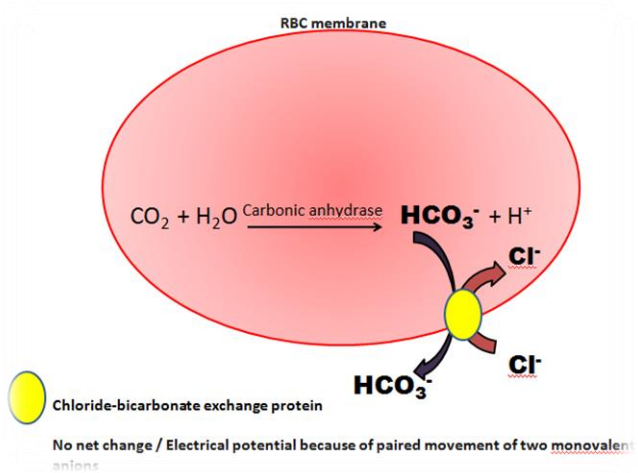
It relates velocity of **enzyme catalyzed reactions to [Substrate].**

Eg: In RBC, **CHLORIDE – BICARBOANATE EXCHANGER (Anion exchange protein)**

- It mediates transport of two anions simultaneously.
- HCO_3^- and Cl^-
- Both anions move in opposite direction.
- Exchange is **eletroneutral**

(no net transfer of charge).



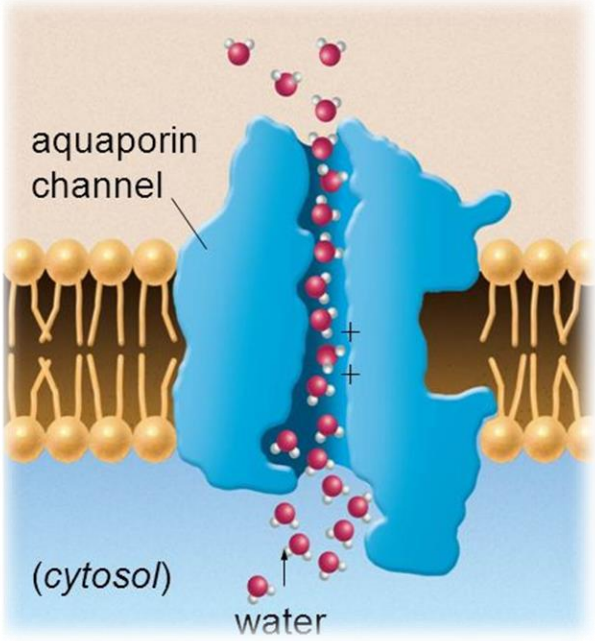


Anion exchanger1 (AE1) or band 3

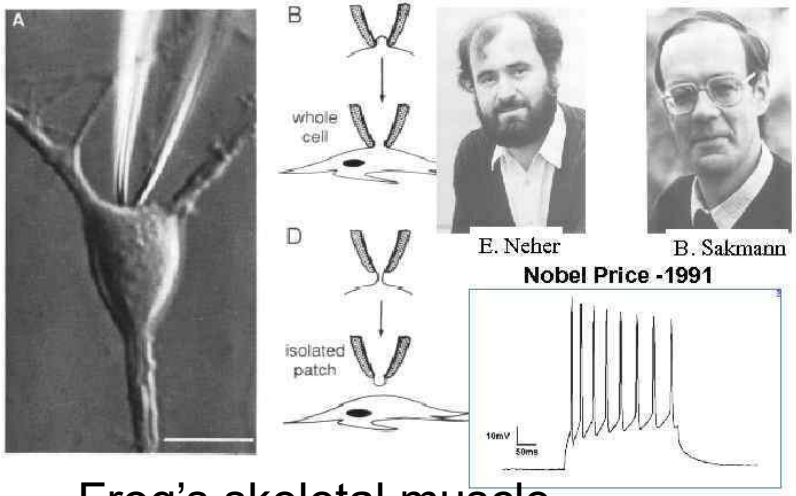
2. Channel mediated proteins:

- Channel proteins form **open pores** through membrane.
- Allows **free diffusion** of molecules (size & charge).
- **Rate of diffusion is more** (* than carrier mediated).
- Specific for **inorganic ion transport** (ION CHANNELS) & highly **selective**.

Eg: Animal cells have **specialized water channel** in pm → **AQUAPORINS.**

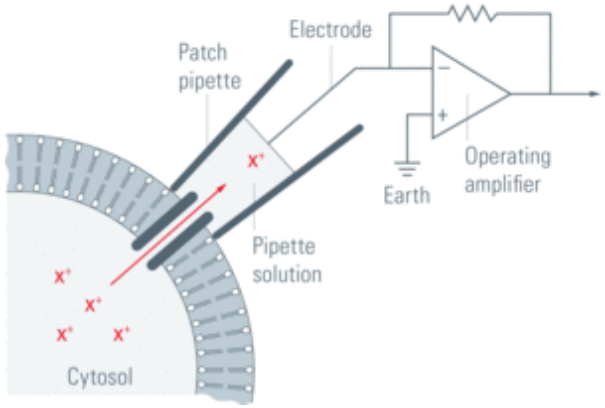


PATCH-CLAMP

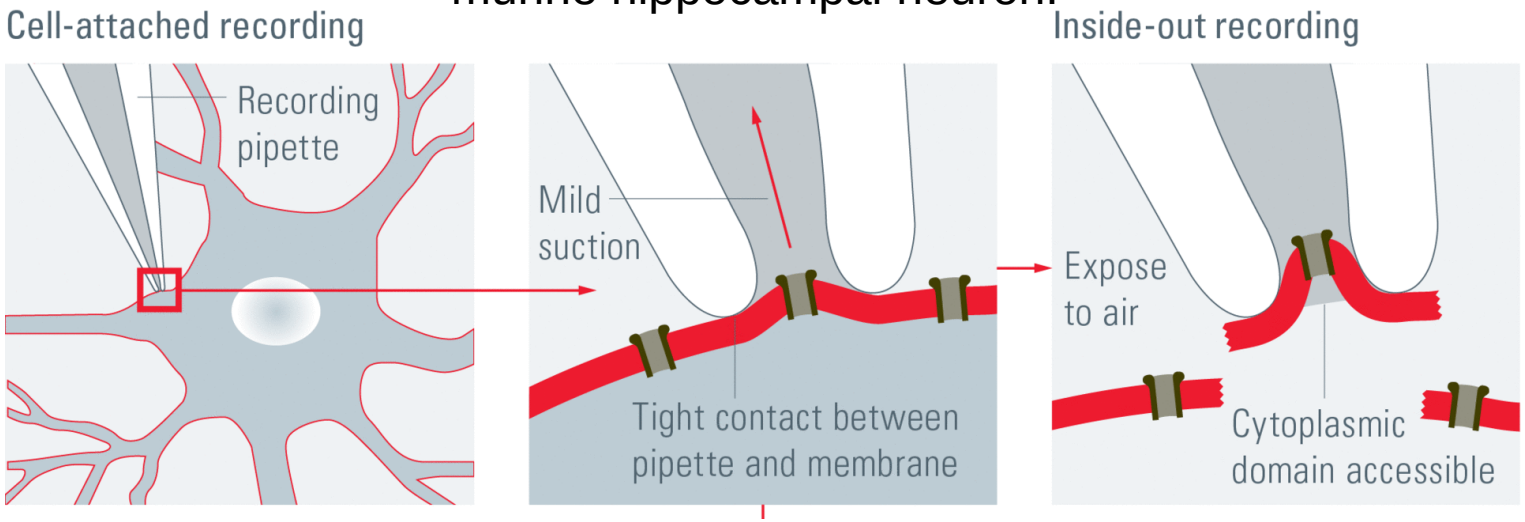


Frog's skeletal muscle

Technique (Membrane potential)

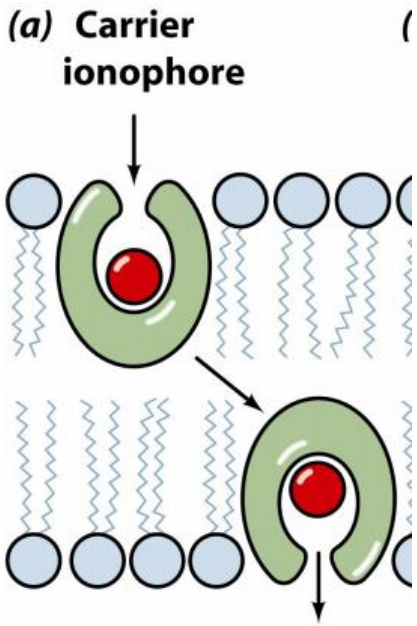


A phase contrast image of a patch pipette attached to the membrane of a cultured murine hippocampal neuron.



Courtesy of Dr. Ainhara Aguado, Ruhr University Bochum, Germany

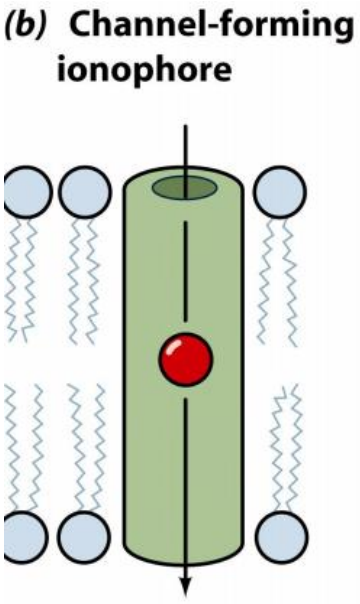
3. IONOPHORES:



- Small **hydrophobic lipid – soluble** molecules.
- It dissolve in lipid bilayers.
- They increase their permeability to specific inorganic ions.
- Ionophores shield the charge of the ion to be transported.
- Thus it **penetrate hydrophobic interior** of lipid bilayer.

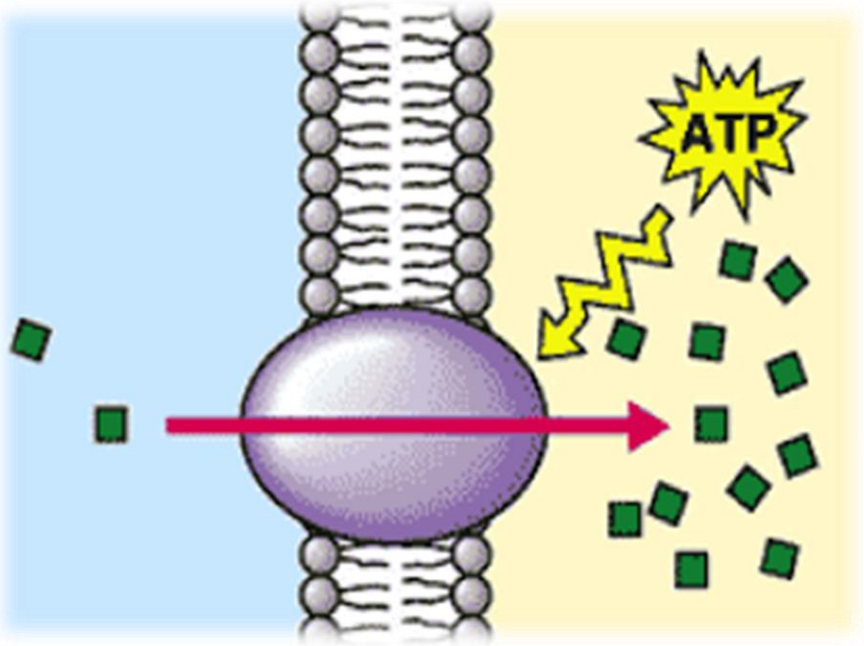
Two Classes of ionophores:

1. **Mobile ion carriers**
2. **Channel formers**



ACTIVE TRANSPORT

- Occurs **against concentration gradient**.
- Mediated by **carrier proteins**.
- **Metabolic energy is utilized** to move ions or molecules against concentration gradient.



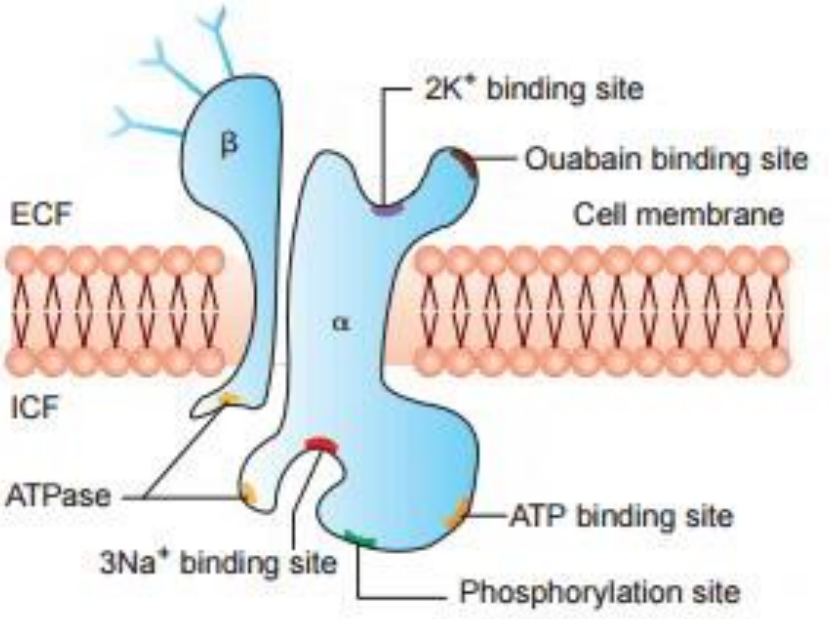
Active transport is of two types:

1. **Primary (direct) active transport**
2. **Secondary (indirect) active transport**

Primary (direct) active transport

Primary active transport utilizes energy in form of ATP to **transport** molecules across a membrane against their concentration gradient.

Therefore, all groups of ATP-powered pumps contain **one or more ATP binding sites** on the **cytosolic domain** of the membrane.



The ATPase Family of Transporters:

- ATP hydrolysis + Auto-phosphorylation of the transporter.
- **Four** primary types of ATPase transporters that function in eukaryotes:
  - **E-type ATPases (E**xtracellular transport**)**
  - **F-type ATPases / $F_0 - F_1$** ATPase **(**Oxidative phosphorylation **F**actor**)**
    - Inner mitochondria (non-photosynthetic eukaryotes)
  - **P-type ATPases (P**lasma membrane **)**
    - Na^+ - K^+ ATPase (pm of Animal cells)
    - H^+ ATPase (pm of fungi & plants)
    - Ca^+ ATPase (Sarcoplasmic reticulum)

    } Electric potential (inside -ve)
  - **V-type ATPases (Endomembranes - V**acuoles**)** – **No** phosphorylation
  - **A-type ATPases (A**rchaeal bacterial transporters**)**

$Na^+ - K^+$ pump

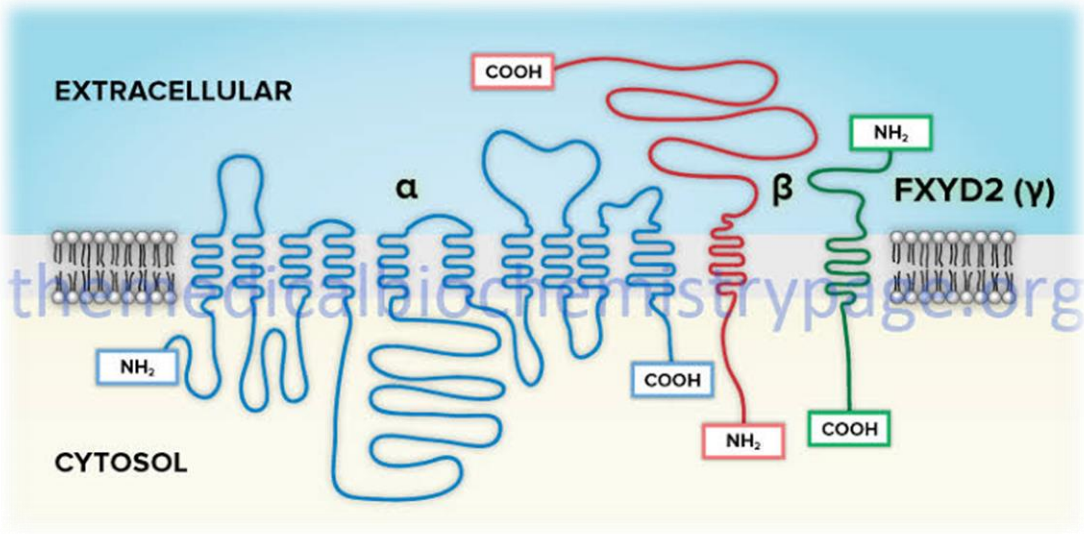
- The extrusion of Na^+ allows cells to **control their water content**.
- Basis for the **electrochemical excitability of nerve cells**.
- Major requirement for ATP production from **glucose oxidation in the central nervous system**.

Eg: **$Na^+ - K^+$ ATPase**

All animal cells maintain lower **K^+** and high **Na^+** (than in surroundings).

- This imbalance is maintained by active transport system in pm, presence of enzyme → **$Na^+ - K^+$ ATPase (breakdown of ATP).**

- For each ATP hydrolysis, 2 **K^+** "moves in" & 3 **Na^+** "moves out".



single transmembrane-spanning proteins termed the FXYD (*fix-id*) proteins

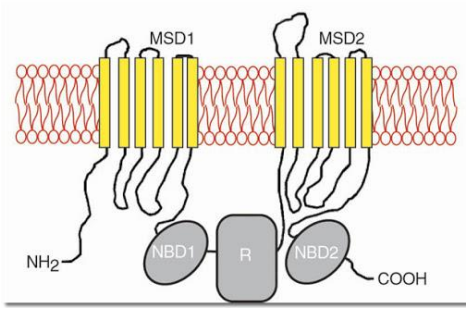
ABC (ATP-Binding Cassette) Family of Transporters:

- The ABC transporters comprise the ATP-binding cassette transporter superfamily (**ATP dependent transporters**) - **(Ions, Sugars and AA).**
- There are **48 known members** of the ABC transporter superfamily and they are **divided into seven subfamilies** based upon phylogenetic analyses.

These seven subfamilies are

1. **ABCA (ABC1) subfamily** - 12 genes (ABCA1–ABCA10, ABCA12, and ABCA13 (ABCA11 and ABCA17 are psuedogenes)).
2. **ABCB (also called TAP/MDR for transporter, ATP cassette)** - 11 genes identified as ABCB1, ABCB4-ABCB11, TAP1 and TAP2 (also known as ABCB2 and ABCB3.
3. **ABCC (also called MRP for multidrug resistance protein)** - 13 genes identified as ABCC1–ABCC6, ABCC8–ABCC13, and CFTR (less commonly identified as ABCC7.
4. **ABCD (also called ALD for adrenoleukodystrophy)-** 4 genes identified as ABCD1–ABCD4.
5. **ABCE (also called OABP for oligoadenylatre-binding protein)-** single gene, ABCE1.
6. **ABCF (also called GCN20), and ABCG (also called White)** - three genes identified as ABCF1–ABCF3.
7. **ABCG subfamily** - five genes identified as ABCG1, ABCG2, ABCG4, ABCG5, and ABCG8.

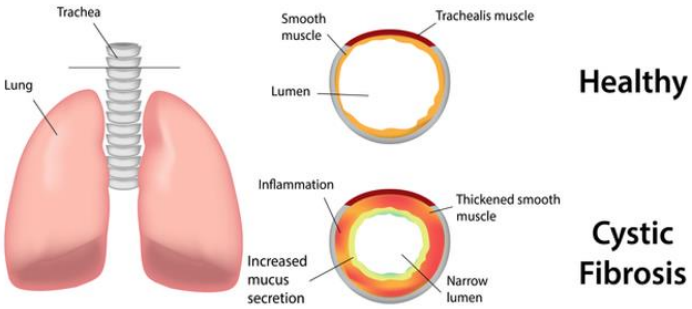
Eg: CFTR (Cystic Fibrosis Transmembrane Conductance Regulator)



- an ABC (**A**TP-**B**inding **C**assette) transporter family.
- It functions as a **Cl^- channel.**
- It requires ATP hydrolysis + cAMP dependent phosphorylation.
- **Defective Cl^- transport → CYSTIC FIBROSIS.**

CYSTIC FIBROSIS

- Genetic disease
- Thick mucus secretion
- It logs air passages in lungs
- Faulty Chloride ion channel → Intracellular [ion] increases.



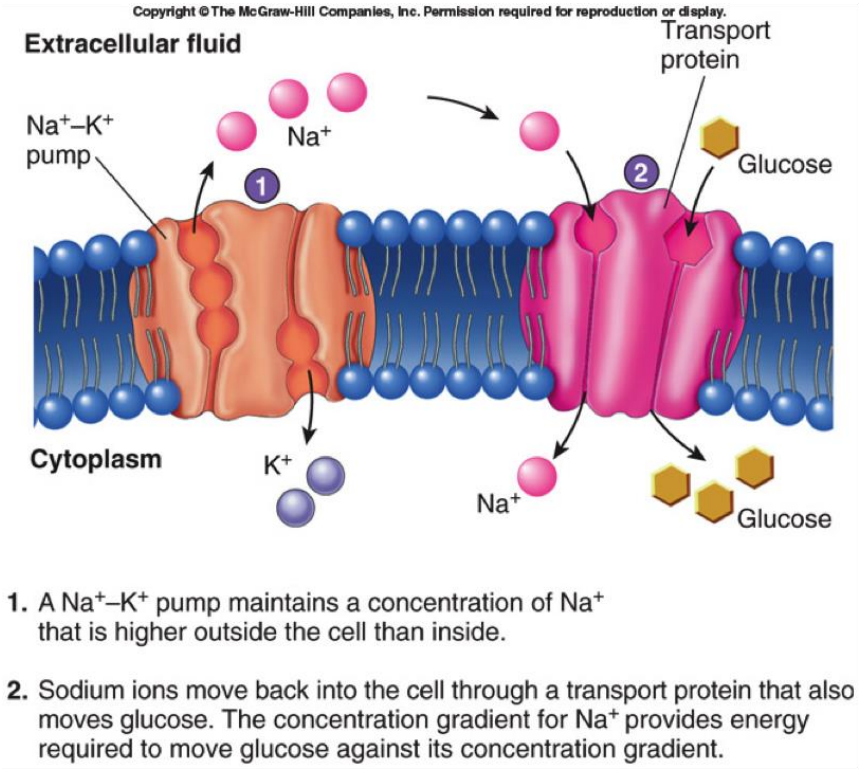
-Q ???

- **How this cause the mucus thick in airways?**

Secondary (indirect) active transport

The energy is derived secondarily from energy that has been stored in the form of ionic concentration differences between the two sides of a membrane.

- It occurs when endergonic transport (uphill) of one solute is **coupled** to exergonic flow (downhill) of different solute that was originally pumped in (uphill) by primary active transport.
- It is either **symport** or **antiport**.



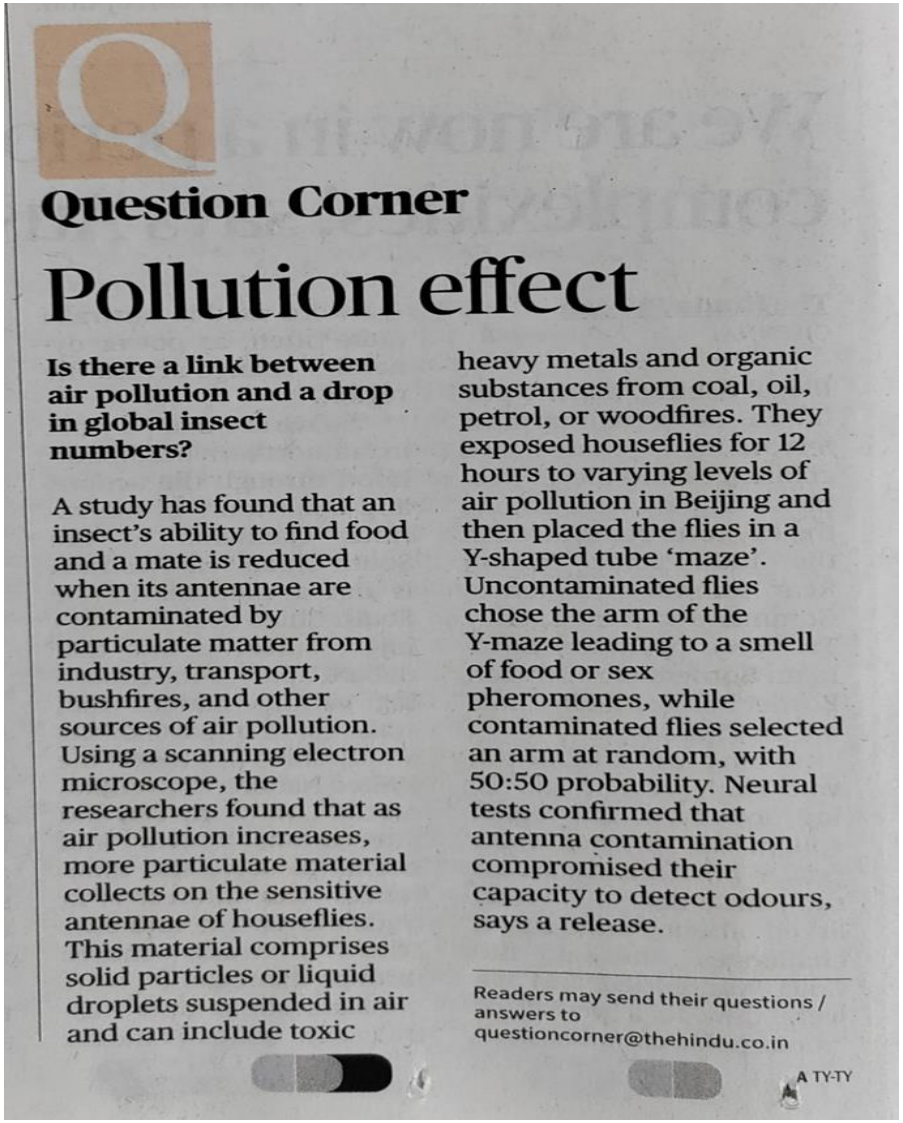
Summary Table: Comparison of Different Types of Transport

	Type of Transport	Type of Protein	Direction X Moves	Source of Energy to transport X	Example(s)	
					Name	Function
1	Simple Diffusion	None	Down its gradient	Gradient of X	Restricted to very small molecules and hydrophobics	How $C0_2$ enters RBC
2	(Facilitated) diffusion through a Channel	Transmembrane channel	down its gradient	gradient of X	water channels in kidney & RBC; many types of ion channels	control vol. of urine & RBC; flux of ions
3	Facilitated Diffusion using a carrier protein; also called 'carrier mediated transport'	Carrier or Permease	down its gradient	gradient of X	Glucose transporter in many plasma membranes	How Glucose exits epithelial cells to body; enters adipose tissue
					RBC anion Exchanger*	Maximizes CO_2 transport by blood
4	Primary (Direct) Active Transport	Pump	up its gradient	ATP	Na^+/K^+ pump	Maintain high $[K^+]$, low $[Na^+]$ in cells
5	Secondary (Indirect) Active Transport	Pump or co-transporter	up its gradient	Not ATP (directly): usually a gradient of some substance other than X	Glucose/Na^+ Co-transport	How Glucose enters epithelial cells from lumen
	* In some older books band 3 protein is called an anion channel. It is now clear it transports ions but is not a channel.					

Question Corner

Pollution effect

Is there a link between air pollution and a drop in global insect numbers?



Question Corner

Pollution effect

Is there a link between air pollution and a drop in global insect numbers?

A study has found that an insect's ability to find food and a mate is reduced when its antennae are contaminated by particulate matter from industry, transport, bushfires, and other sources of air pollution. Using a scanning electron microscope, the researchers found that as air pollution increases, more particulate material collects on the sensitive antennae of houseflies. This material comprises solid particles or liquid droplets suspended in air and can include toxic heavy metals and organic substances from coal, oil, petrol, or woodfires. They exposed houseflies for 12 hours to varying levels of air pollution in Beijing and then placed the flies in a Y-shaped tube 'maze'. Uncontaminated flies chose the arm of the Y-maze leading to a smell of food or sex pheromones, while contaminated flies selected an arm at random, with 50:50 probability. Neural tests confirmed that antenna contamination compromised their capacity to detect odours, says a release.

Readers may send their questions / answers to questioncorner@thehindu.co.in

A TY-TY

The Hindu 16.07.2023

CELL BIOLOGY (22ZOOC12)

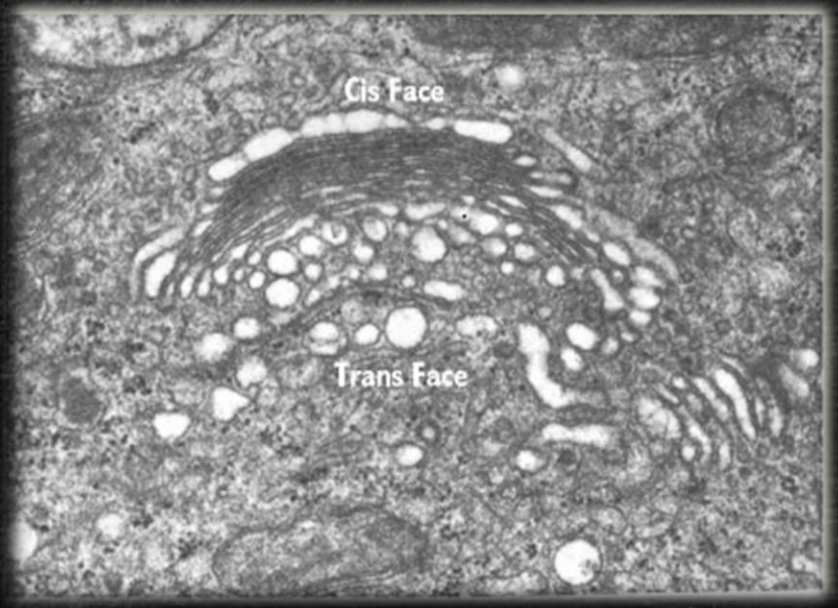
Golgi Complex
Structure and Functions

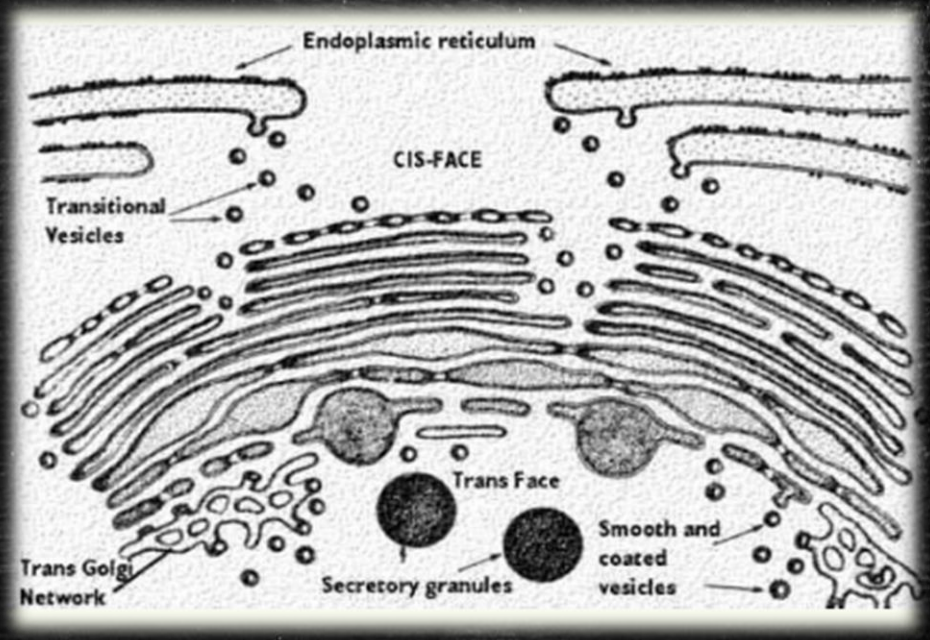
Distribution & Shipping department – Proteins & Lipids (Fats)

Golgi bodies: (Lipochondria, Idiosome or Dalton complex):

- They are **middle man of cell and discovered by Camello Golgi (1898) in cytoplasm** of nerve cell of owl and cat (**silver metallic impregnation technique**).
- They form internal reticular apparatus (*apparato recticulare interno) and take* ***black stain*** **(Sudan III) being rich in lipids.**
- **Dalton and Felix (1954) observed them under** TEM and confirmed their existence.
- In plants, golgi bodies are unconnected and scattered called **dictyosomes.**
- In **fungi, a dictyosome is unicisternal.**
- In **vertebrates** these are found near the nucleus.

- A dictyosome has a stack of usually 3-12 cisternae with swollen ends, tubules and vesicles.
- It shows polarity.
- **Concave or maturing (M) face or trans face / TGN is near cell membrane and Cis or convex or forming (F) face / CSN is towards nuclear membrane.**
- **Lysosomes and secretory vesicle** arise from 'M' face.
- New cisternae are formed from SER.





- They process package and help in transport and release of secretory proteins.
- They also cause glycosidation of lipids and glycosylation of proteins to form glycolipids and glycoproteins.
- Golgi body forms acrosome in sperm, yolk and cortical granules in eggs, secretion of insulin, lactoprotein in mammary glands, cellulose, hemicellulose, mucilage, pectin, cell plate during cell division, root hairs etc.
- They regulate fluid balance of cell.
- All secretory cells are rich in golgi bodies.
- Main enzyme in golgi bodies is nucleoside disphosphatase.
- These bodies arise from SER mainly.

Proteins undergo O-linked glycosylation: N-linked oligosaccharide chains on proteins are altered as proteins pass through Golgi cisternae *en route* from ER.

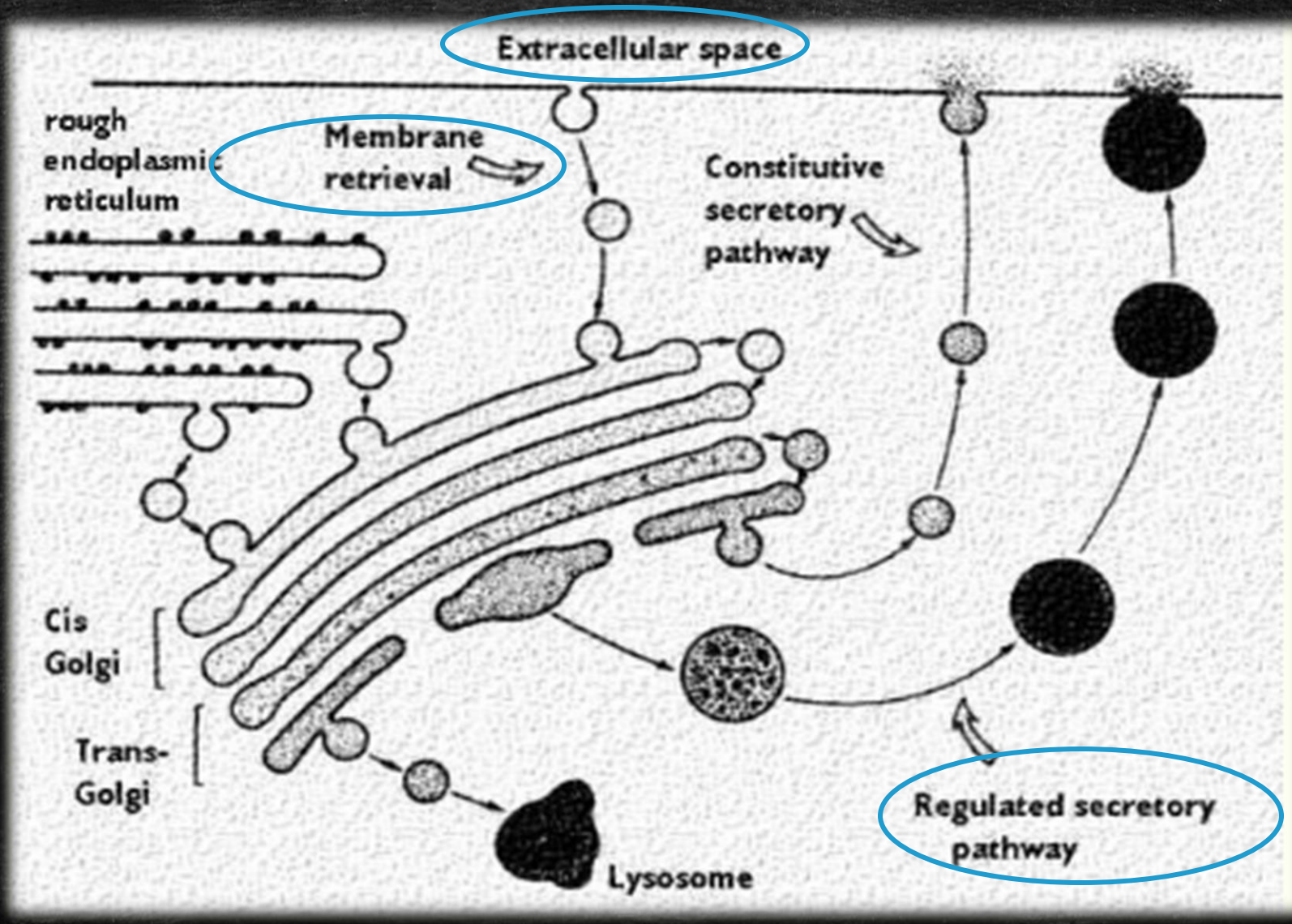
- O-linked oligosaccharides are linked to
  - OH group of Ser / thr via N-acetylgalactosamine (GalNac) and it contains mannose.
  - OH group of hydroxylysine via galactose

So In O-linked glycosylation, first sugar added is N-acetylgalactosamine (GalNac).

Eg: Formation of heavy proteins → Mucins & proteoglycans (secreted and move towards extracellular matrix).

Goblet cells (gut) secrete mucus and have lots of golgi and large vesicles (TFG).

Protein trafficking – Secretion & EM

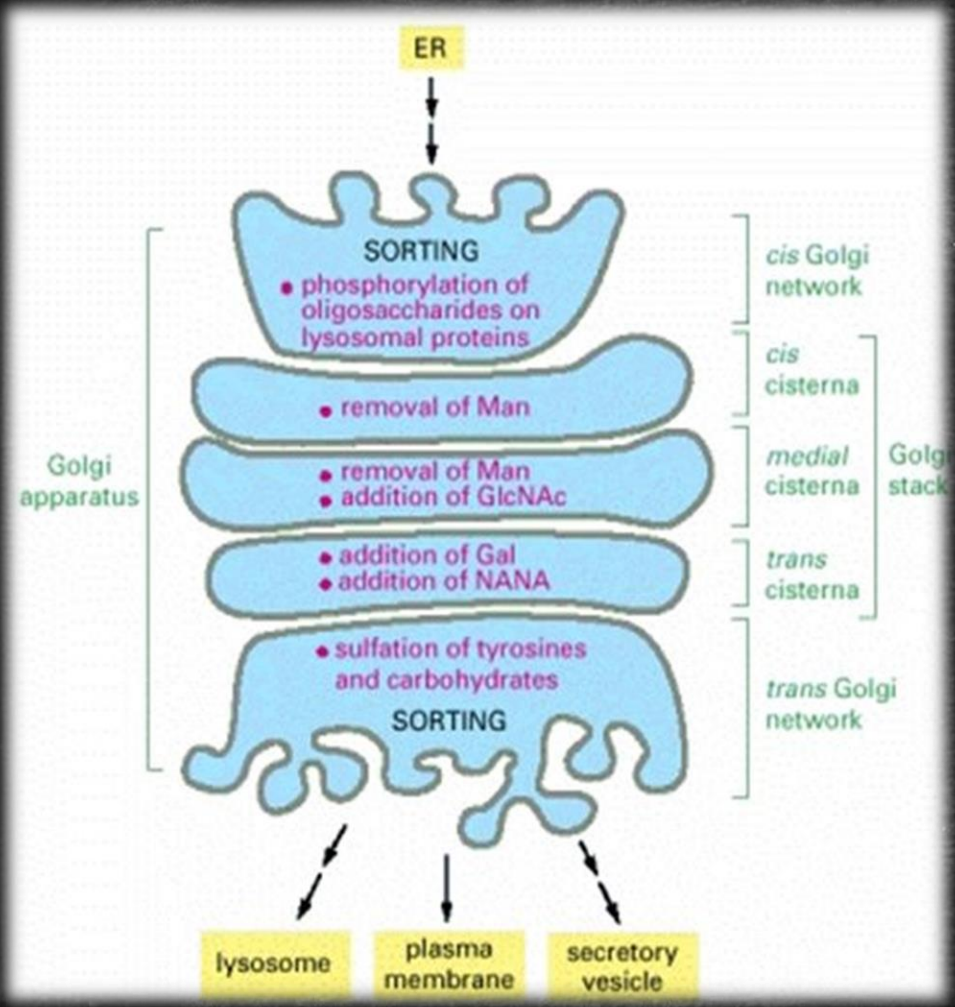


- The Golgi Complex is compartmentalized.
- Phosphorylation occurs in the Cis region.
- In middle regions, different types of carbohydrates (mannose (man), galactose (gal)), etc are added.
- The final sorting is done in the Trans Golgi complex.

Functional differentiation of the Golgi complex (electron microscope with specific techniques that detect enzymes).

- The cis region is rich in lipid-bearing membranes and can be delineated by osmium tetroxide labeling.
- The middle regions label for enzymes that add carbohydrates or other groups.
- The inner, or trans region, is the area where the lysosomes are sorted. Therefore, it is heavily labeled for acid phosphatase.

Functional compartmentalization of the Golgi apparatus



CGN – SORTING
Phosphorylation of Oligosaccharides on Lysosomal proteins.

Cis Cisterna – Removal of Mann

Median Cisterna – Removal of Mann & addition of GlcNAc

Trans Cisterna – Addition of GAL & sialic acid (NANA-N caetylaneuraminic acid)

TGN – SORTING sulfation of Tyrosines and carbohydrates

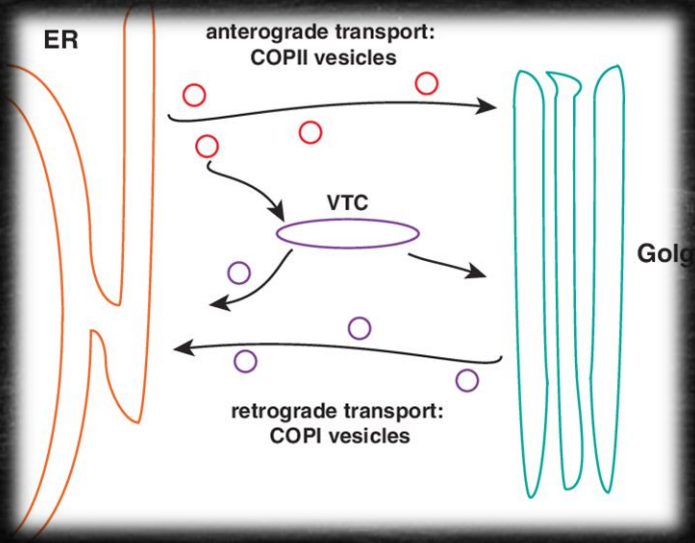
Lipid and polysaccharide metabolism:

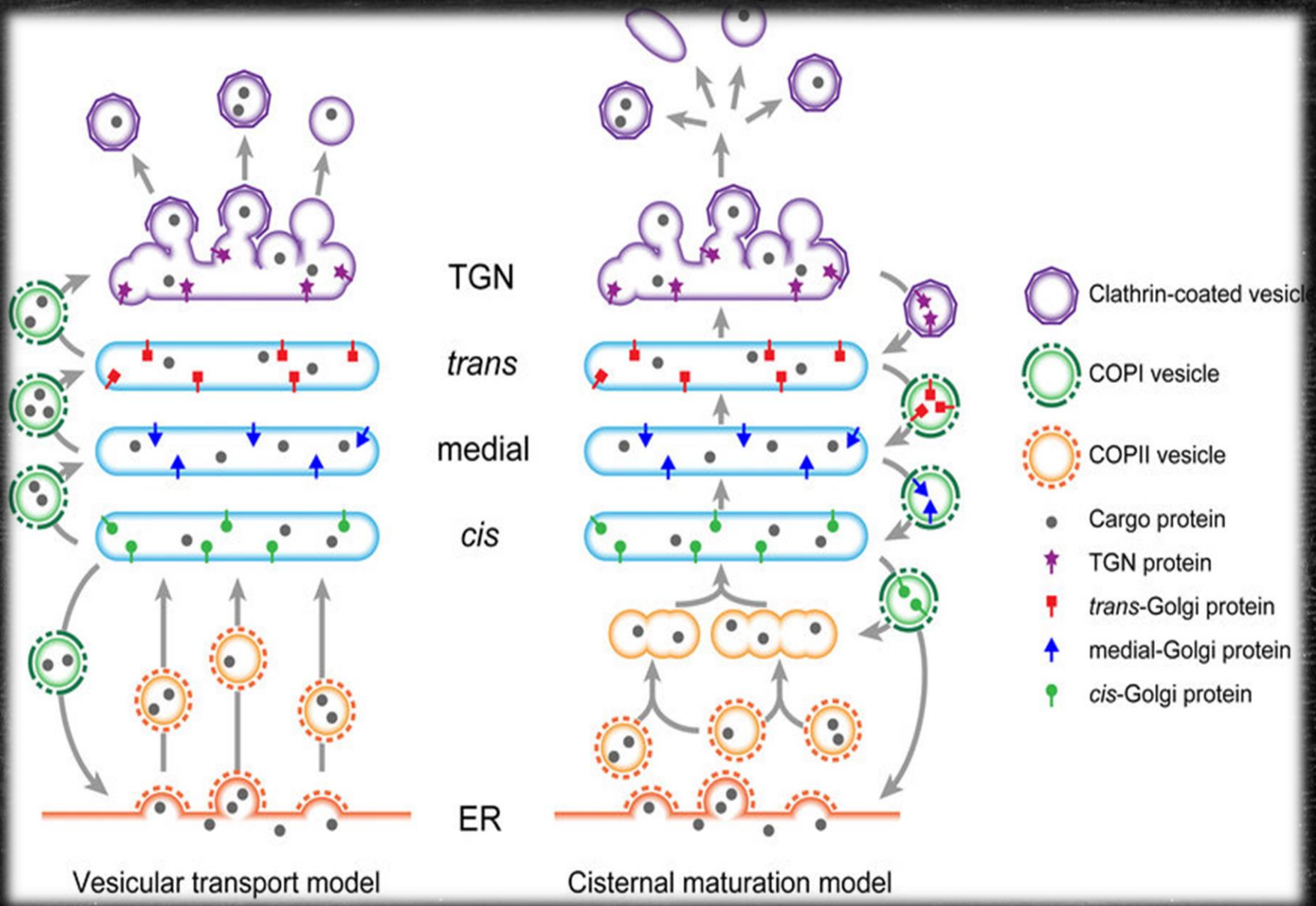
- Synthesis of glycolipids and sphingomyelin. Eg: Ceramide
- Site of carbohydrate metabolism.
- Synthesis of glycosaminoglycans of extracellular matrix.

Transport of proteins through Cisternae: 2 Models

1. Cisternal progression (maturation) model – Dynamic cisternae (Cis→ Medial → Trans) – takes new enzymes.

2. Vesicular transport model – Stationary Cisternae; Vesicles move forward and backward.

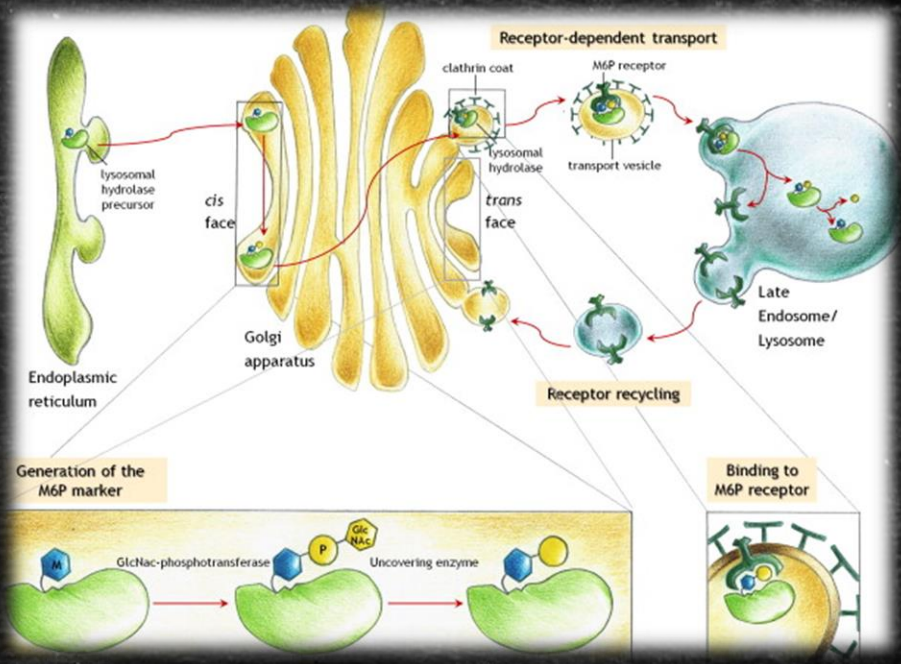



TGN
trans
medial
cis
ER
Clathrin-coated vesicle
COPI vesicle
COPII vesicle
Cargo protein
TGN protein
trans-Golgi protein
medial-Golgi protein
cis-Golgi protein
Vesicular transport model
Cisternal maturation model

Mannose 6 phospahte residues target proteins to lysosomes

pH ~ 6.5 to 7

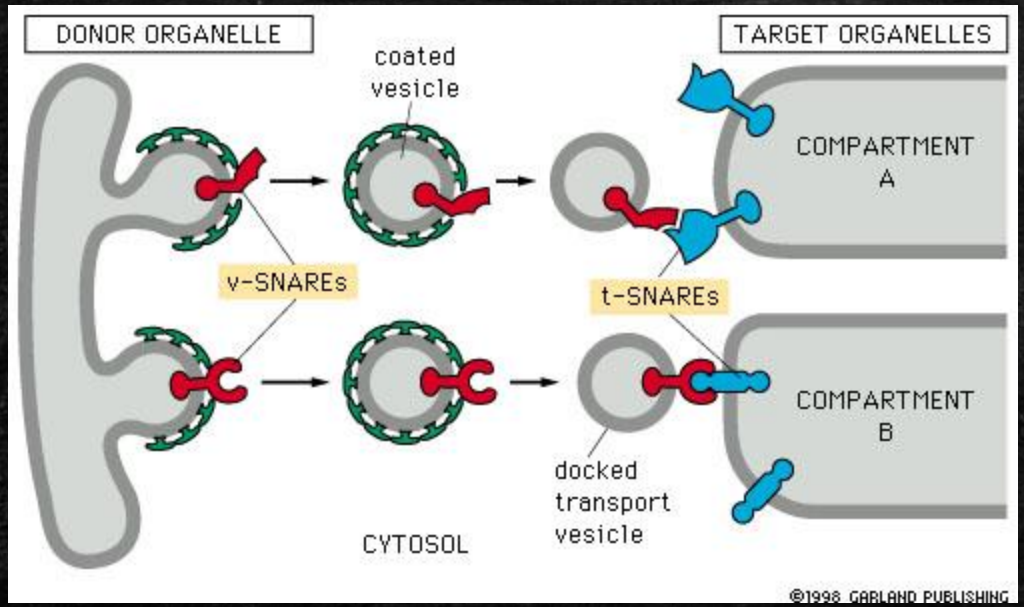
Type signal sequence directing secreted and membrane proteins to specific transport vesicles



Signal sequence	Protein type	Transport step	Vesicle type
Lys-Asp-Glu-Leu (KDEL)	Secreted	G →ER	COP I
Lys-Lys-X-X (KKXX)	Membrane	G →ER	COP I
Di-acidic (Asp-X-Glu)	Membrane	ER → G	COP II
M6P	Secreted	TGN→PM→late endosome	Clathrin

Vesicle fusion:

- Fusion of transport vesicle with the target.
- 2 steps:
  - Recognition: transport vesicle – target membranes.
  - Fusion: vesicle & target membranes
    - transmembrane proteins.
    - fusion between phospholipid bilayers.

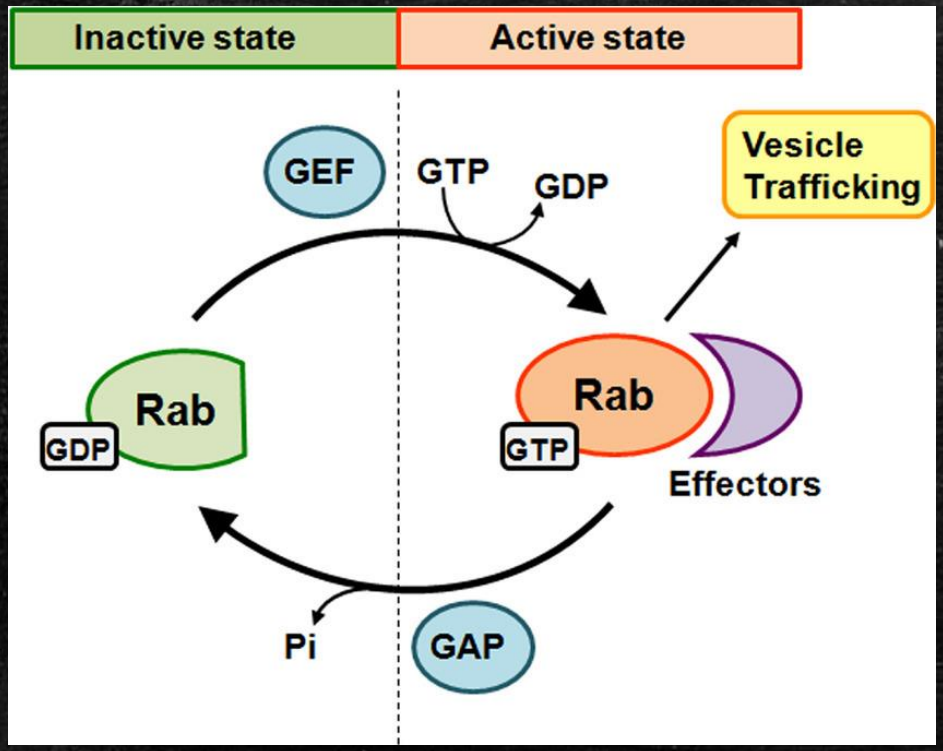


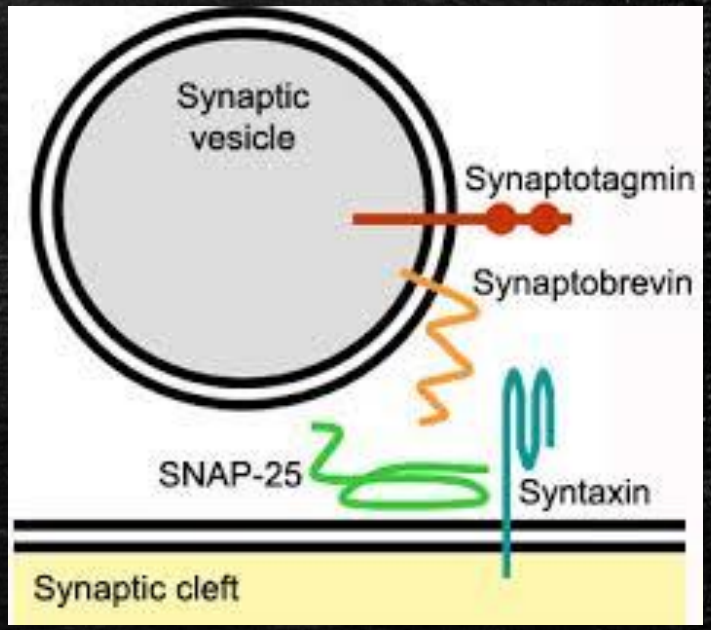
SNARE hypothesis:

Vesicular Fusion → Rothman and his colleagues.

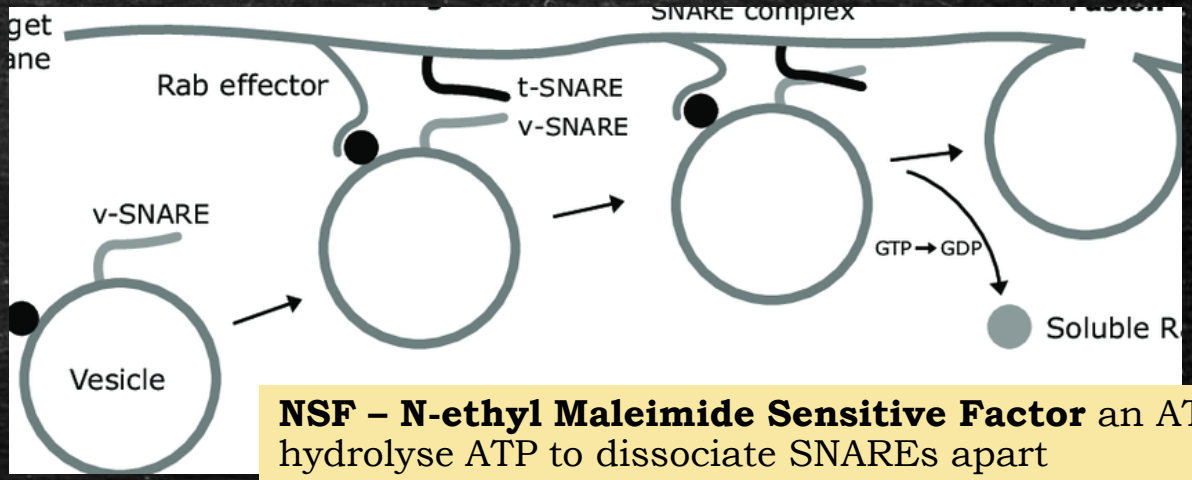
- v-SNAREs – vesicles Eg: Synaptobrevin.
- t-SNAREs – target membranes Eg: Syntaxin & SNAP25.

Recognition – Monomeric GTPases → Rab proteins (varies for secretory or endocytic pathways)

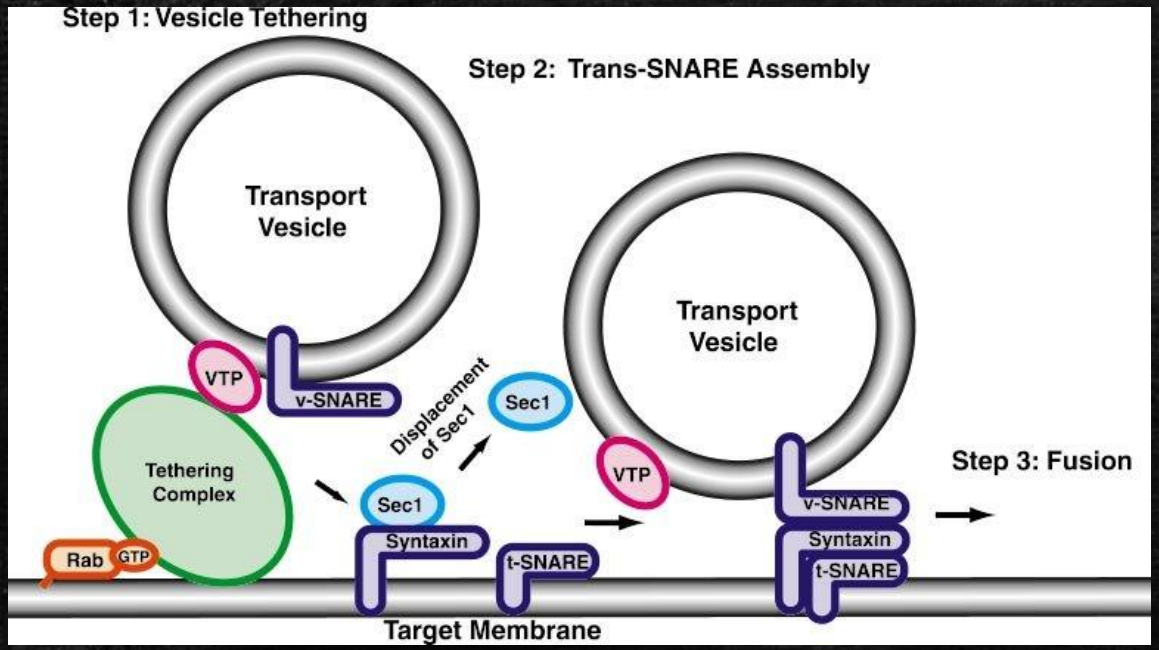




Complementary sets of vesicles SNAREs - determine the selectivity of transport vesicle docking



NSF – N-ethyl Maleimide Sensitive Factor an ATPase hydrolyse ATP to dissociate SNAREs apart



Rab-GDP

CELL BIOLOGY (22ZOOC12)

MITOCHONDRIA

MITOCHONDRIA:

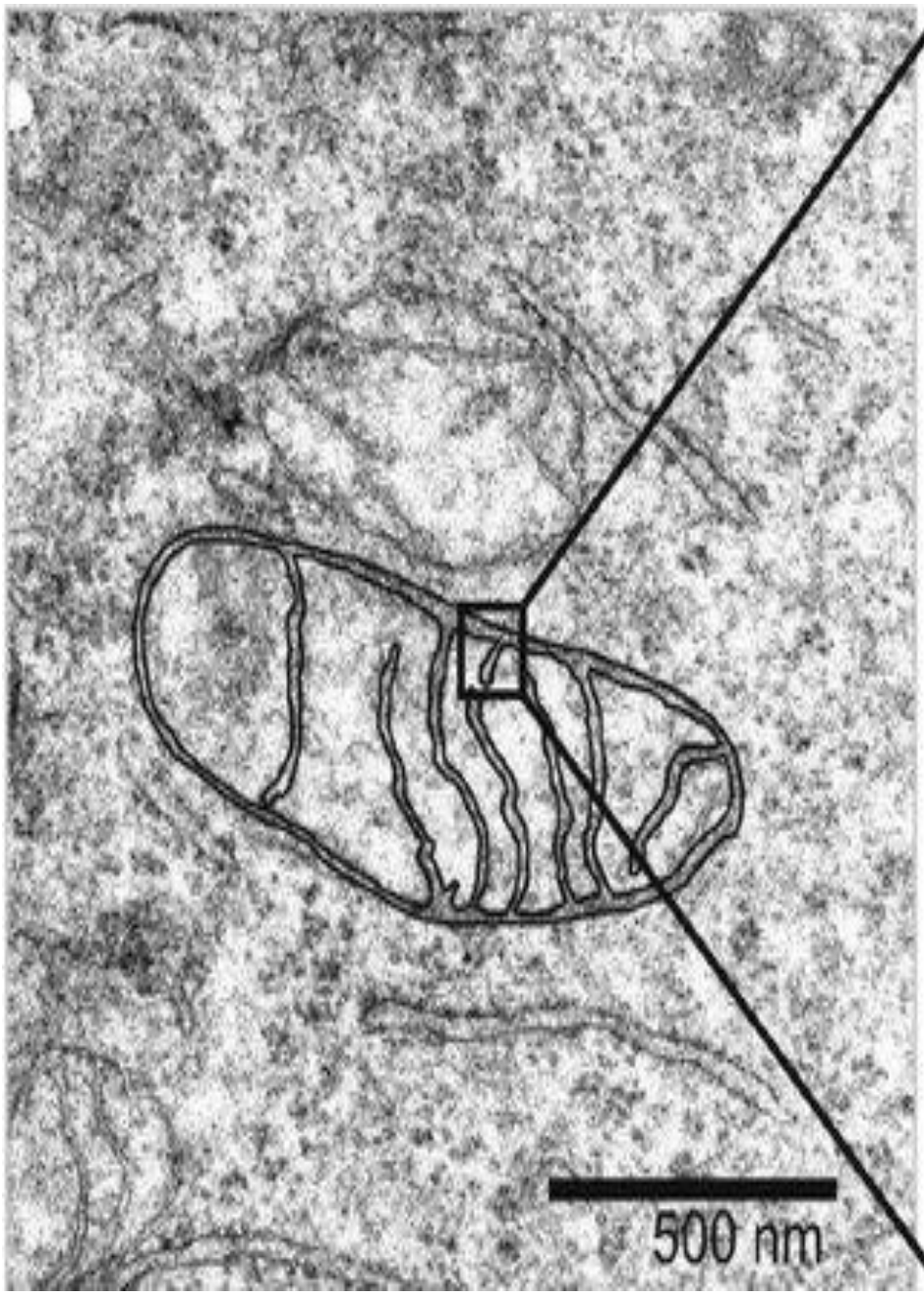
★Energy converting organelles.

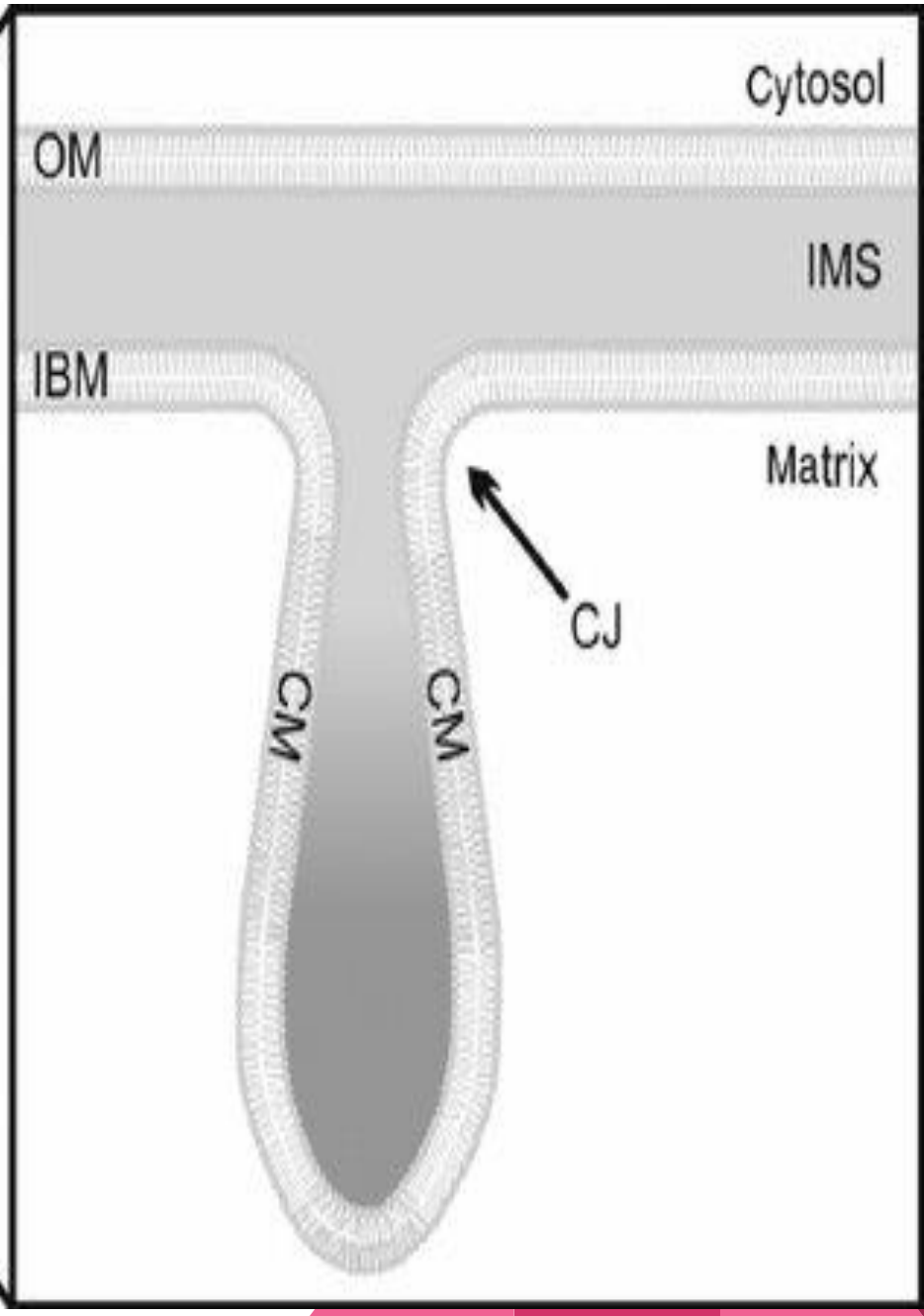
★Virtually present in all eukaryotic cells

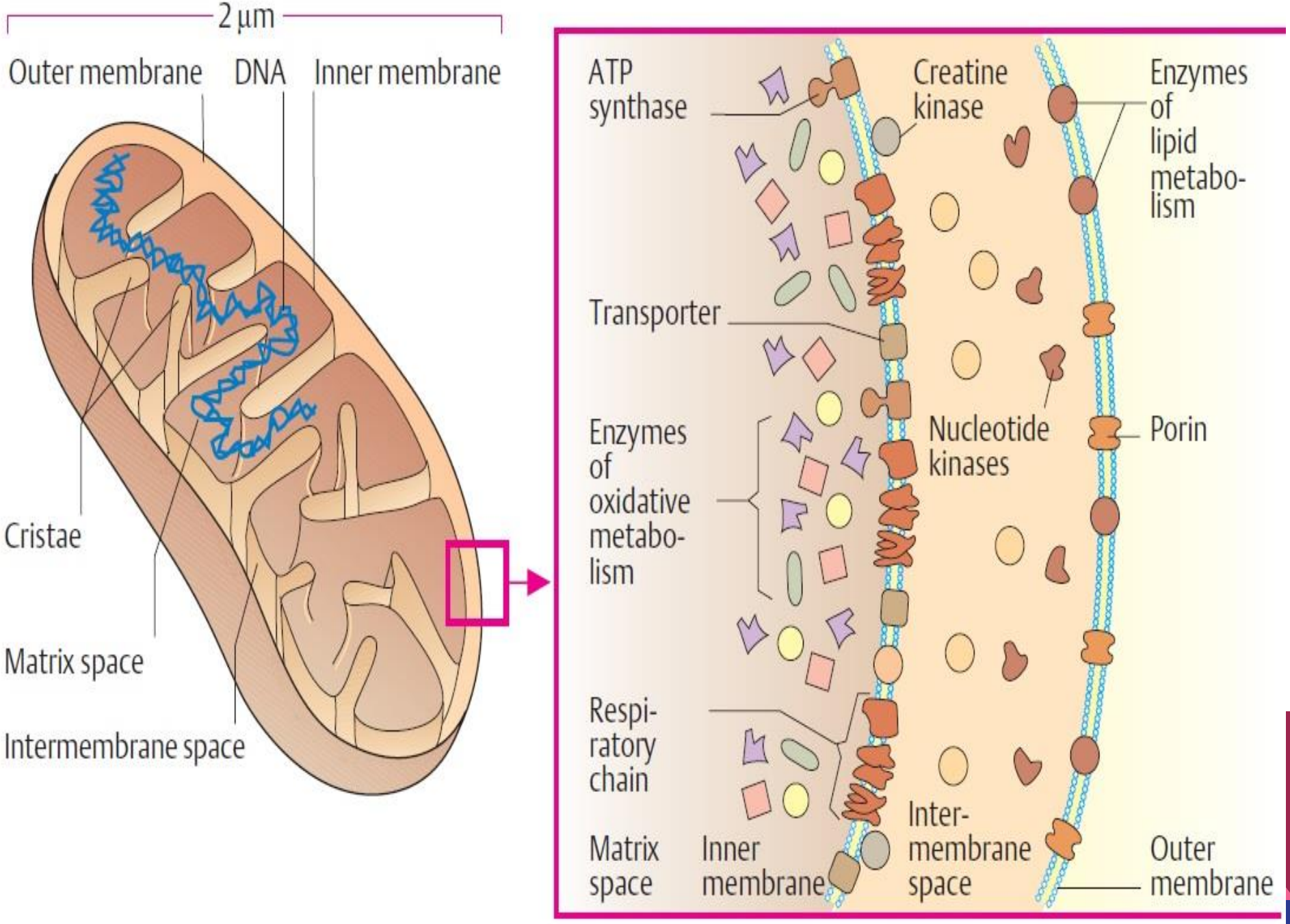
★Double membranous - mobile and plastic organelle.

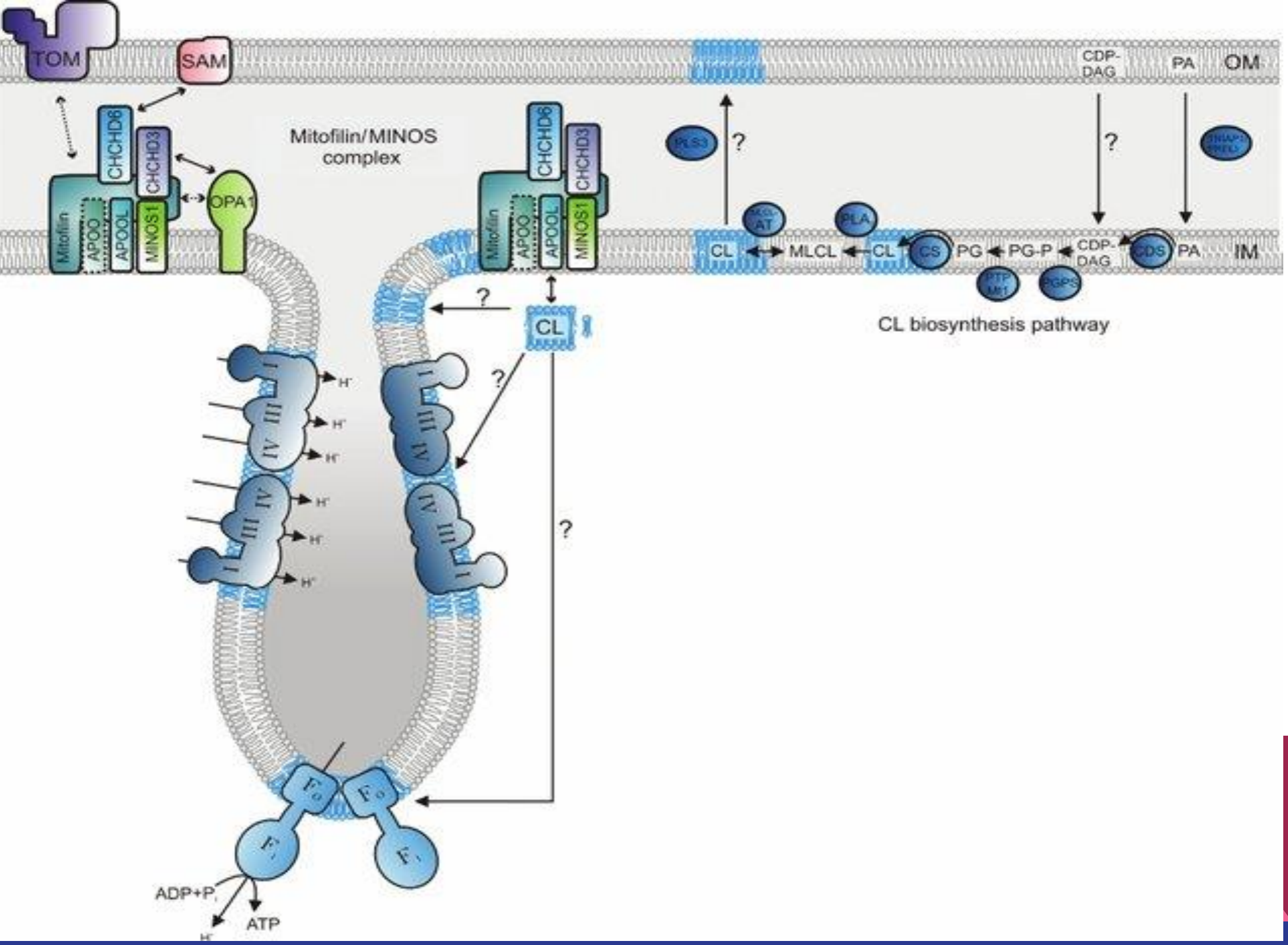
★Outer membrane - smooth - transport proteins - Porin.

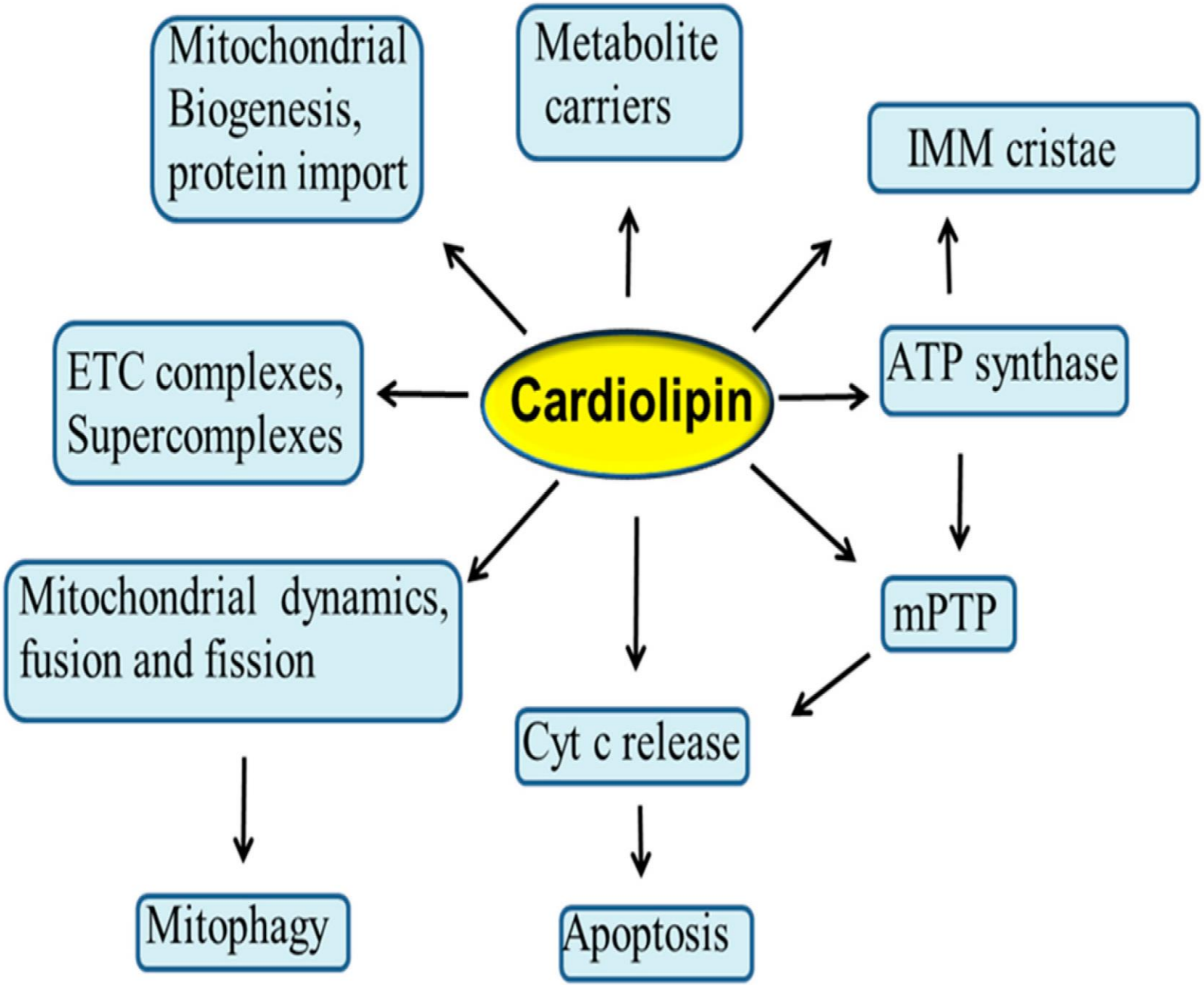
★Inner membrane - highly convoluted - Cristae - Highly impermeable - abundance of phospholipid → Cardiolipin.


500 nm


Cytosol
OM
IMS
IBM
Matrix
CJ
CM
CM


2 µm
Outer membrane
DNA
Inner membrane
Cristae
Matrix space
Intermembrane space
ATP synthase
Creatine kinase
Enzymes of lipid metabo-lism
Transporter
Enzymes of oxidative metabo-lism
Nucleotide kinases
Porin
Respi-ratory chain
Matrix space
Inner membrane
Inter-membrane space
Outer membrane


TOM
SAM
CHCHD6
CHCHD3
Mitofilin
APOO
APOOL
MINOS1
OPA1
Mitofilin/MINOS
complex
CL
?
ADP+P
ATP
CDP-
DAG
PA
OM
IM
PLA
CL
MLCL
CS
PG
PG-P
CDS
PTP
MT
PGP-S
CL biosynthesis pathway


Mitochondrial Biogenesis, protein import
Metabolite carriers
IMM cristae
ETC complexes, Supercomplexes
Cardiolipin
ATP synthase
Mitochondrial dynamics, fusion and fission
mPTP
Cyt c release
Mitophagy
Apoptosis

- ★ Matrix - large internal space - presence of identical copies of mtDNA genome, mitochondrial ribosomes (55S - 80S), tRNAs & enzymes.
- ★ mtDNA - circular, dsDNA (16.6 kb - human), 37 genes, introns are absent, 93 % of coding DNA, maternal inheritance.
- ★ Individual mitochondria contain several copies of mtDNA, without histone protein.
- ★ mtDNA is 10 to 150 times larger than in animals.
- ★ In humans, mtDNA encodes 2 rRNAs, 22 tRNAs and 13 polypeptides.

MITOCHONDRIAL PROTEINS:

★Synthesized by cytosolic and matrix ribosomes.

★Cytosolic ribosomes → proteins are synthesized and translocated into mitochondria by post-translocationally.

★Imported proteins → outer, inner membranes, intermembrane space or matrix.

★Precursor proteins have N-terminal matrix-targeting sequences (hydrophobic aa , basic aa → Arg, Lys, OH containing aa→ Ser, Thr); lacking acidic aa. Hence destined and reaches matrix.

Mt protein Import:

★Active process - Electrochemical gradient (across IMM) required with ATP hydrolysis.

★Requires membrane receptors and translocons.

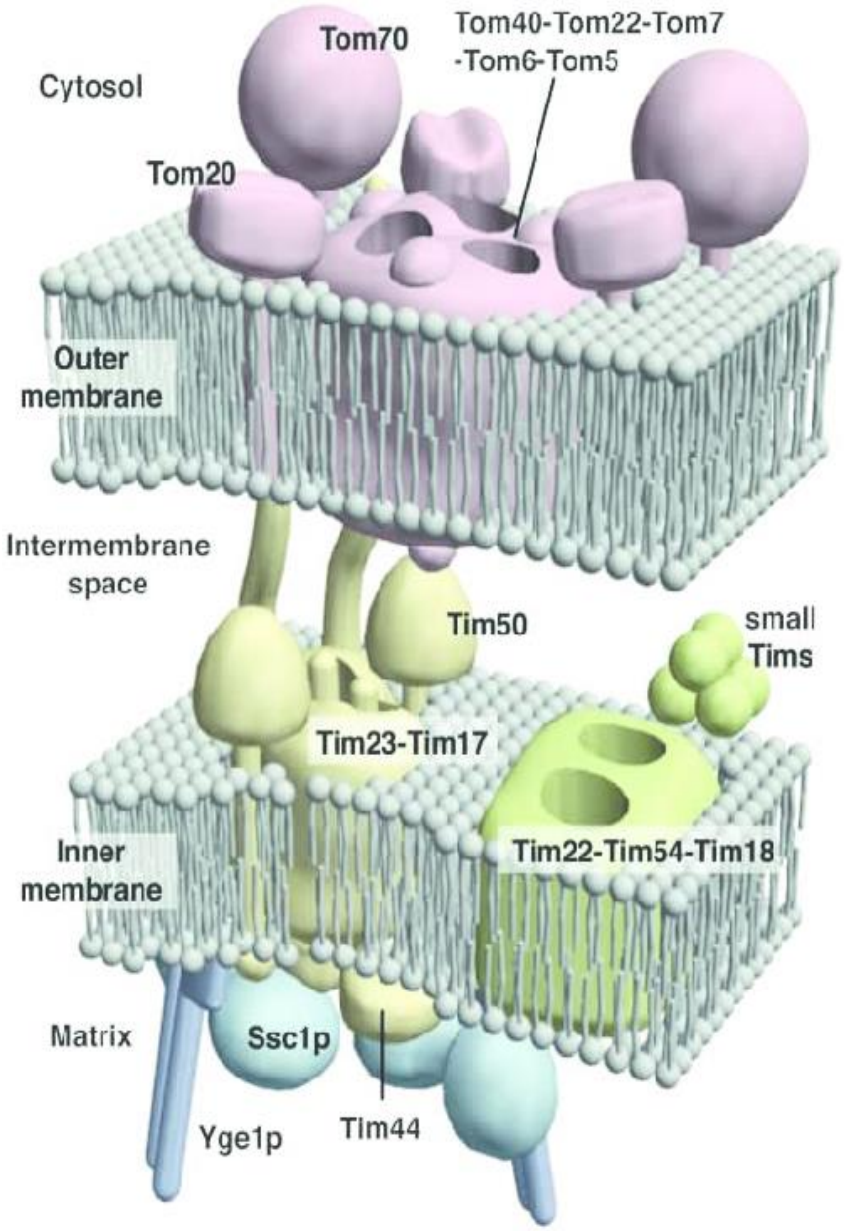
★Translocation complexes

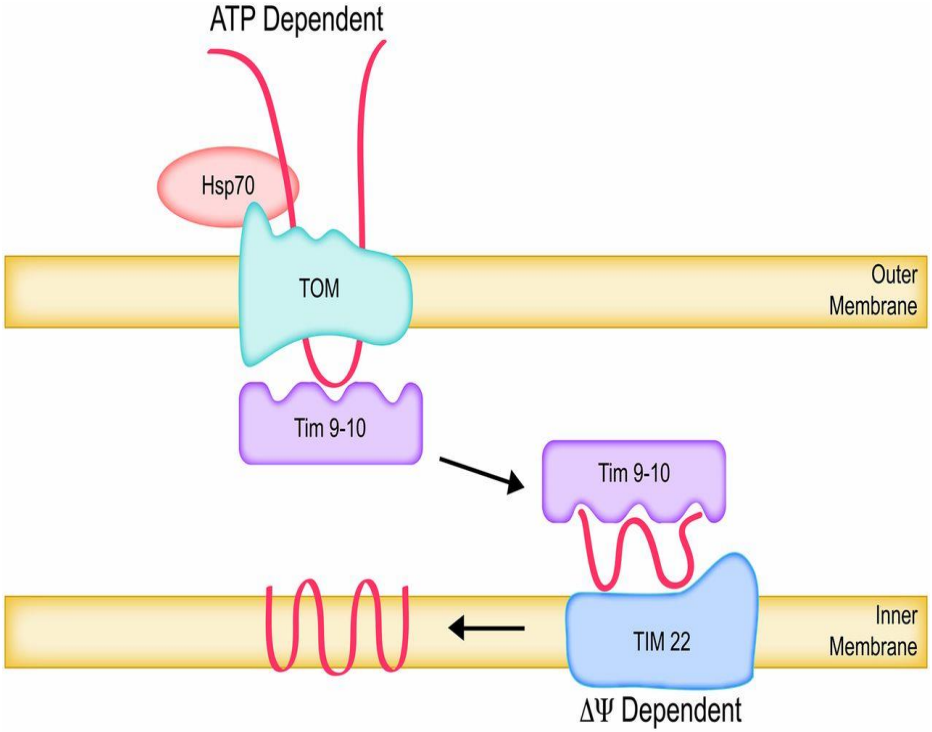
→ TOM (Translocase -of - the outer membrane)

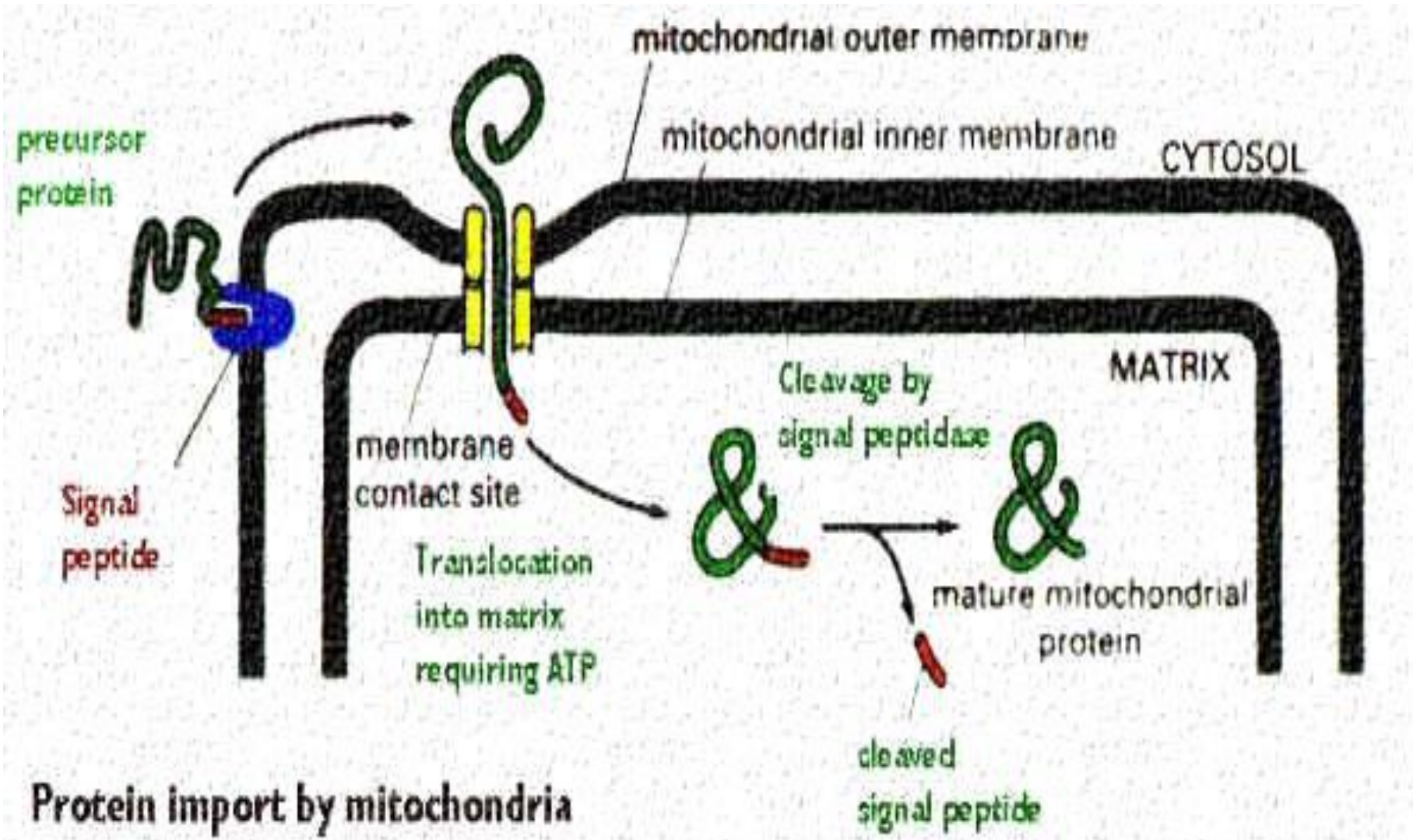
→ TIM (Translocase -of - the inner membrane)

★Proteins remain in unfolded state to pass through the TIM and TOM complexes.

★It is mediated by ATP-dependent binding of Chaperons (Hsp70).


Tom70
Tom40-Tom22-Tom7
-Tom6-Tom5
Cytosol
Tom20
Outer
membrane
Intermembrane
space
Tim50
small
Tims
Tim23-Tim17
Inner
membrane
Tim22-Tim54-Tim18
Matrix
Ssc1p
Yge1p
Tim44


ATP Dependent
Hsp70
TOM
Outer
Membrane
Tim 9-10
Tim 9-10
TIM 22
Inner
Membrane
ΔΨ Dependent



Protein import by mitochondria

REVIEW ARTICLE

Front. Mol. Biosci., 07 December 2017 | https://doi.org/10.3389/fmolb.2017.00083

Protein Translocation into the Intermembrane Space and Matrix of Mitochondria: Mechanisms and Driving Forces


Sandra Backes and

Johannes M. Herrmann*

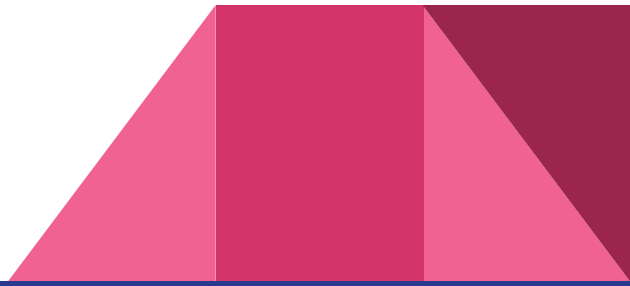
Cell Biology, University of Kaiserslautern, Kaiserslautern, Germany

Mitochondria contain two aqueous subcompartments, the matrix and the intermembrane space (IMS). The matrix is enclosed by both the inner and outer mitochondrial membranes,

https://doi.org/10.3389/fmolb.2017.00083

Origin of mt as cellular organelle:

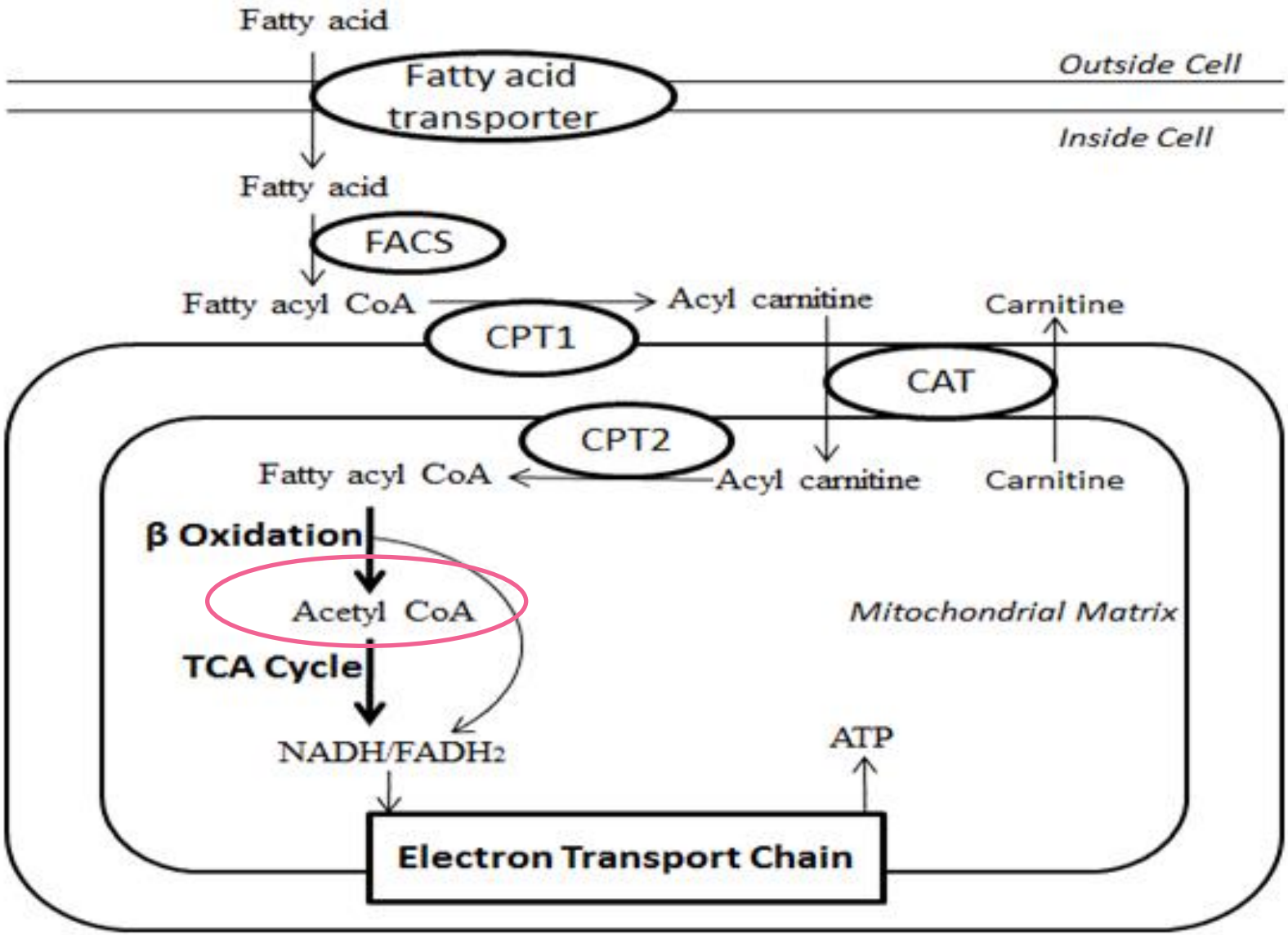
- ★ Endosymbiotic theory - Lynn Margulis (1967) - purple photosynthetic bacteria (1.5bya).
- ★ It’s an organism living within an organism.
- ★ Captured cell was reduced to a functional double membrane organelle and vertically transmitted to subsequent generations (VGT).



Evidences:

- ➔ Self replicating (binary fission).
- ➔ Inner membrane composition is similar in composition to bacteria.
- ➔ Presence of own DNA (mtDNA) - simple circular structure similar to bacterial DNA.
- ➔ mt ribosomes, enzymes and transport systems are similar to bacteria.
- ➔ Size of mt and bacteria are same.
- ➔ mtDNA - have similar structural motifs with bacterial DNA.
- ➔ Protein synthesis in mt is inhibited by a variety of antibiotics that inactivate many bacterial ribosomes, but have little effect on ribosome in cytosol of eukaryotic cells.

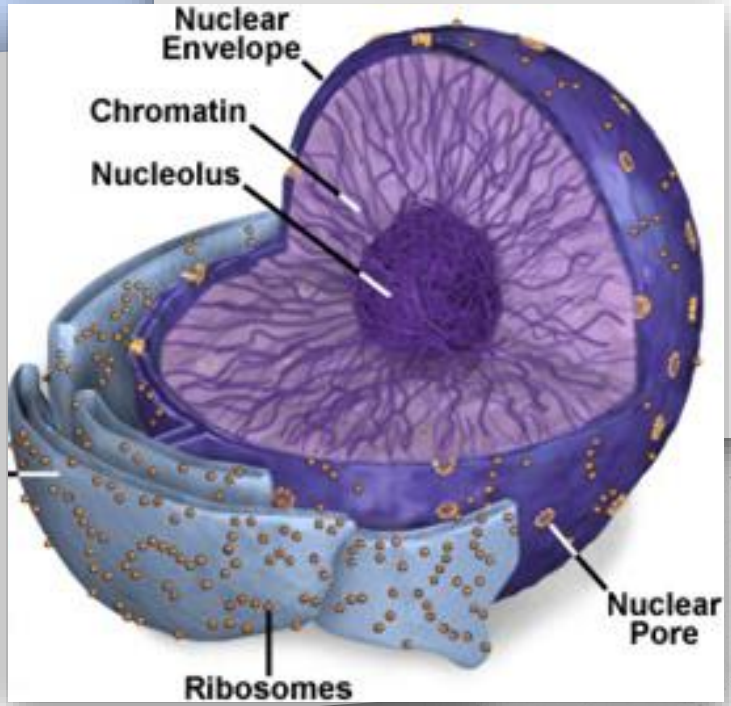
ß – oxidation of fatty acids – (Liver, kidney and Heart tissues)



CELL BIOLOGY (22ZOOC12)

Structure & Function

NUCLEUS



INTRODUCTION:

★ The center for genetic inheritance and gene expression within the cells of eukaryotes.

★ Number: Single, multinuleated (polymorphonuclear), anucleated.

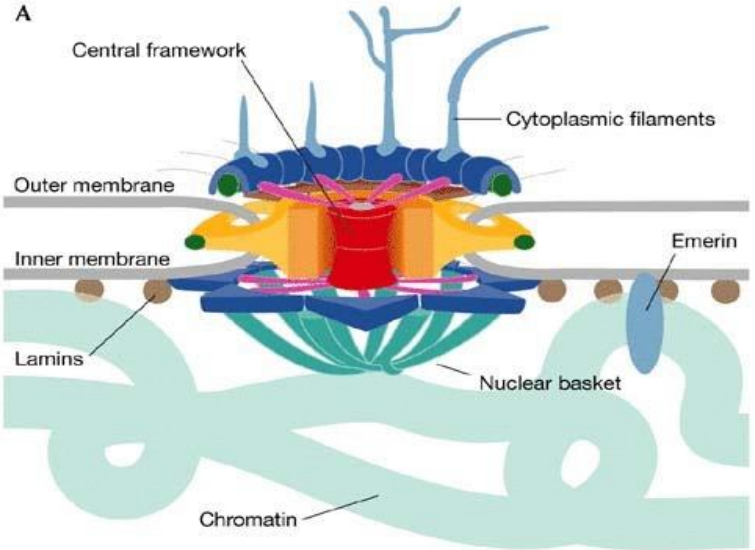
Components:

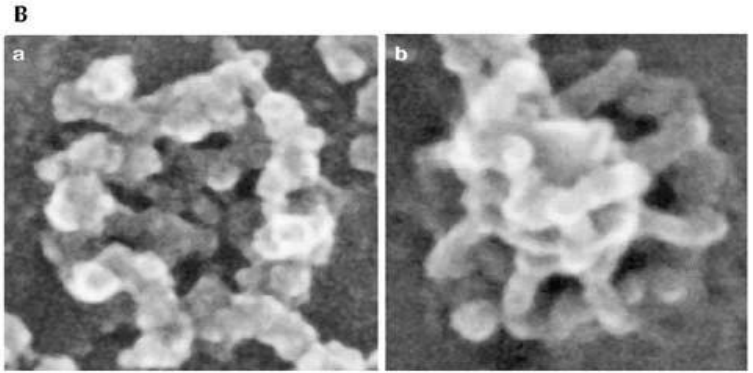
I. Nuclear envelope

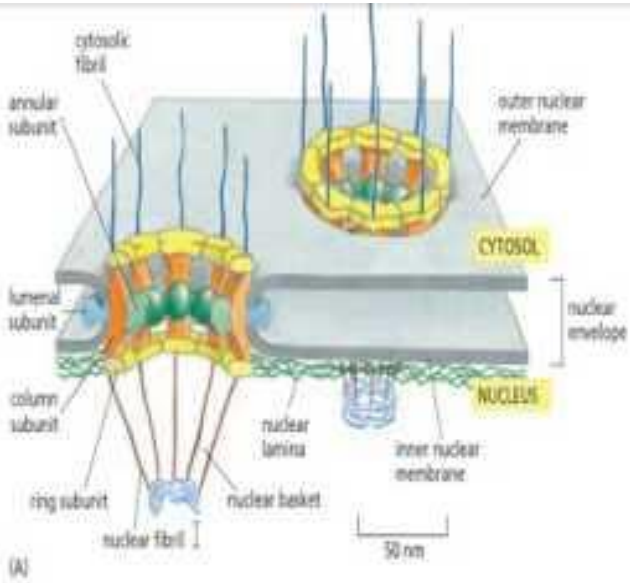
II. Nucleolus

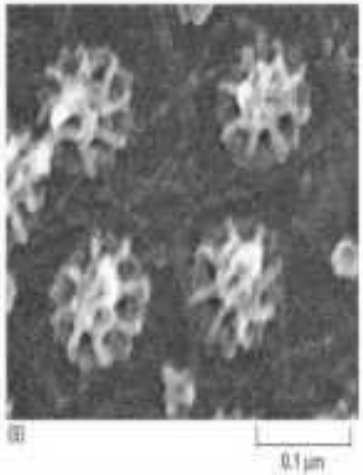
III. Nucleoplasm

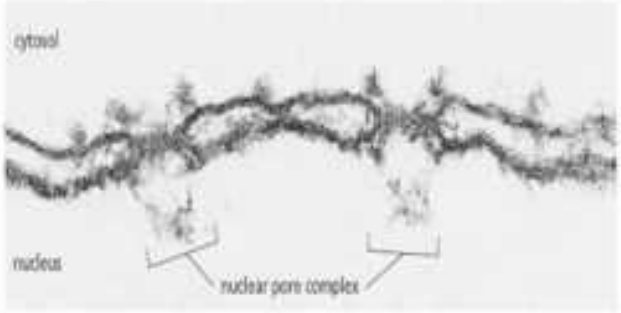
IV. Chromatin

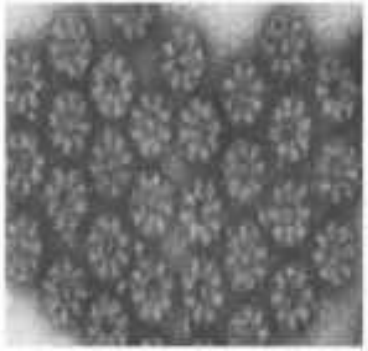

A
Central framework
Cytoplasmic filaments
Outer membrane
Inner membrane
Emerin
Lamins
Nuclear basket
Chromatin


B
a
b


cytosolic fibril
annular subunit
outer nuclear membrane
CYTOSOL
lumenal subunit
nuclear envelope
NUCLEUS
column subunit
nuclear lamina
inner nuclear membrane
ring subunit
nuclear basket
nuclear fibril
50 nm
(A)

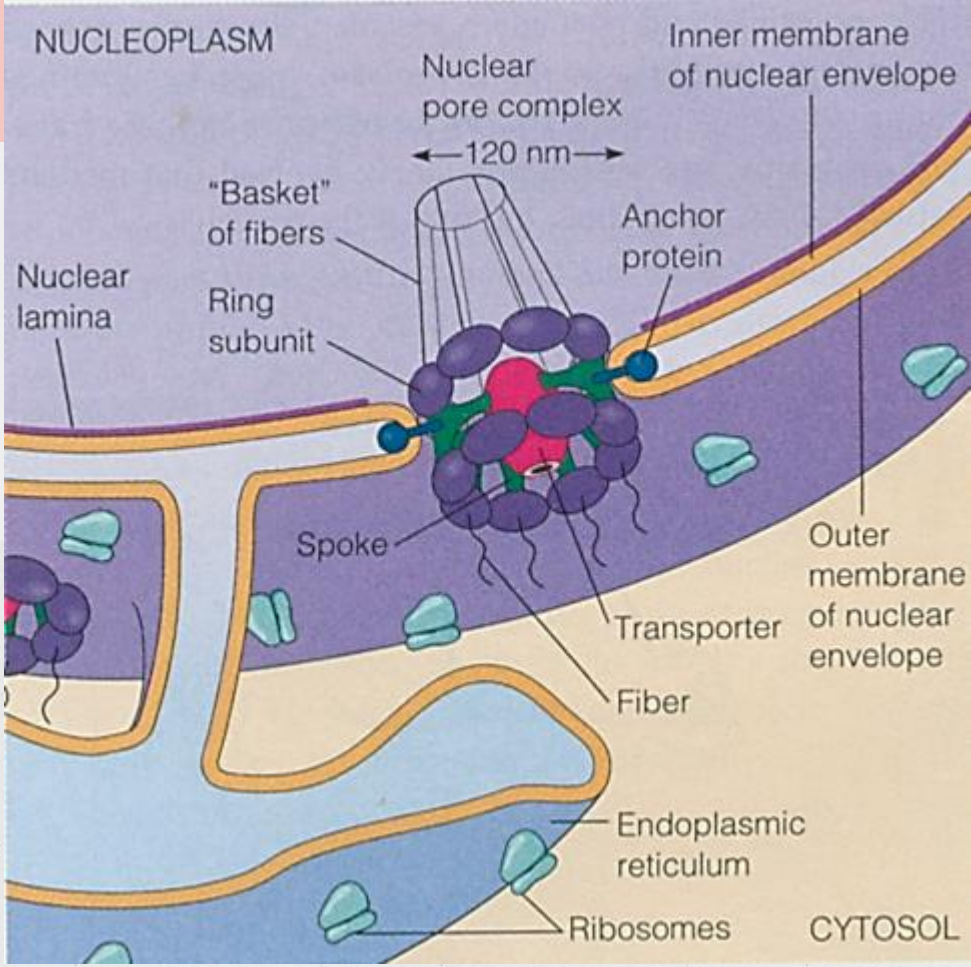

(B)
0.1 μm

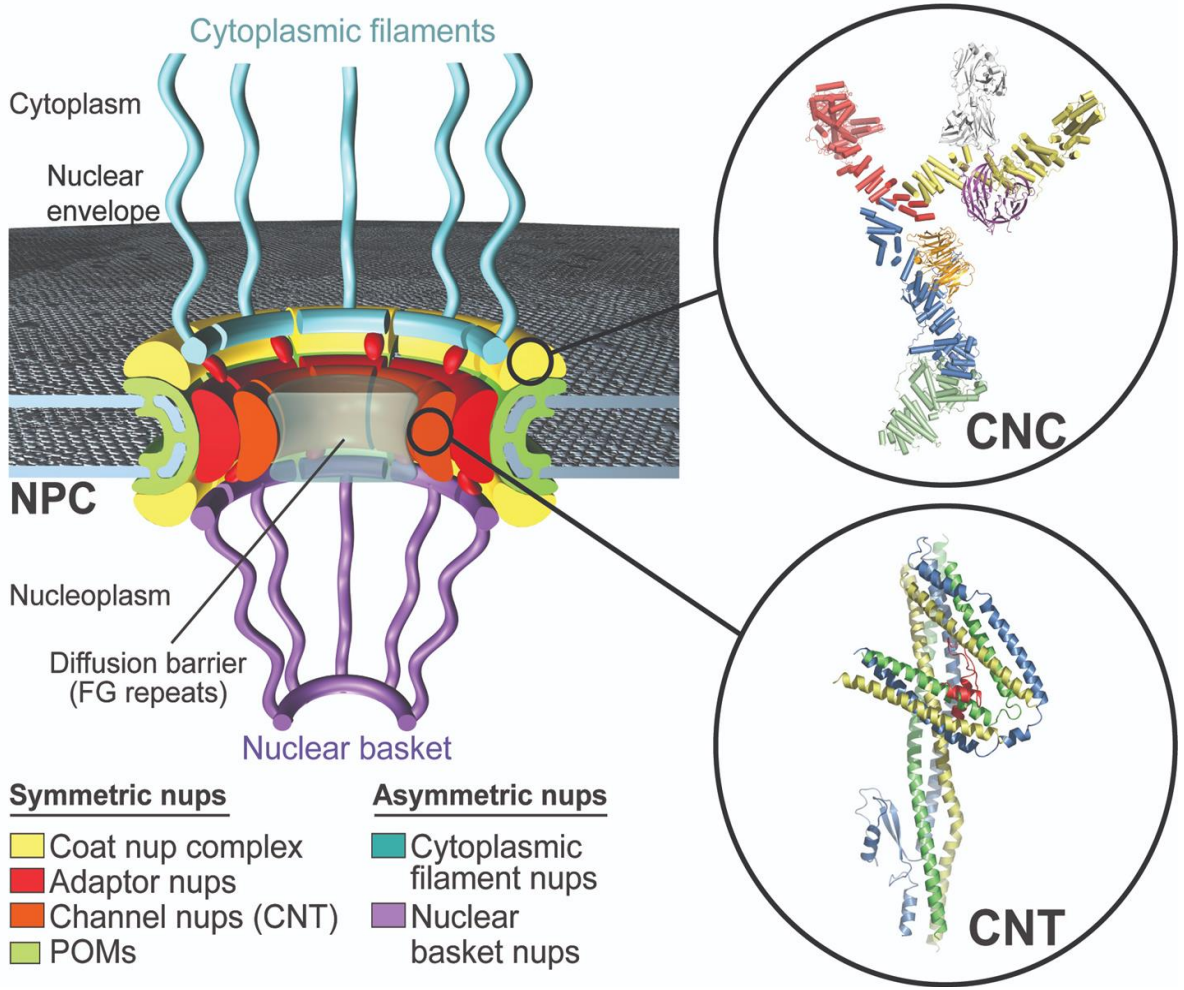

cytosol
nucleus
nuclear pore complex



NUCLEAR ENVELOPE:

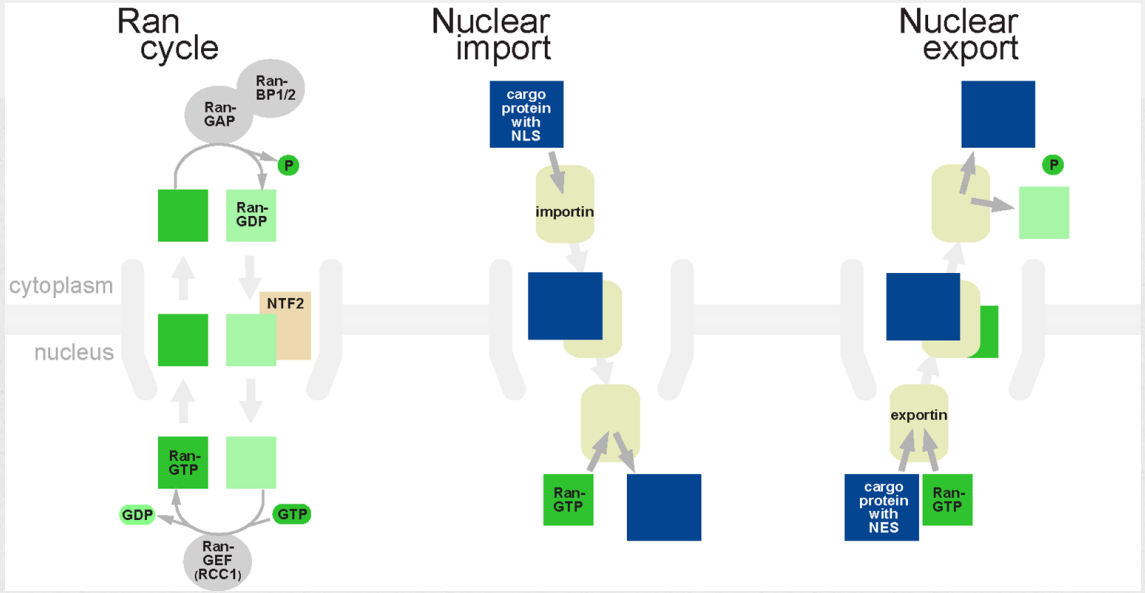
- ★ Double membrane
- ★ Nuclear pores - macromolecular import and export from and to the cytoplasm.
- ★ Outer membrane - continuous with ER.
- ★ Perinuclear space - continuous with the lumen of ER.
- ★ Nuclear lamina - Network of IF (Lamins) on nuclear side of inner membrane; Support and shape.
- ★ Nuclear pore is formed from a complicated structure - Nuclear pore complex (50 - 100 nucleoporins).
- ★ INM and ONM fuse at the nuclear pore complex.




Cytoplasmic filaments
Cytoplasm
Nuclear envelope
NPC
Nucleoplasm
Diffusion barrier (FG repeats)
Nuclear basket
CNC
CNT
Symmetric nups
Coat nup complex
Adaptor nups
Channel nups (CNT)
POMs
Asymmetric nups
Cytoplasmic filament nups
Nuclear basket nups

Transport through nuclear pores:

- ★ Bidirectional transport of molecules.
- ★ RNA molecules and ribosomal subunits - exported to cytosol.
- ★ All proteins that function in nucleus are synthesized in cytosol (imported).
- ★ 50 KDa sized molecules can enter nucleus - passive diffusion (also actively transported).
- ★ Contain specific amino acid sequences - Nuclear - localization signal or Nuclear export signal.

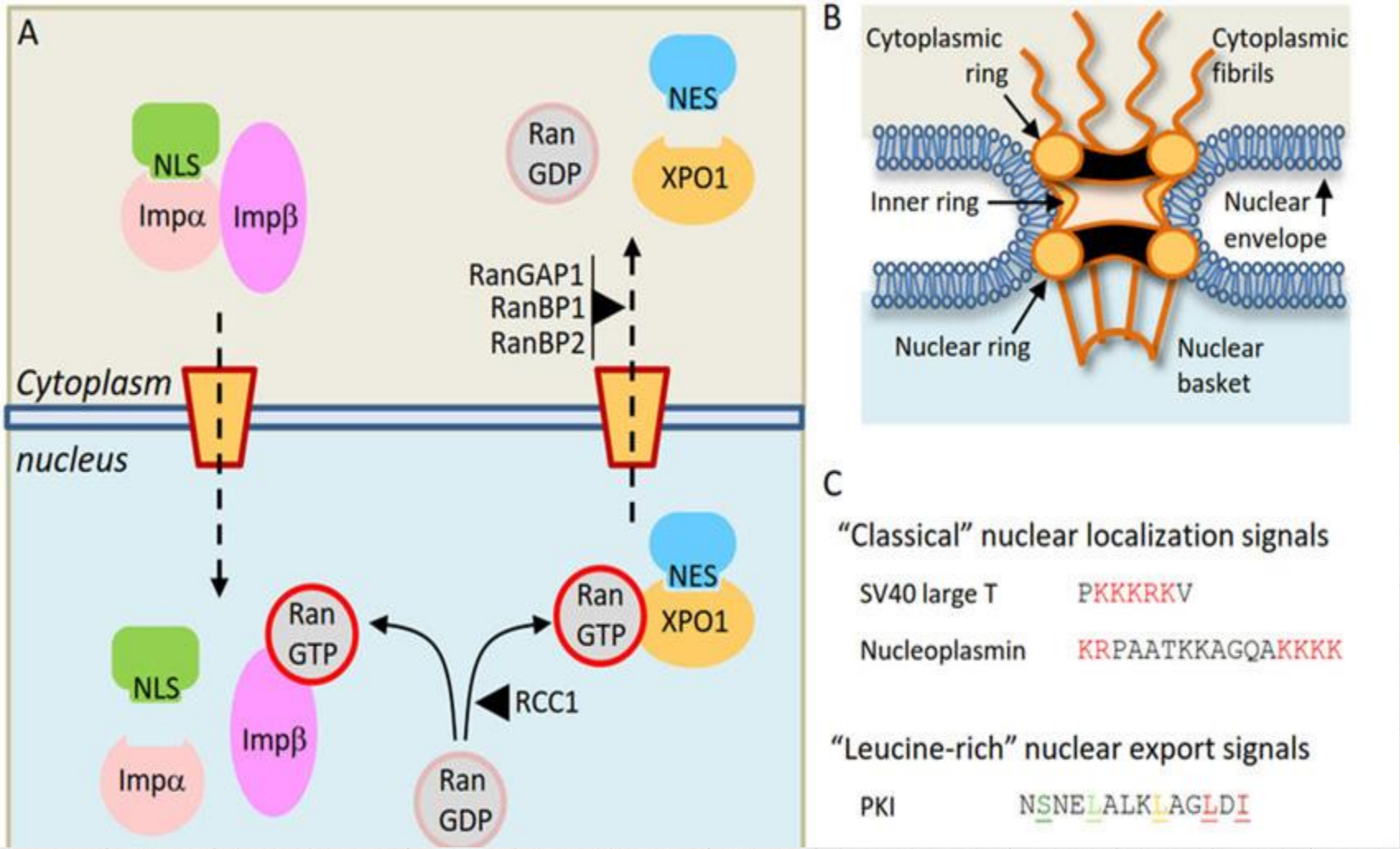


- ★ Nuclear - localization signal acts as binding site for an importin.
- ★ NLS was first identified - viral protein called T-antigen (encoded by SV40 virus- replicate in host nucleus).
- ★ Nuclear proteins are transported post-translationally (conformation).
- ★ NLS are Lys - rich internally located non-cleavable sequences.

- ★ NES are Leu -rich, short sequences, acts as a binding site for an exportin - targets the proteins to cytoplasm.

- ★ Nuclear transport signal → Import or Export
- ★ Transport signal interacts with a specific receptor protein → Exportin or Importin.
- ★ These receptor proteins are homologues to Beta importin family → KARYOPHERINS
- ★ During translocation through pore, importin and exportin interact with Phe-Gly repeat sequences on nucleoporins (subunit of NPC).

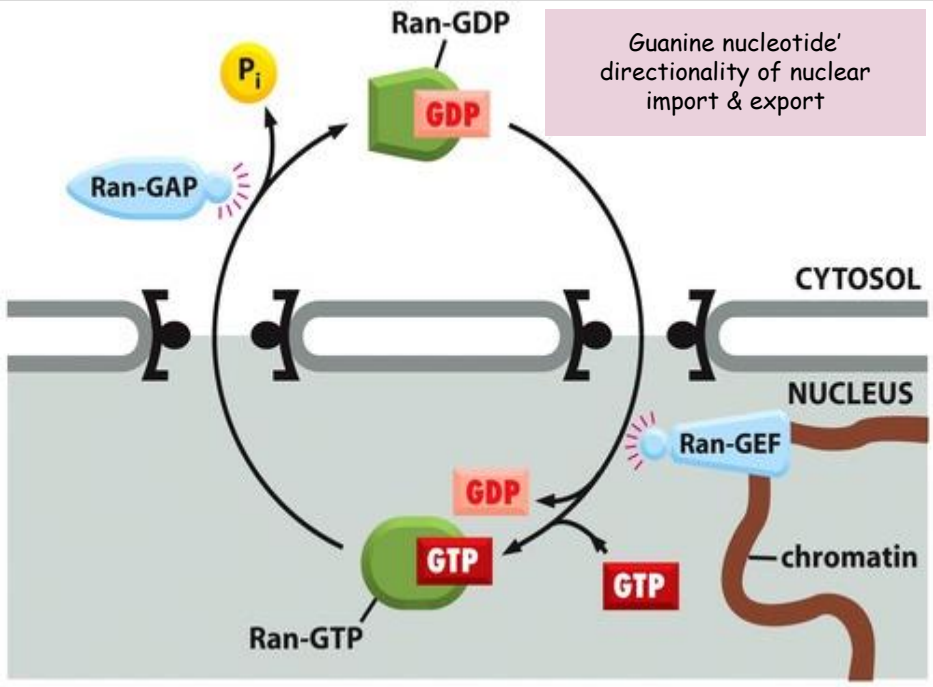
- ★ Ran:
  - Small, monomeric nuclear GTPases.
  - Regulator in interaction of complex with NPCs and its tranaslocation.
  - Ran shuttles across nuclear envelope through nuclear pores, but conc. in nucleus (nuclear transport factor - 2 mediated active import)


A
NLS
Impα
Impβ
Ran
GDP
NES
XPO1
RanGAP1
RanBP1
RanBP2
Cytoplasm
nucleus
Ran
GTP
NES
XPO1
NLS
Impβ
Impα
RCC1
Ran
GTP
Ran
GDP
B
Cytoplasmic
ring
Cytoplasmic
fibrils
Inner ring
Nuclear
envelope
Nuclear ring
Nuclear
basket
C
"Classical" nuclear localization signals
SV40 large T PKKKRKV
Nucleoplasmin KRPAATKKAGQAKKKK
"Leucine-rich" nuclear export signals
PKI NSNELALKLAGLDI

- ★ [Ran-GTP] occurs in nucleus; generated by nucleotide exchange, catalyzed by GEF.
- ★ [Ran-GDP] occurs in cytoplasm.
- ★ This difference is because of asymmetric distribution of two proteins;
  - GEF : Guanine nucleotide exchange factor (GEF).
  - GAP : GTPase activating protein.

Nucleus contains Ran-GEF, which stimulates replacement of GDP by GTP, thus converting

Ran-GDP → Ran GTP



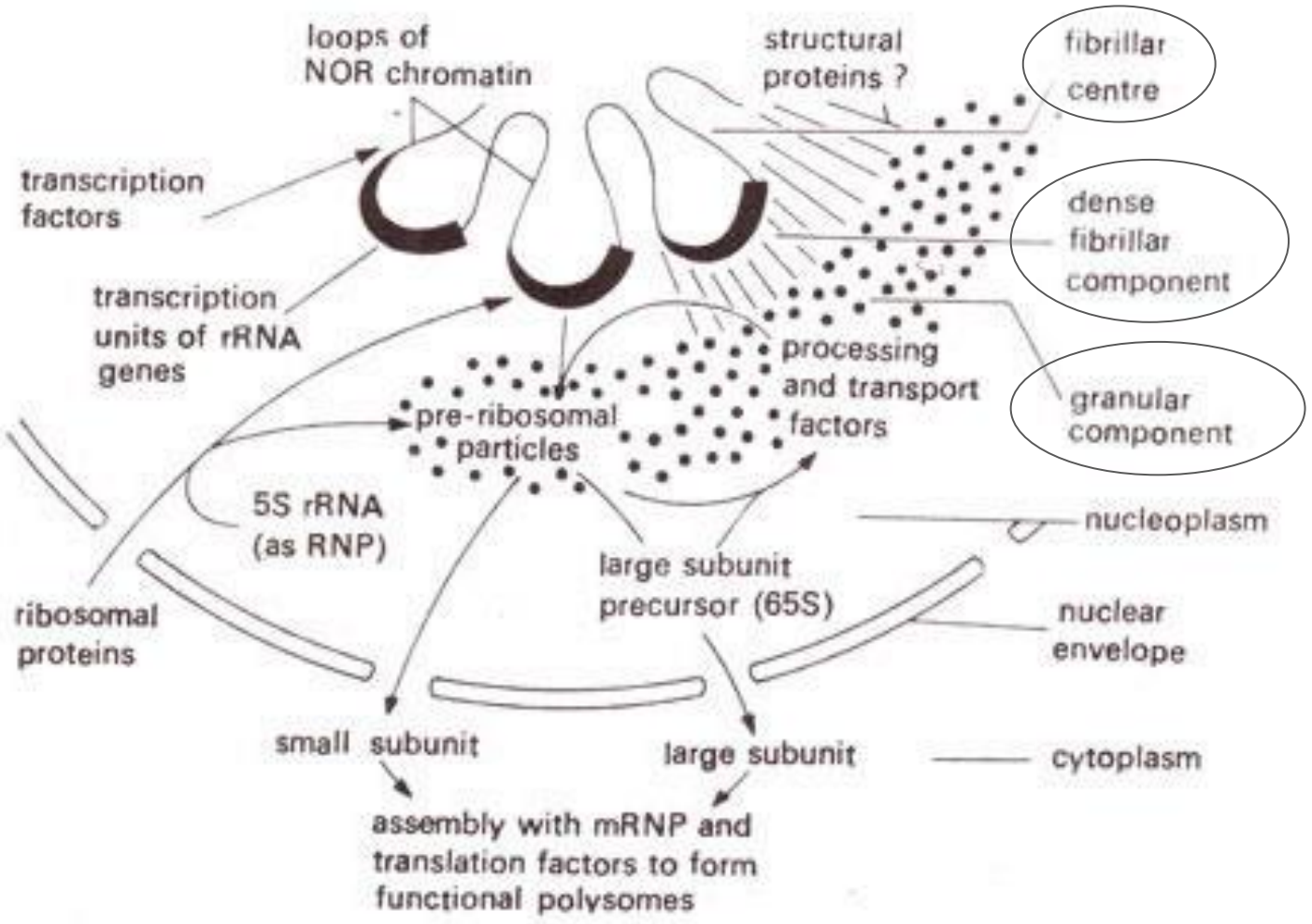
Cytoplasm contains Ran-GDP, which causes GTP to be hydrolysed to GDP. Hence Ran-GDP is localized on surface of cytoplasmic side of NPC. "Export complexes - stable: Ran-GTP; Import complexes -stable:Ran-GDP"

NUCLEOLUS:

- ★ Non - membrane bound, dynamic body.
- ★ It disappears - late Prophase; Reappears at Nucleolar Organizing Region (NOR) of Chr at Telophase.
- ★ Each nucleolus is produced by a Nucleolar Organizing Region (NOR) present on a Nucleolar Organizing chromosome (All eukaryotic cells have one such chr.).
- ★ Number of nucleoli may be 1 - 100 / nucleus (1 to 4 in common).
- ★ Eg: Xenopus oocytes - 1000 nucleoli / nucleus.

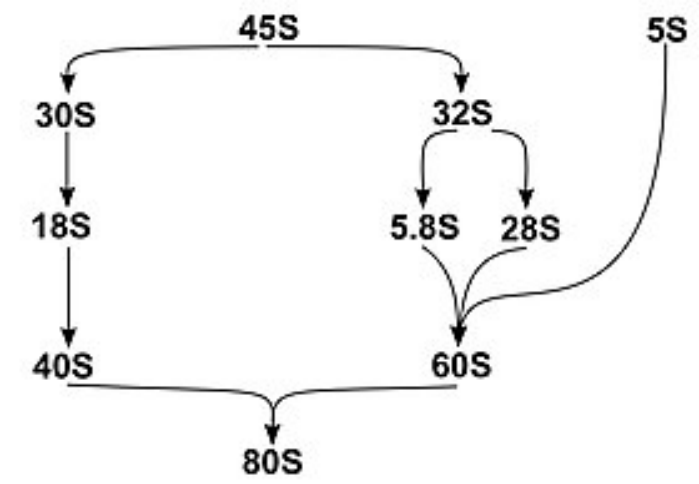
Functions:

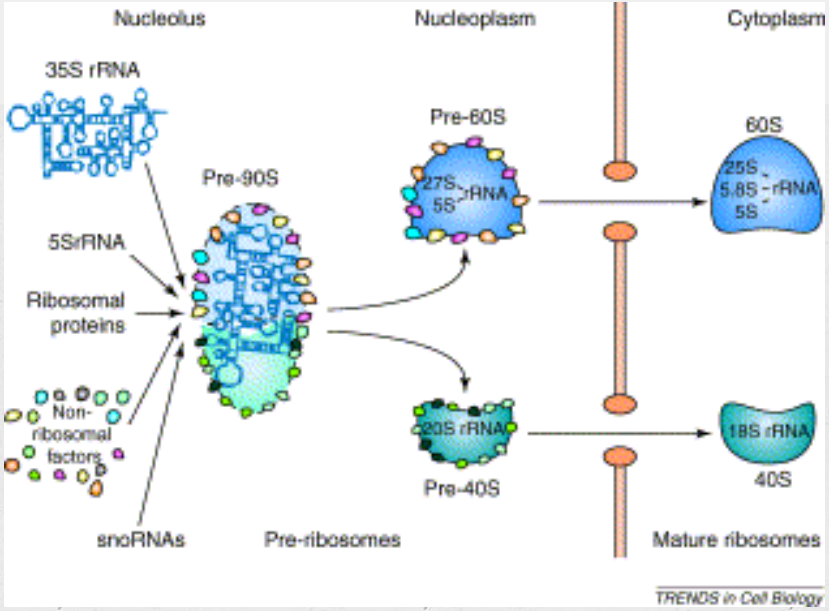
- ★ Nucleolus → Site of transcription of rRNA and assembly of ribosomes.
- ★ So [rRNA and proteins].
- ★ Matured are released into cytoplasm through nuclear pore.


loops of
NOR chromatin
structural
proteins ?
fibrillar
centre
transcription
factors
dense
fibrillar
component
transcription
units of rRNA
genes
processing
and transport
factors
granular
component
pre-ribosomal
particles
5S rRNA
(as RNP)
nucleoplasm
large subunit
precursor (65S)
ribosomal
proteins
nuclear
envelope
small subunit
large subunit
cytoplasm
assembly with mRNP and
translation factors to form
functional polysomes

Ribosomal subunit production:

- ★ Nucleolus is the most conspicuous structure within interphase nucleus.
- ★ Responsible for synthesizing a large nascent precursor rRNA (pre-rRNA).
- ★ It is 45S (mammals) → processed and cleaved → 18S, 5.8S and 28S.
- ★ Concomitant assembly of these RNAs with incoming ribosomal proteins → smaller and larger subunits (pass into cytoplasm).





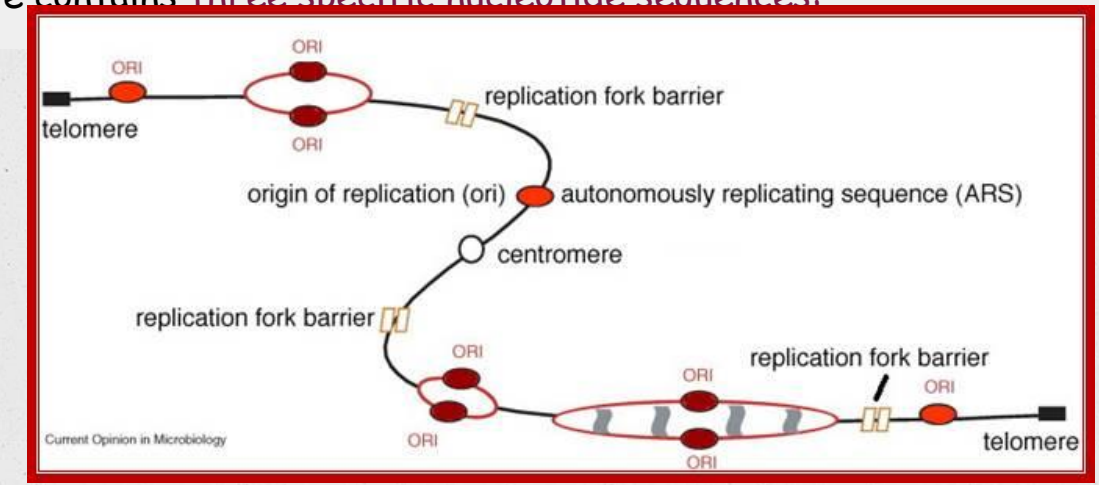
NUCLEOLAR ORGANIZER:

- ★ They are secondary constrictions within mitotic chromosomes of eukaryotes.
- ★ Primary constriction occurs at centromere.
- ★ The number of sec. constrictions within certain chromosomes of *D. funebris* is proportional to number of nucleoli. (....Acc. to Heitz).
- ★ Named these regions - sine acid thymonucleinico.
- ★ McClintock → coined Nucleolar Organizer (satellite seg at maize chr. No.6 that give rise to nucleolus).

So, during late telophase, RNA Pol I transcribes rRNA genes and the newly synthesized rRNA likely nucleates the assembly of nuleolus by recruiting prenucleolar bodies.

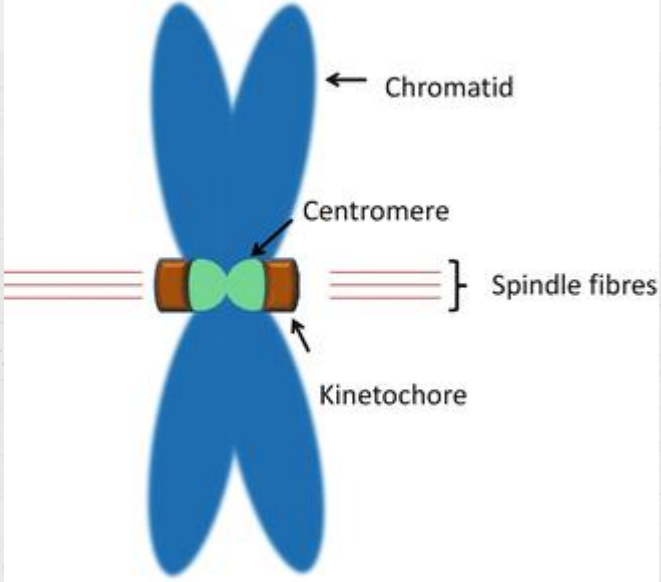
Chromosome (Gr: Color; body):

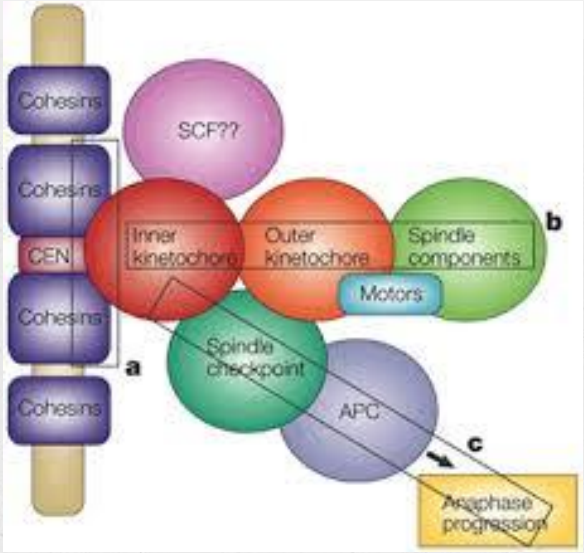
- ★ An organized structure of DNA and protein, found in nucleus of eukaryotic cells.
- ★ It is a single piece of coiled DNA containing coding and non-coding sequences.
- ★ They have the property of being very strongly stained by specific dyes.
- ★ A chromosomal DNA molecule contains three specific nucleotide sequences:
  - Centromere
  - Telomere
  - Ori



1. Centromere:

- ★ The constricted region of linear chr. Is the centromere.
- ★ It is not located in the center of chr. And also exceptionally in chr. Ends.
- ★ Either side of centromere → chromosome's arms.
- ★ Function is to keep chromosomes properly aligned during complex division processes.
- ★ Important sites for holding sister chromatids and as attachment sites for Mts of mitotic spindle.





2. Telomere:

- ★ Repetitive stretches of DNA at the ends of linear chr,
- ★ They protect the ends of chr (lllr to shoelaces) from unravelling.

3. Origin of replication:

- ★ Replication origin is a particular sequence in achr. to initiate replication.

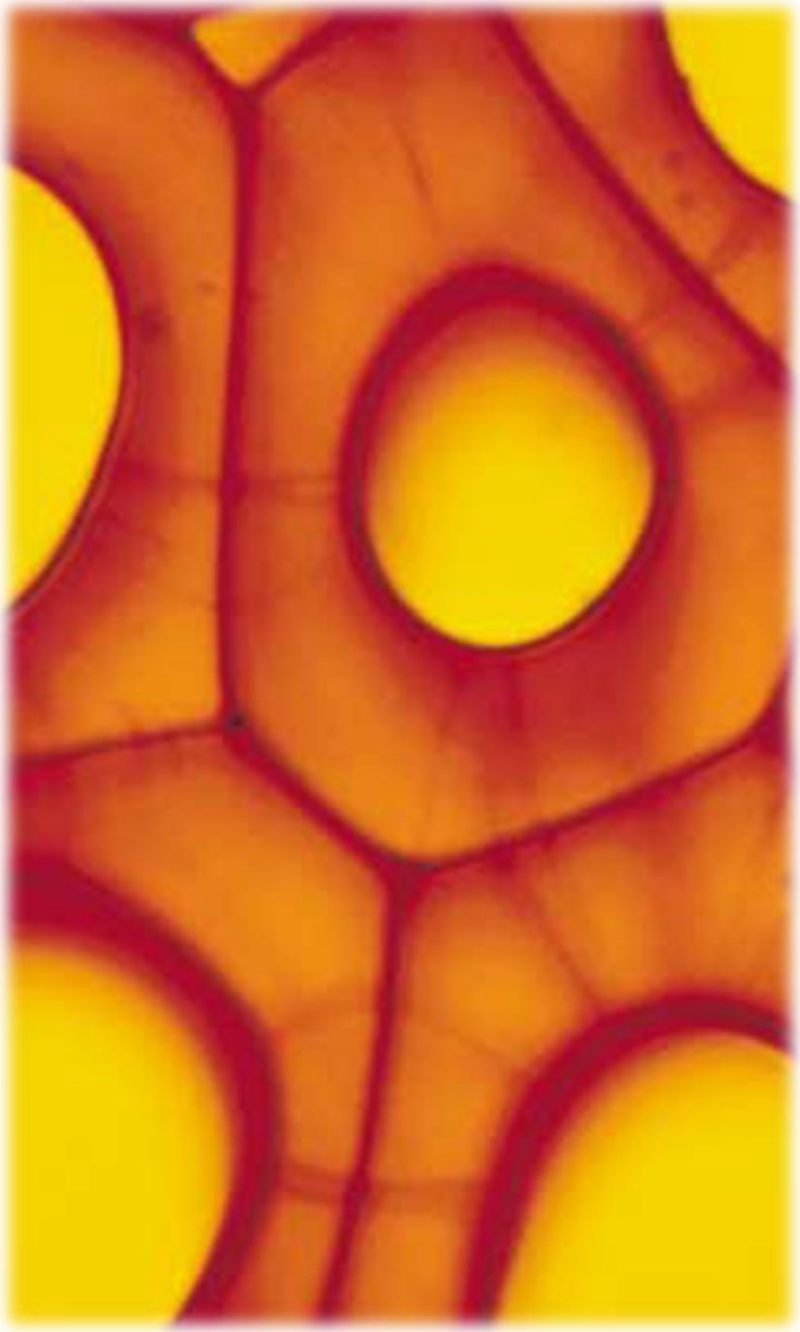
Chromosome number:

- ★ All eukaryotic cells have multiple linear chromosomes (2 to less than 50,..... also reach 1000s).
- ★ Majority of eukaryotic cells are dilploid; having two copies of Chr.

Table 23–2 Normal chromosome number in different organisms*

Bacteria	1
Fruit fly	8
Red clover	14
Garden pea	14
Yeast	16
Honeybee	16
Corn	20
Frog	26
Hydra	30
Fox	34
Cat	38
Mouse	40
Rat	42
Rabbit	44
Human	46
Chicken	78

* For all eukaryotic organisms listed, the diploid chromosome number is shown.



CELL BIOLOGY (22ZOOC12)

Cell → Cell Interactions

- **Intercellular Attachment**

Cell adhesion molecules (CAMs)

Introduction

- The development of highly specialized groups of cells (**tissues** → blood and muscle) in multicellular is unique.

- Remarkably, each cell within a tissue performs the functions of that tissue and no other, even though all cells of the body are derived from a single fertilized cell and contain the same genetic information.

To understand on how do cells sense where they are?

How do they "know" which type of tissue they belong to?

Cell surface proteins mediate cell-cell interactions

1. The expression of cell identity.

Cells possess on their surfaces a variety of tissue-specific identity markers that identify both the tissue and the individual.

2. Intercellular adhesion.

Cells attach themselves to one another with protein links. Some of the links are very strong, others more transient.

3. Tight junctions.

Adjacent cells form a sheet when connected by tight junctions, and molecules are encouraged to flow through the cells, not between them.

4. Anchoring junctions.

The cytoskeleton of a cell is connected by an anchoring junction to the cytoskeleton of another cell or to the extracellular matrix.

5. Communicating junctions.

Many adjacent cells have direct passages that link their cytoplasms, permitting the passage of ions and small molecules.

Cell Communicating Mechanisms

Mechanism	Structure	Function	Example
INTRACELLULAR RECEPTORS	No extracellular signal-binding site	Receives signals from lipid-soluble or noncharged, nonpolar small molecules	Receptors for NO, steroid hormone, vitamin D, and thyroid hormone
CELL SURFACE RECEPTORS			
Chemically gated ion channels	Multipass transmembrane protein forming a central pore	Molecular "gates" triggered chemically to open or close	Neurons
Enzymic receptors	Single-pass transmembrane protein	Binds signal extracellularly, catalyzes response intracellularly	Phosphorylation of protein kinases
G-protein-linked receptors	Seven-pass transmembrane protein with cytoplasmic binding site for G protein	Binding of signal to receptor causes GTP to bind a G protein; G protein, with attached GTP, detaches to deliver the signal inside the cell	Peptide hormones, rod cells in the eyes
PHYSICAL CONTACT WITH OTHER CELLS			
Surface markers	Variable; integral proteins or glycolipids in cell membrane	Identify the cell	MHC complexes, blood groups, antibodies
Tight junctions	Tightly bound, leakproof, fibrous protein "belt" that surrounds cell	Organizing junction: holds cells together such that material passes *through* but not *between* the cells	Junctions between epithelial cells in the gut
Desmosomes	Intermediate filaments of cytoskeleton linked to adjoining cells through cadherins	Anchoring junction: "buttons" cells together	Epithelium
Adherens junctions	Transmembrane fibrous proteins	Anchoring junction: "roots" extracellular matrix to cytoskeleton	Tissues with high mechanical stress, such as the skin
Gap junctions	Six transmembrane connexon proteins creating a "pipe" that connects cells	Communicating junction: allows passage of small molecules from cell to cell in a tissue	Excitable tissue such as heart muscle
Plasmodesmata	Cytoplasmic connections between gaps in adjoining plant cell walls	Communicating junction between plant cells	Plant tissues

1. Tissue-Specific Identity Markers (Cell identity)

- Every cell contains a specific array of marker proteins on its surface.
- These markers identify each type of cell in a very precise way.

Eg: Glycolipids –

Most tissue-specific cell surface markers are glycolipids (lipids with carbohydrate heads) → A, B and O blood types.

Eg: **MHC Proteins –**

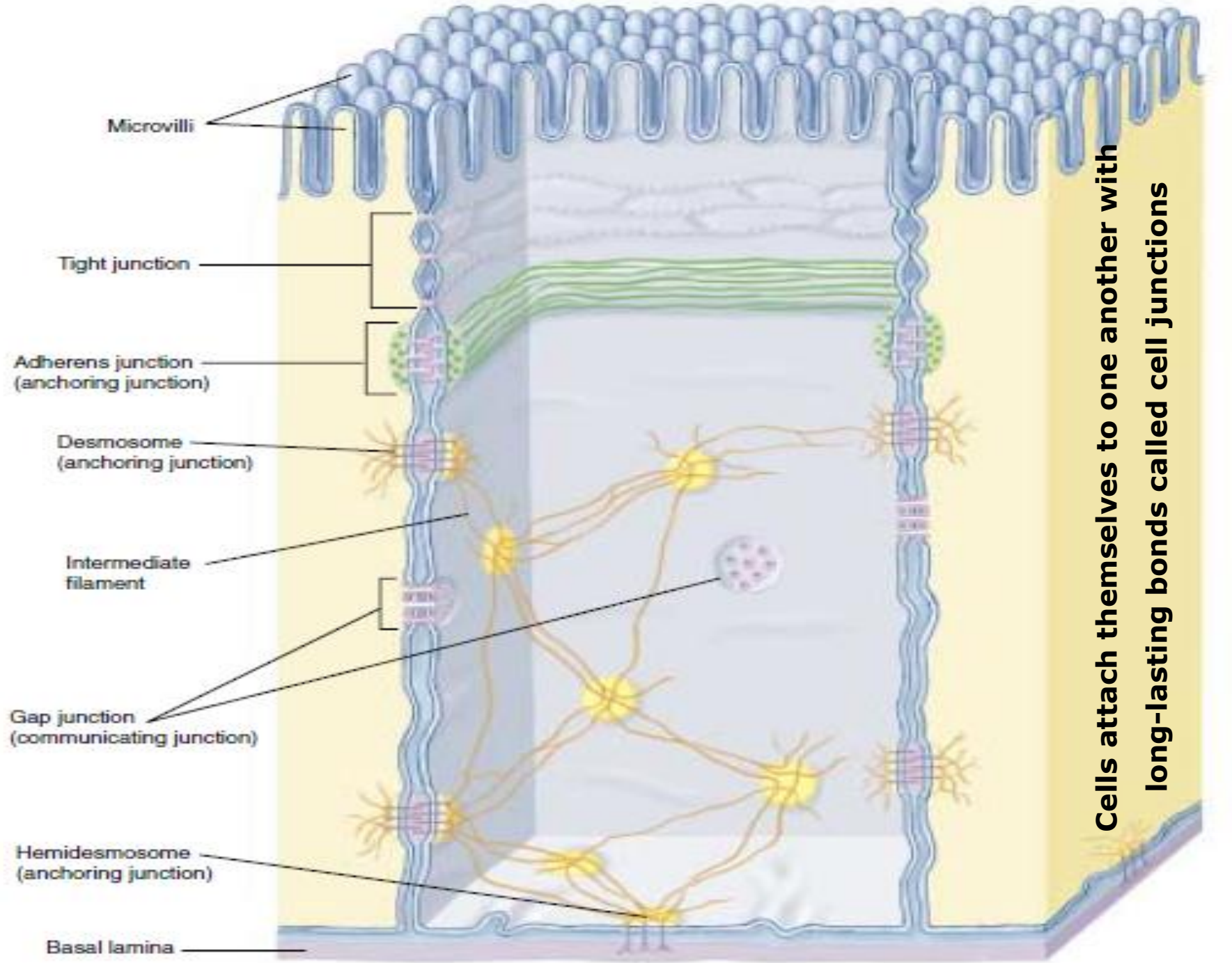
Differentiate self and non-self cells.

2. Intercellular Adhesion:

Most cells are in physical contact with other cells as members of **organized tissues.**

These cells and the mass of other cells clustered around them form **permanent connections** with each other called **cell junctions.**

Eg: In lungs, heart, or gut.


Microvilli
Tight junction
Adherens junction
(anchoring junction)
Desmosome
(anchoring junction)
Intermediate
filament
Gap junction
(communicating junction)
Hemidesmosome
(anchoring junction)
Basal lamina
Cells attach themselves to one another with
long-lasting bonds called cell junctions

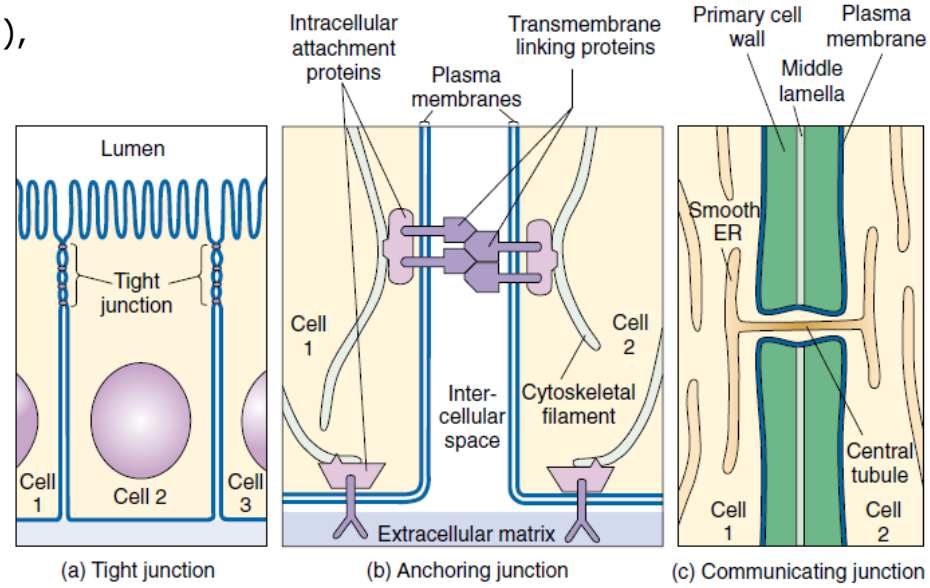
3. Cell junctions:

Cell junctions are divided functionally into three categories:

- **Tight junctions (Leakproof sheets)**
- **Anchoring junctions**
- **Communicating junctions.**

▪ **Occluding junctions** - connect the plasma membranes of adjacent cells in a sheet.

It prevent small molecules from leaking between the cells and through the sheet (wall within the organ), keeping molecules on one side or the other.



(a) Tight junction (b) Anchoring junction (c) Communicating junction

Tight junctions connect the plasma membranes of adjacent cells into sheets

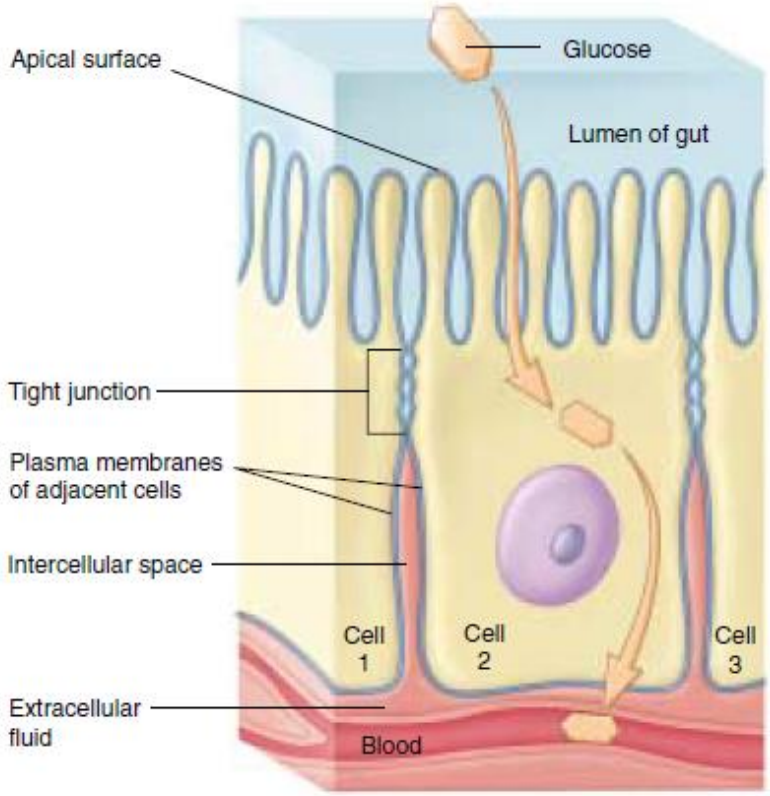
- Tight junctions connect the plasma membranes of adjacent cells in a sheet, preventing small molecules (water) from leaking between the cells and through the sheet.

Creating Sheets of Cells - Digestive tract

- The cells that line an animal's digestive tract are organized in a sheet only one cell thick.

- One surface of the sheet faces the inside of the tract and the other faces the extracellular space where blood vessels are located.
- Tight junctions encircle each cell in the sheet between neighboring cells are so securely attached that there is no space between them for leakage.

- Hence, nutrients absorbed from the food in the digestive tract must pass directly through the cells in the sheet to enter the blood.



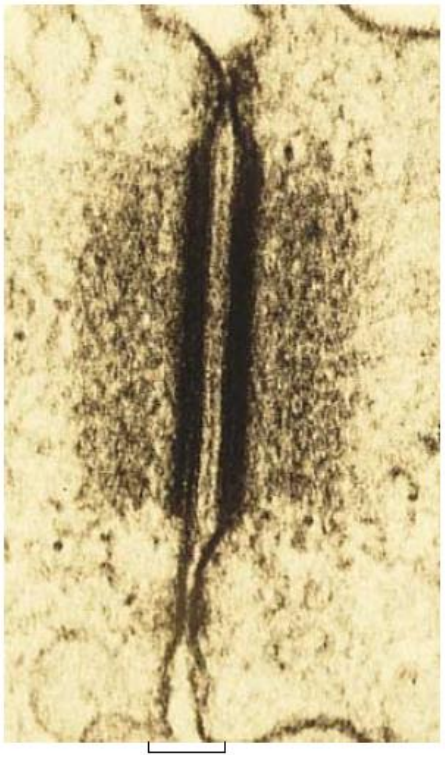
4. Anchoring junctions:

- Anchoring junctions mechanically attach the cytoskeleton of a cell to the cytoskeletons of other cells or to the extracellular matrix.

- They are commonest in tissues subject to **mechanical stress**, such as muscle and skin epithelium.

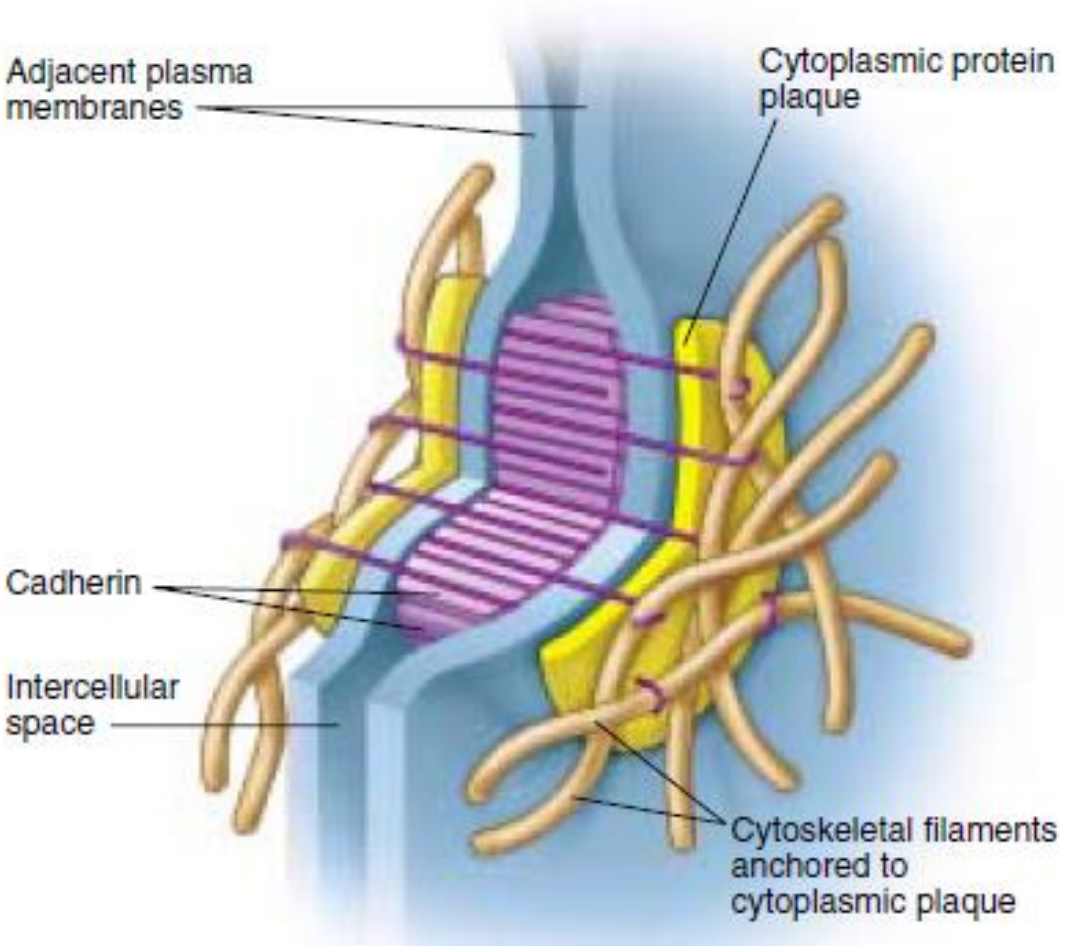
Eg: Desmosomes

- Cadherin and Intermediate Filaments (IF)

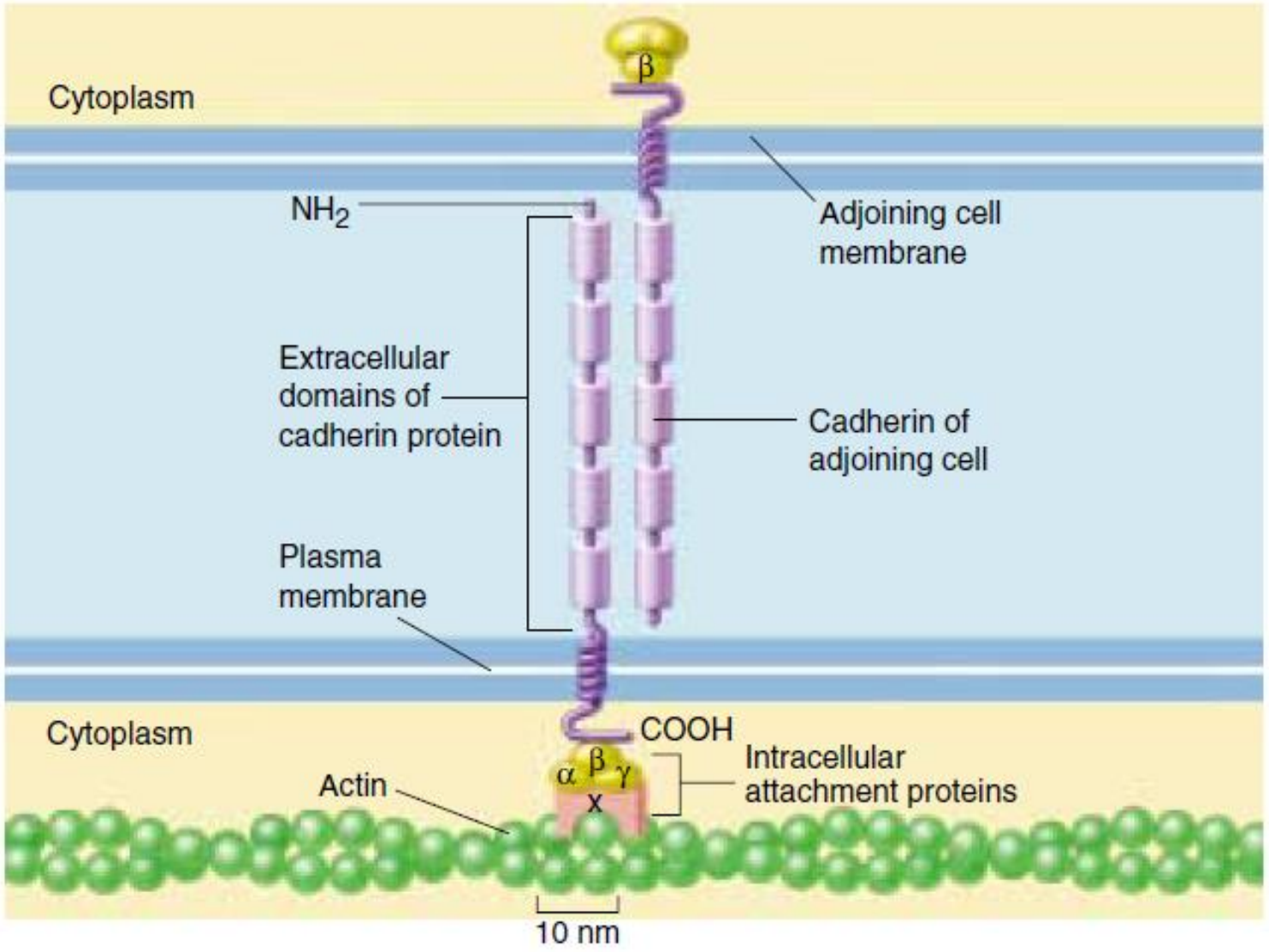


Cadherin proteins create adhering link between adjoining cells.

- **Desmosomes** connect the cytoskeletons of adjacent cells.
- **Hemidesmosomes** anchor epithelial cells to a basement membrane.
- Proteins called **cadherins** (single-pass transmembrane glycoproteins) create the critical link (**stable**).



Actin-linking cadherins



❖ **Cadherin and Actin Filaments:**

Cadherins can also connect the actin frameworks of cells in cadherin-mediated junctions. They are **less stable** links.

- Many kinds of actin-linking cadherins occur in **different tissues**, as well as in the **same tissue at different times**.
- **Gene-controlled regulation in cadherin expression.**

Eg: During vertebrate development, the **migration of neurons** in the embryo is associated with changes in the type of cadherin expressed and these migrating cells reach their destination.

❖ **Integrin-Mediated Links:**

▪ Anchoring junctions called **adherens** junctions connects the actin filaments of one cell with those of neighboring cells or with the extracellular matrix.

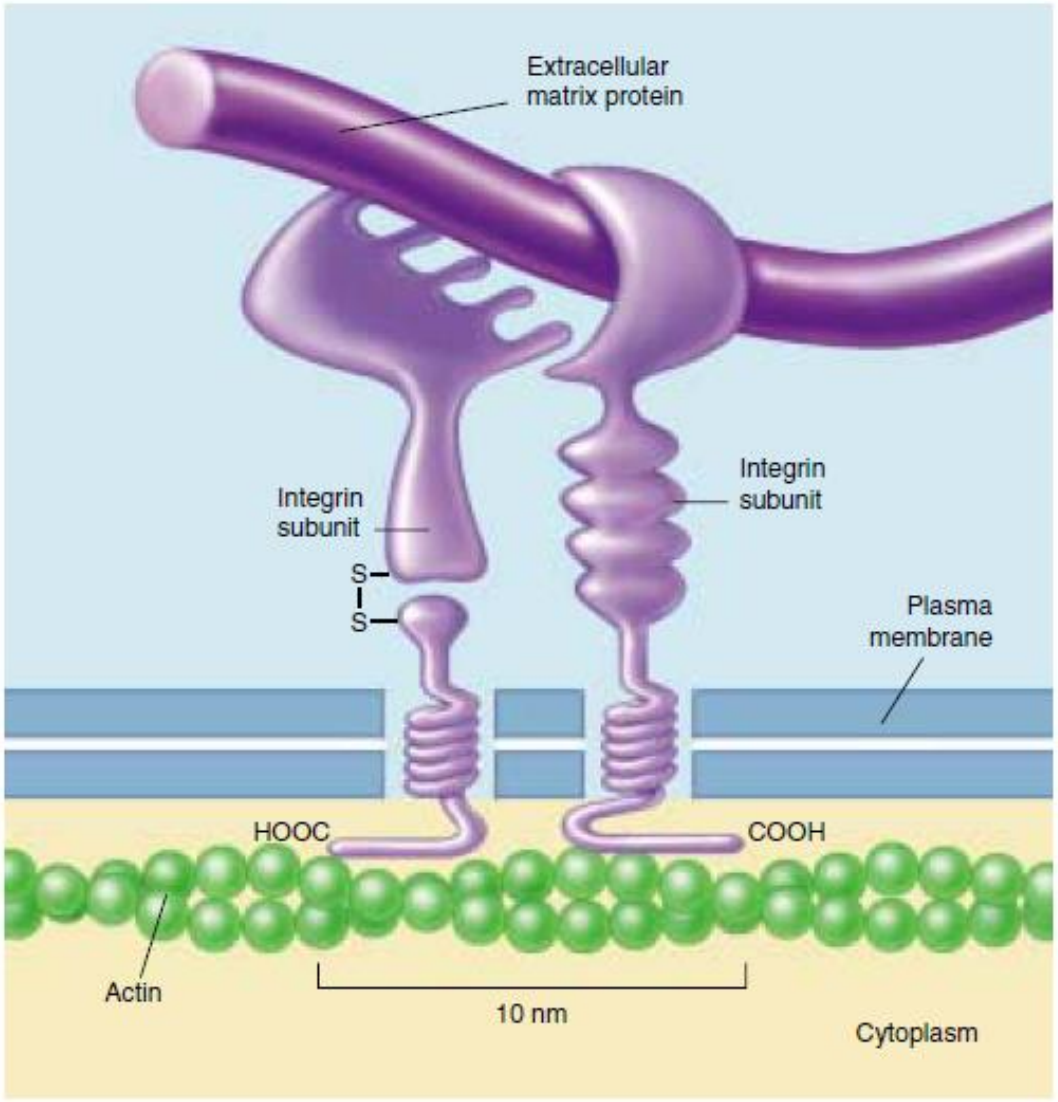
The linking proteins in these junctions are members of a large superfamily of cell surface receptors called **integrins**.

▪ Integrin is a **transmembrane protein** composed of **two different glycoprotein subunits (heterodimer)** that extend outward from the plasma membrane.

▪ Together, these subunits bind a protein component of the extracellular matrix, like two hands clasping a pole.

▪ There appear to be many different kinds of integrin (identified 20), each with a slightly different shaped **"hand."**

An integrin-mediated junction



These adherens junctions link the actin filaments inside cells to their neighbors and to the extracellular matrix.

5. Communicating Junctions:

(Gap junction – animal cell; Plasmodesmata – plant cell)

- Many cells communicate with adjacent cells through direct connections, called **communicating junctions.**

- A **chemical signal passes directly** from one cell to an adjacent one.

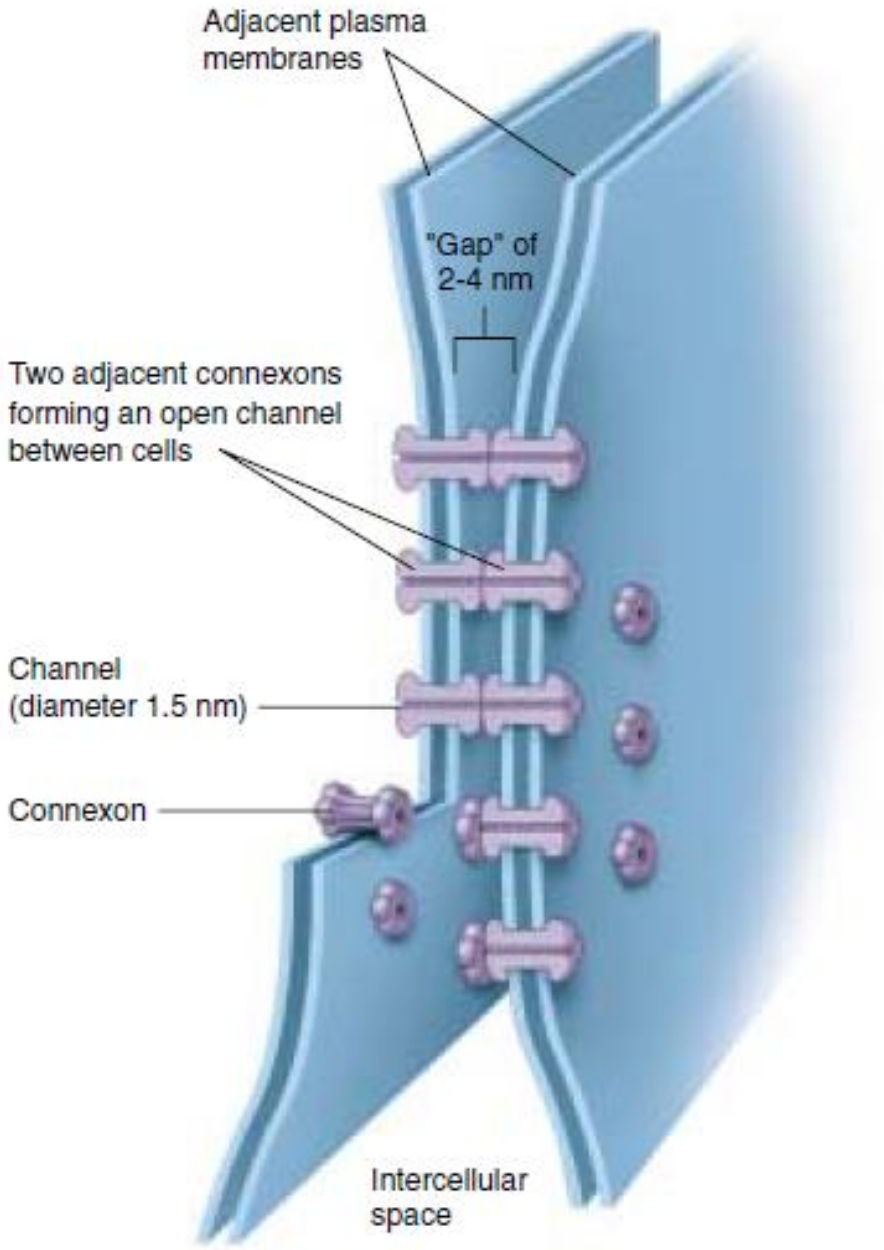
- It establish **direct physical connections** that link the cytoplasms of two cells together. Hence **permitting small molecules or ions** to pass from one to the other.

❖ Gap Junctions in Animals:

Communicating junctions called gap junctions are composed of structures called **connexons.**

A gap junction forms when the **connexons** of two cells align perfectly, creating an open channel spanning the plasma membranes of both cells.

- It provide passageways to permit small substances → simple sugars and amino acids.
- It **prevent** the passage of larger molecules such as proteins.
- Gap junction channels are **dynamic** structures. It can open or close in response to a variety of factors (Ca++ and H+ ions).



Gap junctions.

Connexons create passageways that connect the cytoplasms of adjoining cells.

- Connexon is a complexes of **six identical transmembrane proteins,** arranged in a circle to create a channel through the plasma membrane that protrudes several nanometers (4nm) from the cell surface.

- It hold the plasma membranes of the paired cells about 4 nanometers apart (opposite to tight junction).

An important function:

When a cell is damaged, its plasma membrane often becomes leaky. Ions in high concentrations outside the cell, such as Ca++, flow into the damaged cell and shut its gap junction channels (seals). This isolates the cell and so prevents the damage from spreading to other cells.

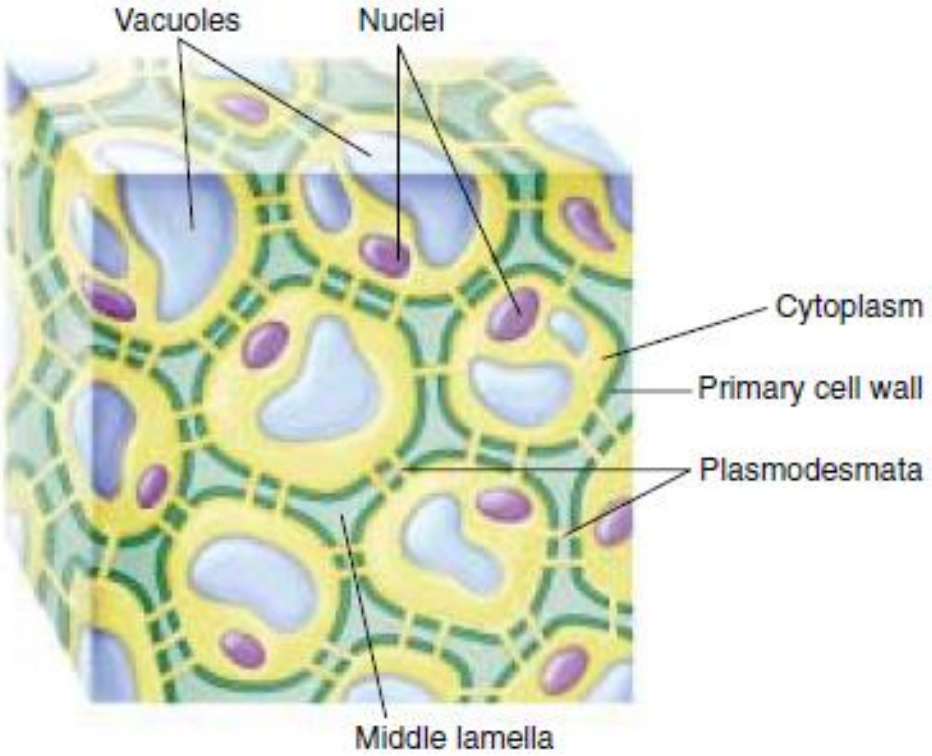
❖ Plasmodesmata in Plants

- Cell walls separate every cell from all others.
- Cell – cell junctions occur only at holes or gaps in the walls, where the plasma membranes of adjacent cells can come into contact with each other.

Cytoplasmic connections that form across the touching plasma membranes → plasmodesmata.

- It permit the **controlled passage** of small molecules or ions between cells.

Similar to gap junctions in animal cells, but the central tubule of plasmodesmata Connects the ER of the two cells.



Summary:

- Tight junctions and desmosomes enable cells to adhere in tight, leakproof sheets, holding the cells together such that materials cannot pass between them.

- Gap junctions (in animals) and plasmodesmata (in plants) permit small substances to pass directly from cell to cell through special passageways.