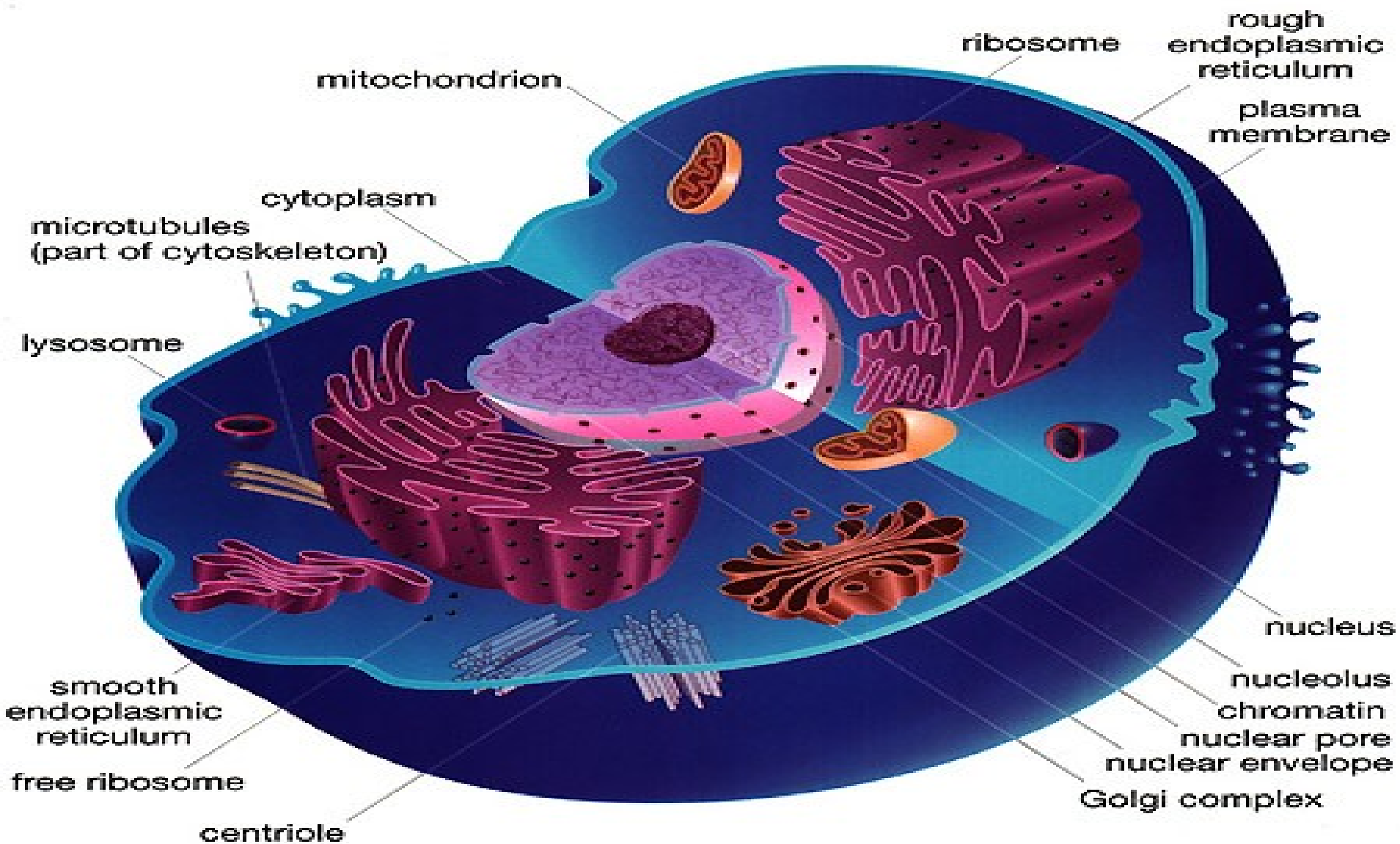


COMPARTMENT ORGANIZATION

- The cell is the smallest functional unit of living organisms and tissues.
- One universal feature of all cells is the plasma or cell membrane or plasma lemma.
- The plasma membrane forms the external boundary of the cell, and serves as **the interface between the machinery in the interior of the cell and the extracellular fluid (ECF) that bathes all cells.**
- In addition they form **a system of internal membranes which set various membrane closed compartments within the cell.**
- These membrane systems of the cell can be viewed as a single entity, even though they are highly distinctive and localised in their activities.

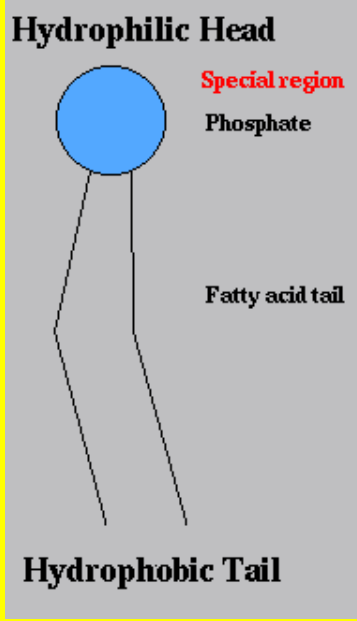
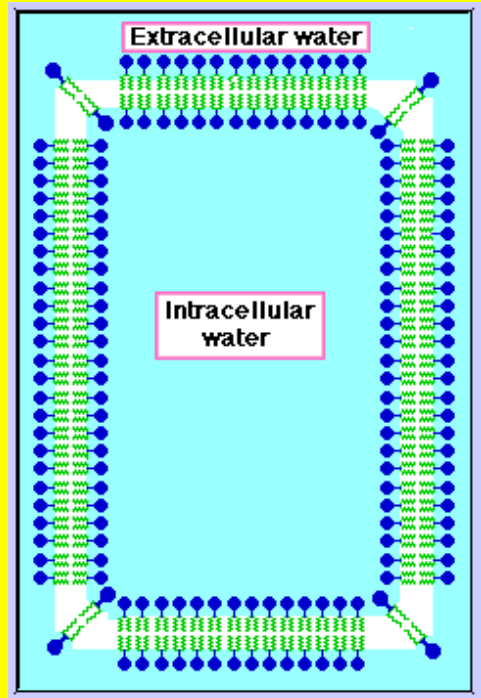
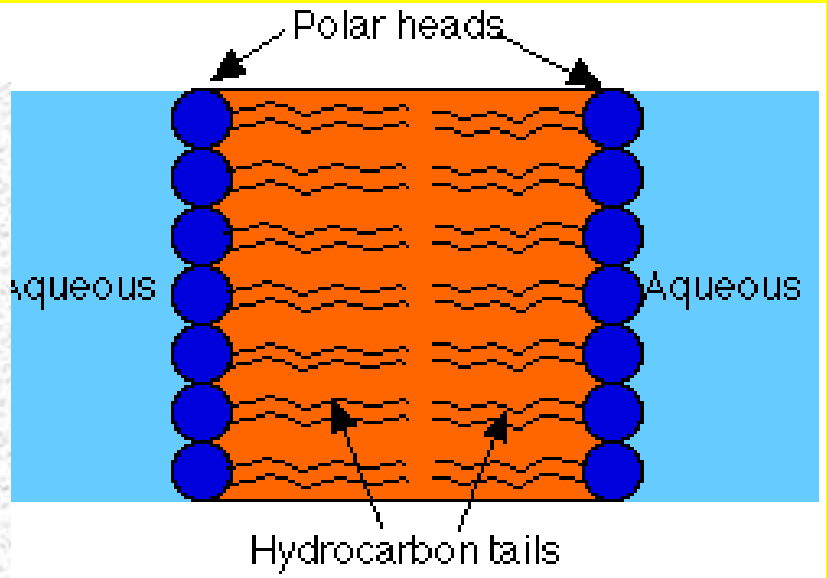
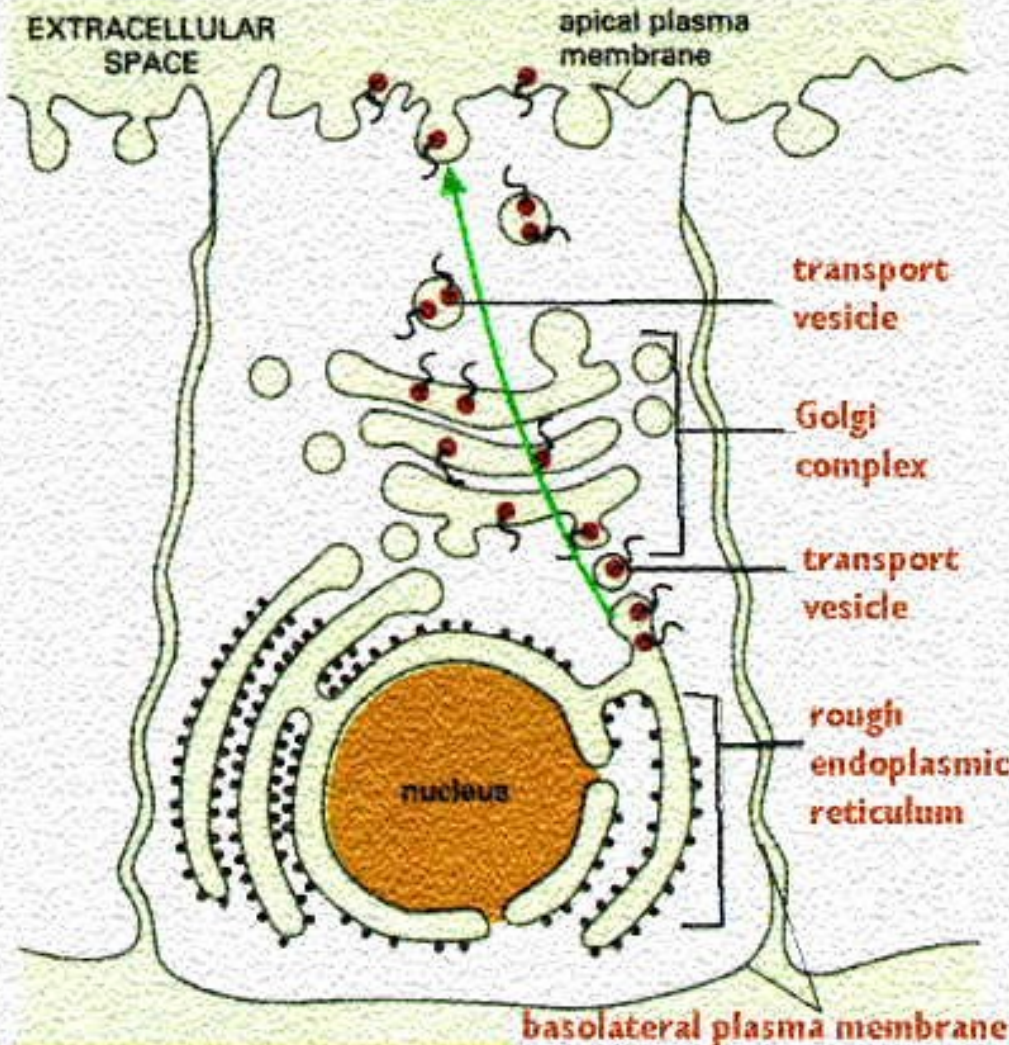
- All membranes have the same basic structure.
- Cell membranes are built from phospholipids and proteins, which float in a double layer of lipids.
- Electron microscopic examinations of cell membranes have led to the development of the lipid bilayer model (also referred to as the fluid mosaic model).



BIOLOGY: Life on Earth, Fifth Edition
by Teresa Audesirk and Gerald Audesirk

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Animal cell



- In our discussion of the cell today, we are only interested in details of the structure of the cell or its contents, in so far as it allows us to grasp **the principle that the cell membrane, and its extension within the cell effect compartmentalization of the cell;** and..... **in so doing, greatly enhances the capability of the cell to perform myriads of chemical reactions simultaneously within the relatively small compass of space which constitutes the cell.**

- **Cell membranes are vital because they separate the cell from the outside world.**
- **Cells also have internal membranes that also separate compartments inside the cell to facilitate and protect important processes and events.**
- **We will see later that the internal membranes of the cell form compartments for specialized biochemical activities.**

- **The cell or plasma membrane performs the following 3 main functions.**
- **1. Isolation of the cells's contents from the external environment.**
- **2. Regulation of the exchange of essential substances in and out of the cell by controlling the entry of ions and nutrients, the elimination of wastes, and the release of secretory products.**
- **3. Communication with other cells so that the plasma membrane can change in response to it's surroundings.**

- The plasma membrane is said to be selectively permeable.
- In the simplest case, nutrients must be allowed to enter the cell and waste products to exit.
- **Another critical task is to maintain an ionic composition inside the cell that is very different from that outside the cell.**

- The following internal fluid compartments are of interest to us in Clinical Medicine.
- **Extracellular Compartment**
 - plasma
 - cerebrospinal fluid
 - interstitial fluid
- **Intracellular Compartment**

- One important role of the cell membrane is to regulate the composition of the internal fluids such that they are constantly at conditions compatible with life- e.g pH
- The composition of this fluid in the **intracellular compartment** also reflects the condition or state of the cells.
- The plasma membrane must also regulate the composition of the fluid surrounding the cells, i.e the fluid in the **extracellular compartment** .

- Consequently, cellular contents are delineated by **a selectively permeable plasma membrane, which is essentially a barrier that renders the cell as a compartment apart from other cells.**
- The cell membrane functions as a semi-permeable barrier, **allowing a very few molecules across it while fencing the majority of organically produced chemicals inside the cell.**
- In the process, the cell membrane **permits control over the internal environment of the cell and also acts as a physical barrier that separates the inside of the cell from the surrounding extracellular fluid.**

- The cell membrane is the first part of the cell affected by changes in the extracellular fluid.
- It also contains a variety of receptors that allow the cell to recognize and respond to specific molecules in its environment.
- Any alteration in the cell membrane may affect all cellular activities.
-

- Biological membranes form a continuous sheet-like barrier around cells or **intracellular compartments**.
- By restricting the diffusion of most substances **between compartments** and facilitating the movement of others through a wide variety of protein pores and transport systems, membranes present highly selective permeability barriers that allow the **control of the chemical environment in cellular compartments and between cells and its surroundings**.

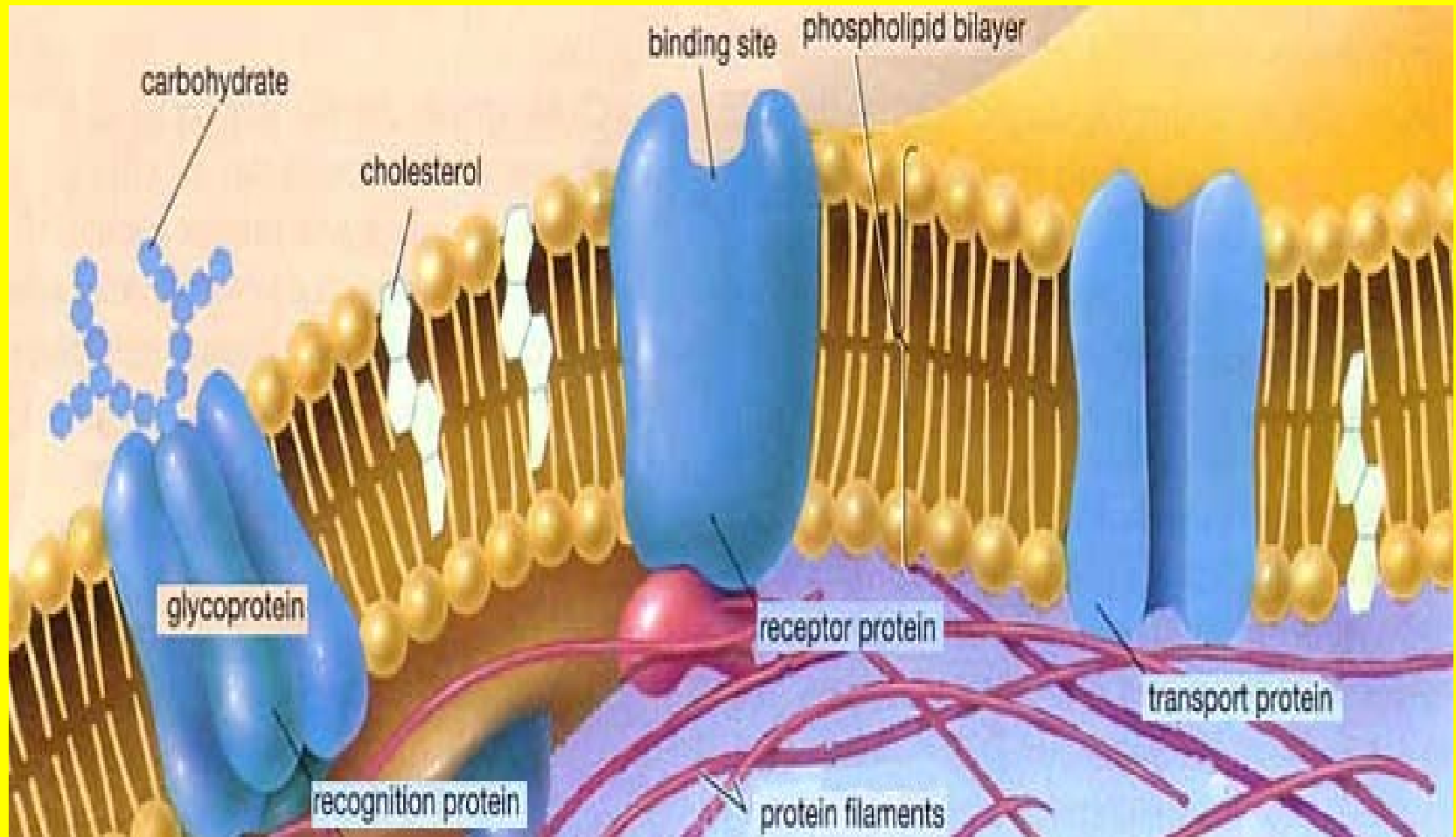
- Membranes have important roles in biological communication by **allowing for the flow of information between compartments in the cell and between cells and their environment.**
- The presence of specific molecules in membranes allows **recognition of stimuli in the form of chemical signals** (eg hormones, local mediators and neurotransmitters), **electrical events** (changes in membrane potential) and **light** (retina) as well **secondary signal generation in response to recognition of the primary stimulus**, which may be physical, chemical, or electrical.
- **Different membranes have specialized functions** (table 4.1) but they are all roles of **compartmentalization.**

Membrane	Function
Plasma membrane	<p>Separates the cytoplasm from the extracellular environment by forming a continuous barrier around the cell. This limits diffusion of substances into and out of the cytoplasm, protecting the cell from adverse fluctuation in conditions and therefore permitting control of the intracellular environment.</p> <p>Punctuated by transport proteins which enable permeability to be regulated.</p> <p>Mediates direct communication between cells e.g. contact inhibition, cell adhesion.</p> <p>Contains receptor and effector mechanisms for chemical signalling between cells, e.g. hormones, growth factors, local chemical mediators, neurotransmitters</p>
Mitochondrial membrane	Mediates energy conservation by oxidative phosphorylation (c.f. photosynthesis in chloroplasts)
Rough endoplasmic reticulum	Biosynthesis of membrane, secretory and some organellar proteins
Nerve cell – axonal	Conduction of electrical signals
Nerve cell – presynaptic	Release of neurotransmitter
Nerve cell – postsynaptic	Response generation to neurotransmitter

Table 4.1 Some specialized functions of different cellular membranes

Proteins embedded in the membrane

The phospholipid bilayer has many proteins embedded in it. **These proteins regulate the movement of materials in and out of the cell and allow the cell to communicate with its environment.**

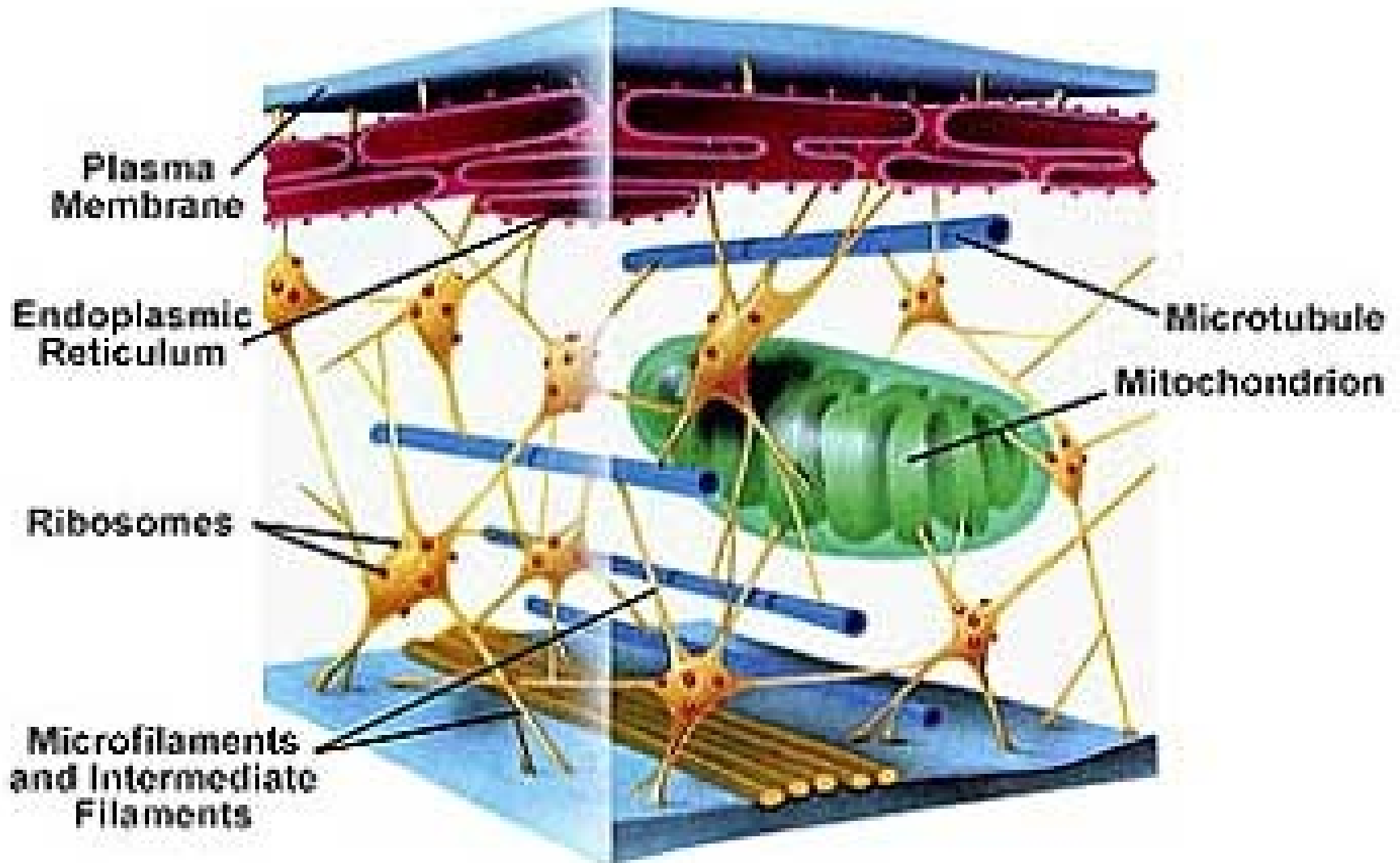


- We cannot stress enough that all the membranes of the cell act mainly as permeability barriers to all charged and hydrophilic molecules. The movement of molecules and ions across a membrane is mediated, and regulated, by specific membrane transport systems.
- We will not today discuss the various transport mechanisms used by cells. These and other activities of the cell will be discussed in great detail in subsequent lectures.
- However, we do wish to stress now that these transport mechanisms could not be effective or efficient without compartmentalization.
- We will also at this time list the important roles that these transport mechanisms play in the function of the cell.

- **Transport processes have important roles including:**
- **• maintenance of intracellular pH**
- **• maintenance of ionic composition**
- **• regulation of cell volume**
- **• concentration of metabolic fuels and building blocks**
- **• extrusion of waste products of metabolism and toxic substances**
- **• generation of ionic gradients necessary for the electrical excitability of nerve and muscle**

- The cytoskeleton is a complex crosslinked network of structural proteins, unique to eukaryotic cells, that **extends from specialized contacts in the plasma membrane to form an intracellular cytoskeleton.**
- It is a dynamic three-dimensional structure that fills the cytoplasm, and is **involved in cell to cell and cell to substratum interactions.**
- This structure acts as both muscle and skeleton, for movement and stability, and maintenance of basic cell shape. It also has important dynamic functions where it contributes to cellular processes **such as the movement of organelles, endocytosis, secretion and cell division.**
- The primary types of fibers comprising the cytoskeleton are microfilaments, microtubules, and intermediate filaments.
- **The important function of compartmentalization involving the cytoskeleton involves these tubules.**

The cytoskeleton



- **The cytoskeleton also microcompartmentalizes the cytoplasm by restricting free diffusion, and may help to localize individual enzymes catalysing sequential reactions into the same cell regions such that metabolites may be channelled from one enzyme to the next, increasing the efficiency of the pathway overall.**
- **The best example of this is probably the enzyme complex palmitate synthase involved in the adding of acetyl coA units in the cytosol in the synthesis of fatty acids.**
- **We will demonstrate this in a minute.**

- Membrane compartmentalization selectively inhibits the passage of most water-soluble substances.
- The fact that there are within the cytoplasm many **membrane-lined compartments (organelles)**, each with different internal solute concentrations facilitates this process.
- **Such compartmentalization prevents the dilution of metabolic intermediates and cofactors in multistep biochemical reactions, and protects sensitive reactions from the intrusion of extraneous substances.**

- **LOCATION SPECIFICITY**
- **ENZYMES MAY BE PRESENT IN SPECIFIC ORGANELLES**
- Many enzymes are localized in specific organelles within the cell as shown. Such compartmentalization serves to isolate the reaction substrate or product from other competing reactions, to provide a favorable environment for the reaction, and to organize the thousands of enzymes present in the cell into purposeful pathways.
- **The spatial arrangement and compartmentalization of enzymes, substrates, and cofactors within the cell are of cardinal significance.**
- **In liver cells, for example, the enzymes of glycolysis are located in the cytoplasm, whereas enzymes of the citric acid cycle are in the mitochondria.**

The intracellular location of some important biochemical pathways.

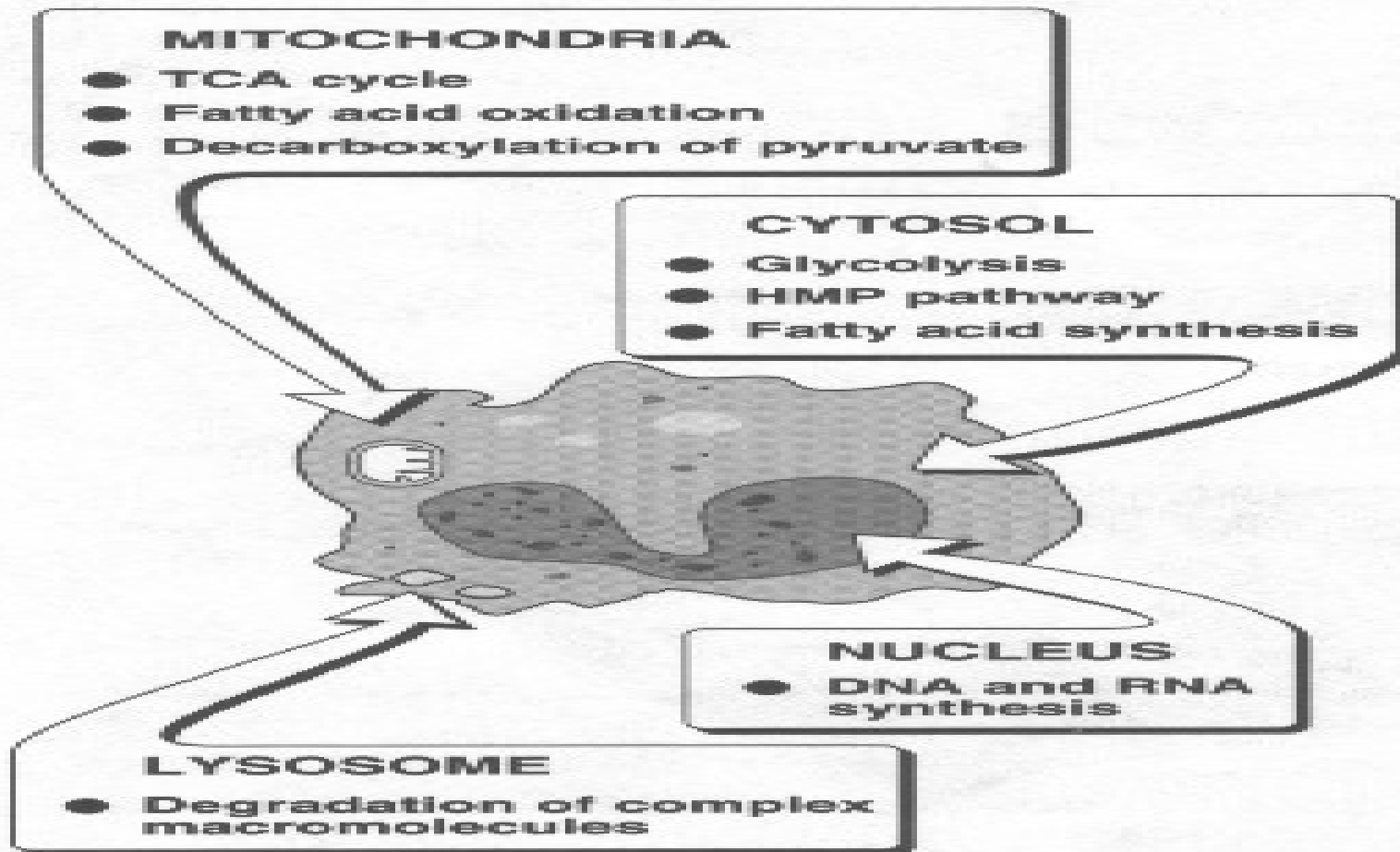


Figure 4.3

The intracellular location of some important biochemical pathways.

HUMAN PALMITATE SYNTHASE

DOMAINS

1.- Malonyl transferase

2.- Acyl transferase

3.- Condensing

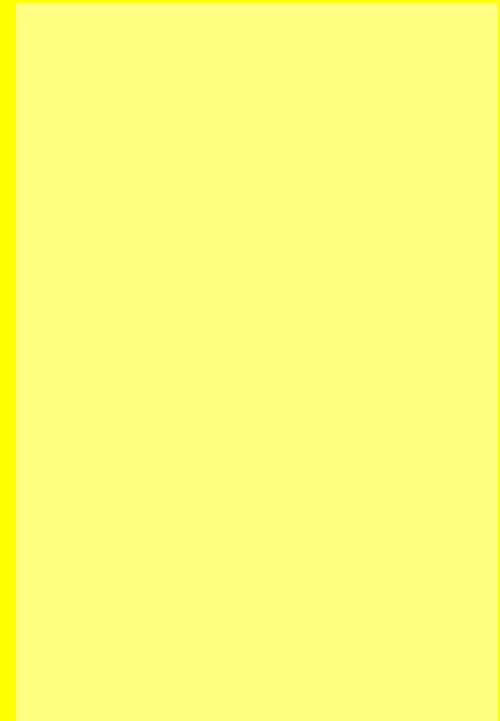
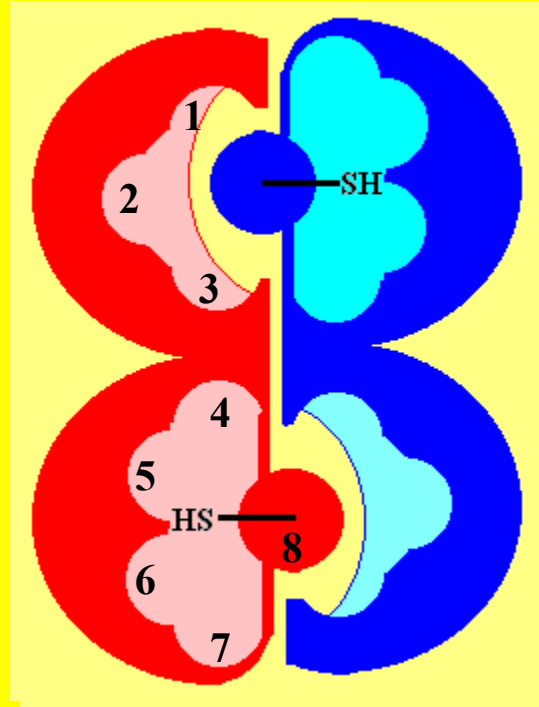
4.- β -ketoacyl-reductase

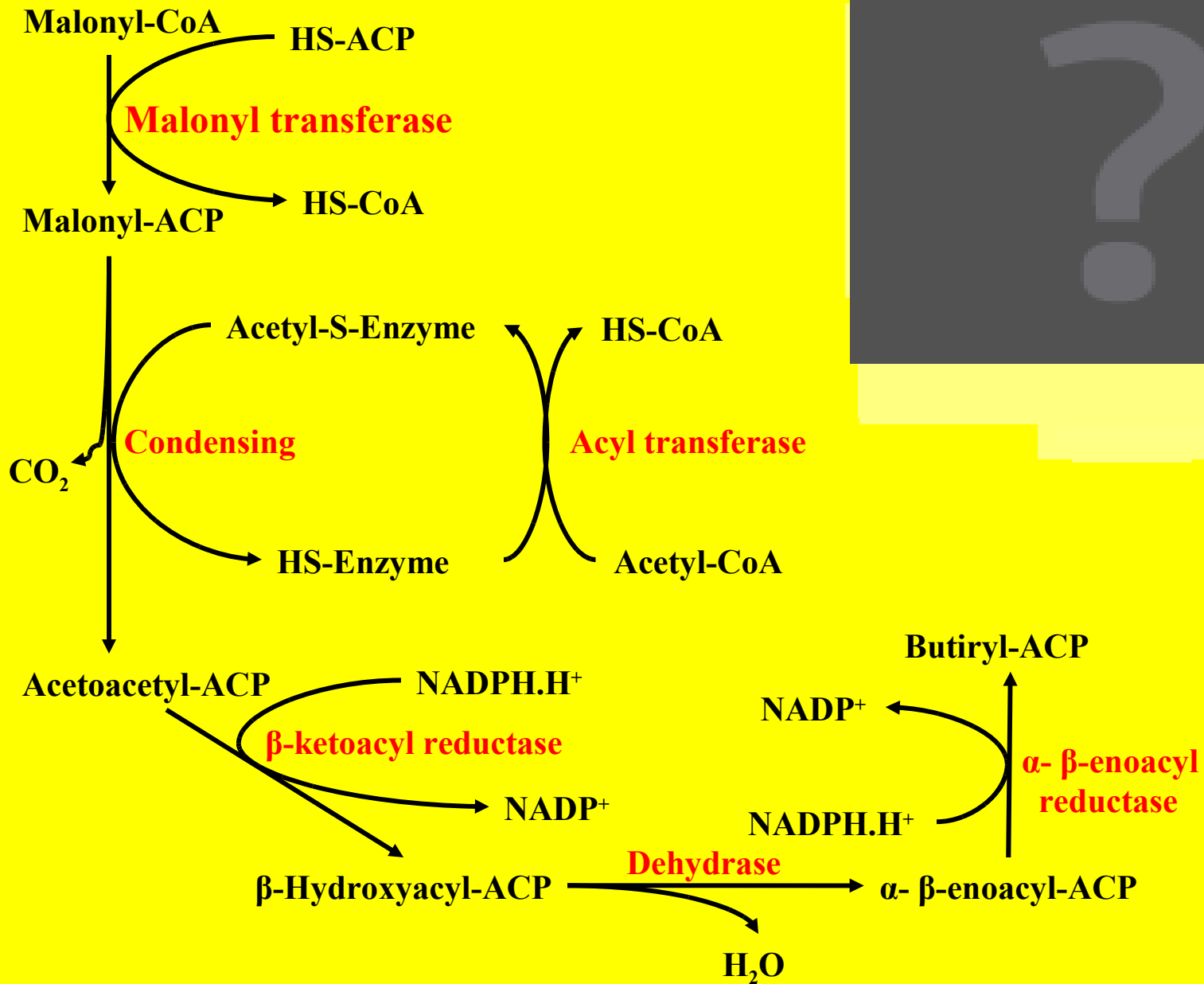
5.- Dehydrase

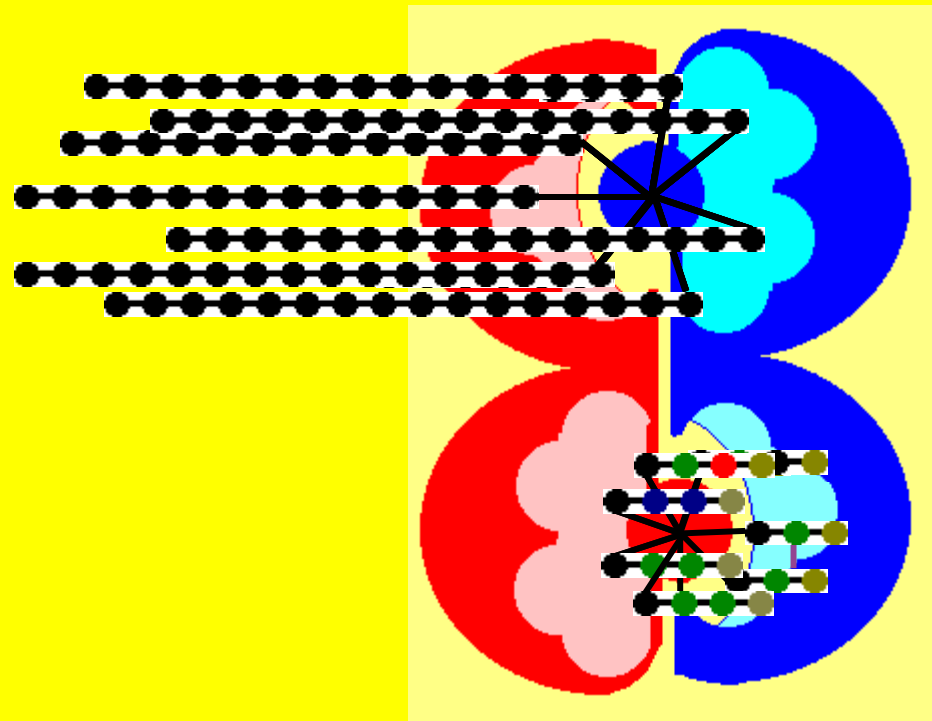
6.- α - β -enoacyl reductase

7.- Palmitate release

8.- Acyl carrier protein (ACP)





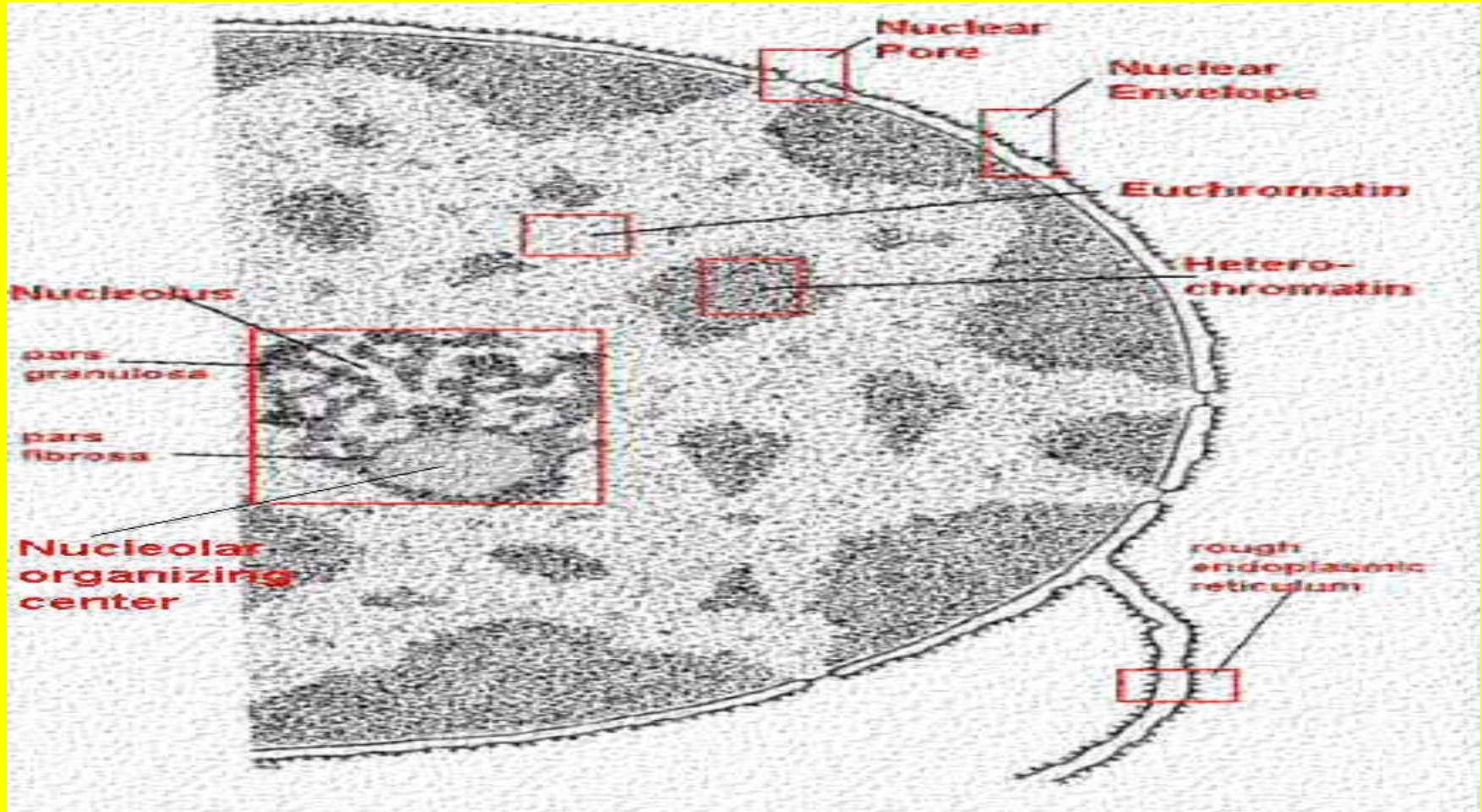


- The cytosol or cytoplasm is the largest compartment in eukaryotic cells and constitutes about half the cell volume.
- It contains *inter alia* water and dissolved ions, metabolites, building blocks, proteins and ribonucleic acids and **is the location of many metabolic pathways.**
- For example the cytosol or cytoplasm is the location for the following metabolic pathways.....Glycolysis, Gluconeogenesis (OAA \rightarrow Glucose), Pentose Phosphate Pathway, all except first 2 steps of urea cycle.
- In contrast, the following metabolic pathways **occur in the mitochondria:** TCA, Gluconeogenesis (Pyruvate \rightarrow OAA, pyruvate Carboxylase), beta oxidation, first 2 steps of urea cycle.
- **Compartmentalization allows reactions to occur simultaneously in both the cytosol and the mitochondria.**

- There is a further degree of **molecular compartmentalization** in the cytosol or cytoplasm.
- Eukaryotic cells of higher organisms, including humans, have a **membrane-bounded nucleus**, and other **membrane-bounded** structures, termed **organelles, which compartmentalize cellular functions and permit greater cellular specialization and diversity.**
- **With the exception of mature red blood cells, every eukaryotic cell contains a nucleus; the contents of which, are surrounded by a nuclear envelope, which consists of a double layer of inner and outer nuclear membranes separated by the periplasmic space.**

The nucleus is surrounded by a nuclear envelope, which consists of a double layer of inner and outer nuclear membranes separated by the periplasmic space.

The nucleus communicates with the cytoplasm via the nuclear pore, and with the ribosomes via the ER.



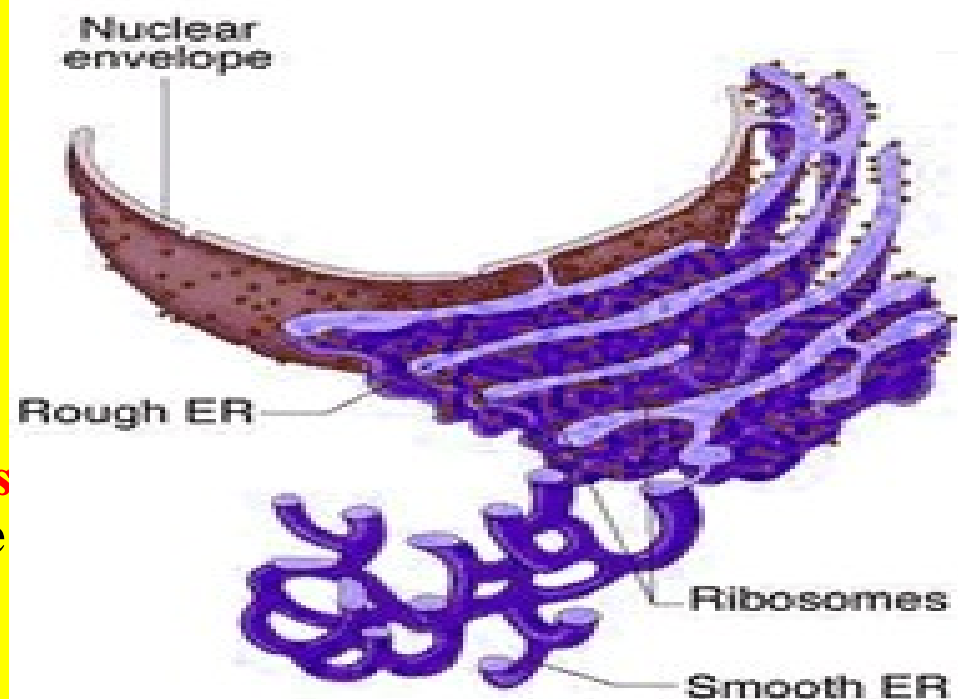
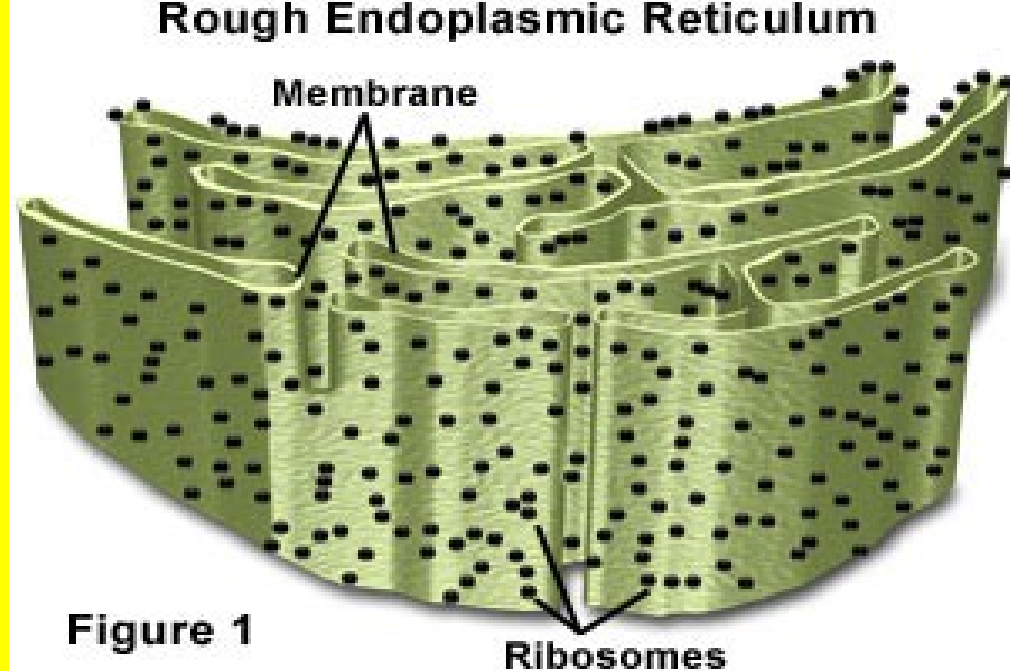
- **The periplasm is a continuum with the ER lumen since the outer nuclear membrane forms a continuum with the rough endoplasmic reticulum (ER) which bear ribosomes involved in the synthesis of nuclear components.**
- **Some of these nascent proteins are translocated into the space between the inner and outer nuclear membrane, termed the periplasmic or perinuclear space, which forms a continuum of the ER lumen.**
- **The structure of the nucleus is thus designed to participate in compartmentalization of cellular functions and protein targeting and protein secretion.**

- **The nucleus compartmentalizes reactions involving the synthesis of DNA, RNA and ribonuclear protein complexes.**
- **This separates nuclear mRNA transcription from cytoplasmic translation into protein, which, in turn, permits post-transcriptional processing of mRNA and an additional level of complexity in the organization and expression of the genetic code.**

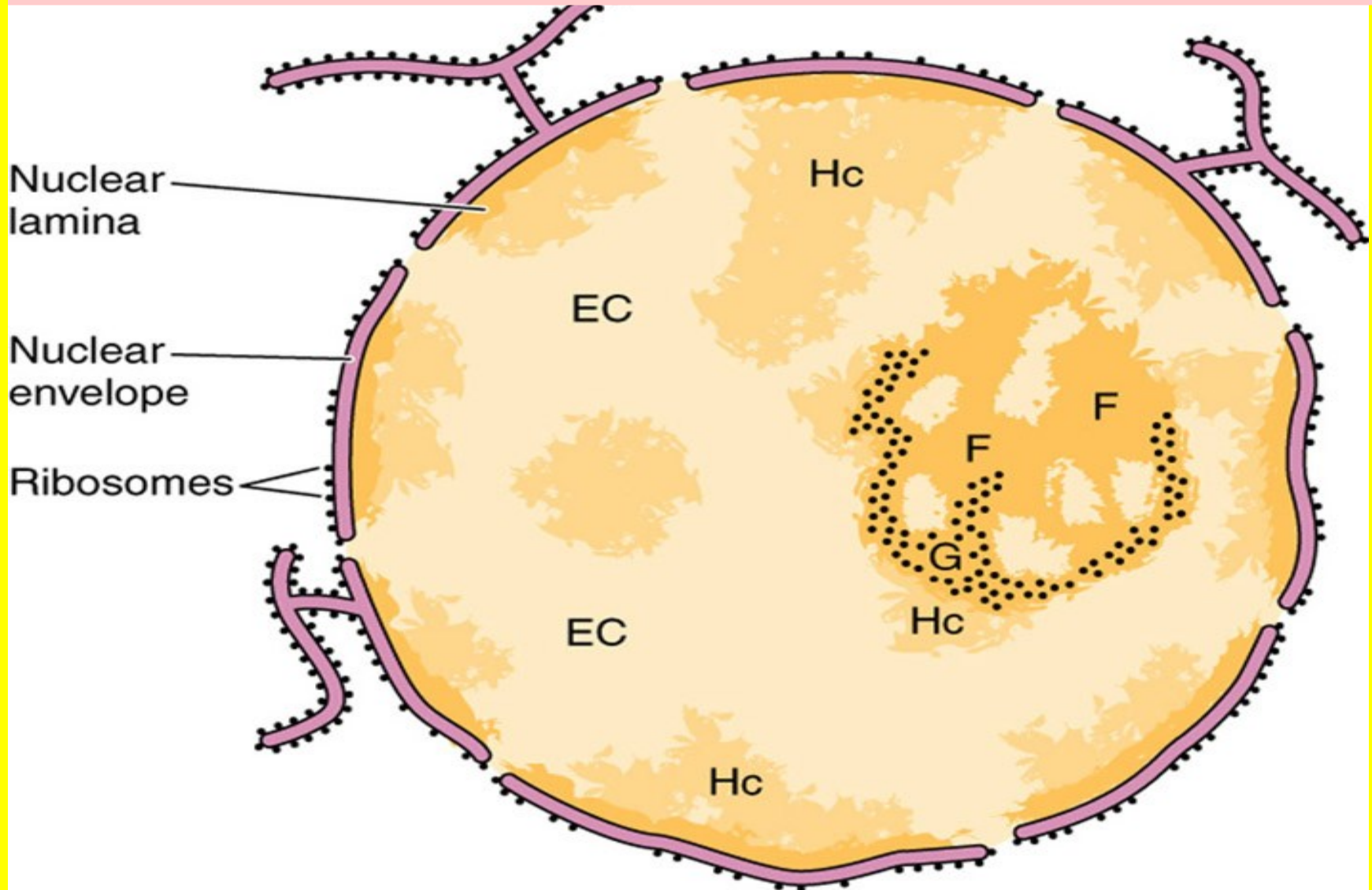
- The nuclear contents in the nucleoplasm are surrounded by a double layer of membrane, which makes up the nuclear envelope. Punctuating the nuclear envelope are selective **nuclear pore structures** (7 nm diameter). (Fig. 5.3).
- These complex structures are involved in the communication between the nucleoplasm and cytoplasm by allowing the uninhibited two-way passage of small molecules and proteins up to a molecular weight of 60 kDa.
- However, nuclear proteins much larger than this cut-off size can pass through the nuclear pores following synthesis in the cytoplasm, as can preformed ribosomal complexes and mRNA passing into the cytoplasm.
- Here again, we see the selectivity in transport of substances within the cell that is afforded **by compartmentalization by intracellular membranes.**

- They also provide the exit route for newly formed ribosomes and a selective mechanism for the import of larger proteins bearing appropriate nuclear targeting signal motifs, i.e protein secretion.
- The selective transport of large molecules is receptor-mediated, requiring recognition of nuclear targeting signal motifs by the nuclear pore complex.
- Several short primary sequences that target proteins to the nucleus have been identified but no common structural similarity exists between them, suggesting that a family of receptor proteins for different signals must be involved.
- The selective transport process is also energy-dependent.
- In the absence of ATP, import of nuclear proteins is inhibited even though the proteins cluster over the nuclear pore complexes.

- The endoplasmic reticulum (ER) is an anastomosing network (reticulum) of flattened sacs and tubules (cisternae) of membrane within the cytoplasm, all of which interconnect to form a common lumen.
- This membrane structure may represent up to half of the total cell membrane in an actively secreting cell.
- It forms a continuation of the nuclear envelope such that the ER lumen is continuous with the periplasmic space of the nuclear envelope and is often arranged in concentric flattened layers around the nucleus.
- This structure allows **communication between the nucleus of the cell** [where the DNA template is kept], **with the cytoplasm-** into which proteins are secreted.

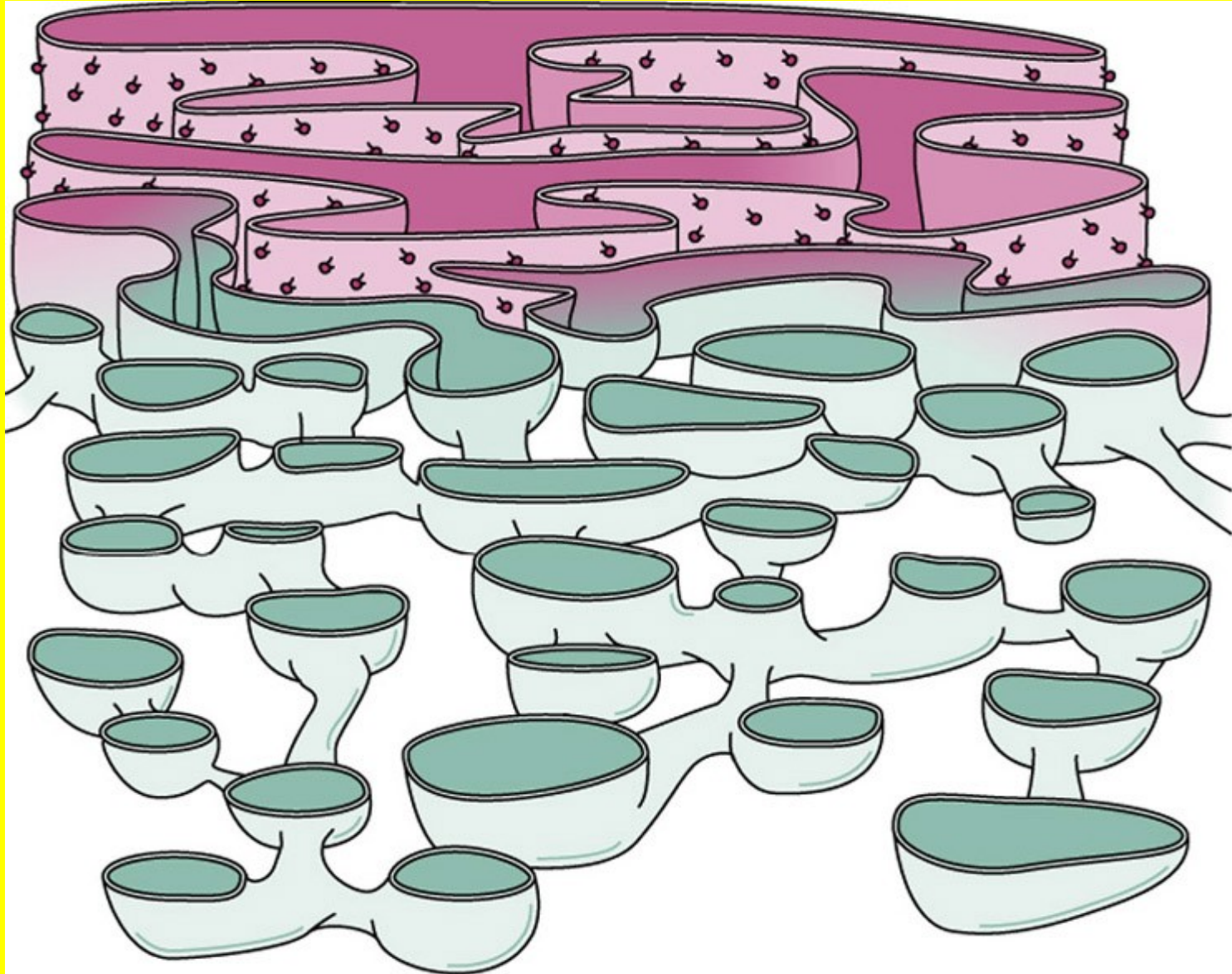


RER Shows continuity with nuclear envelope's outer membrane. This allows the RER and the nucleus to communicate, and facilitate the transfer of genetic information into the synthesis of proteins.



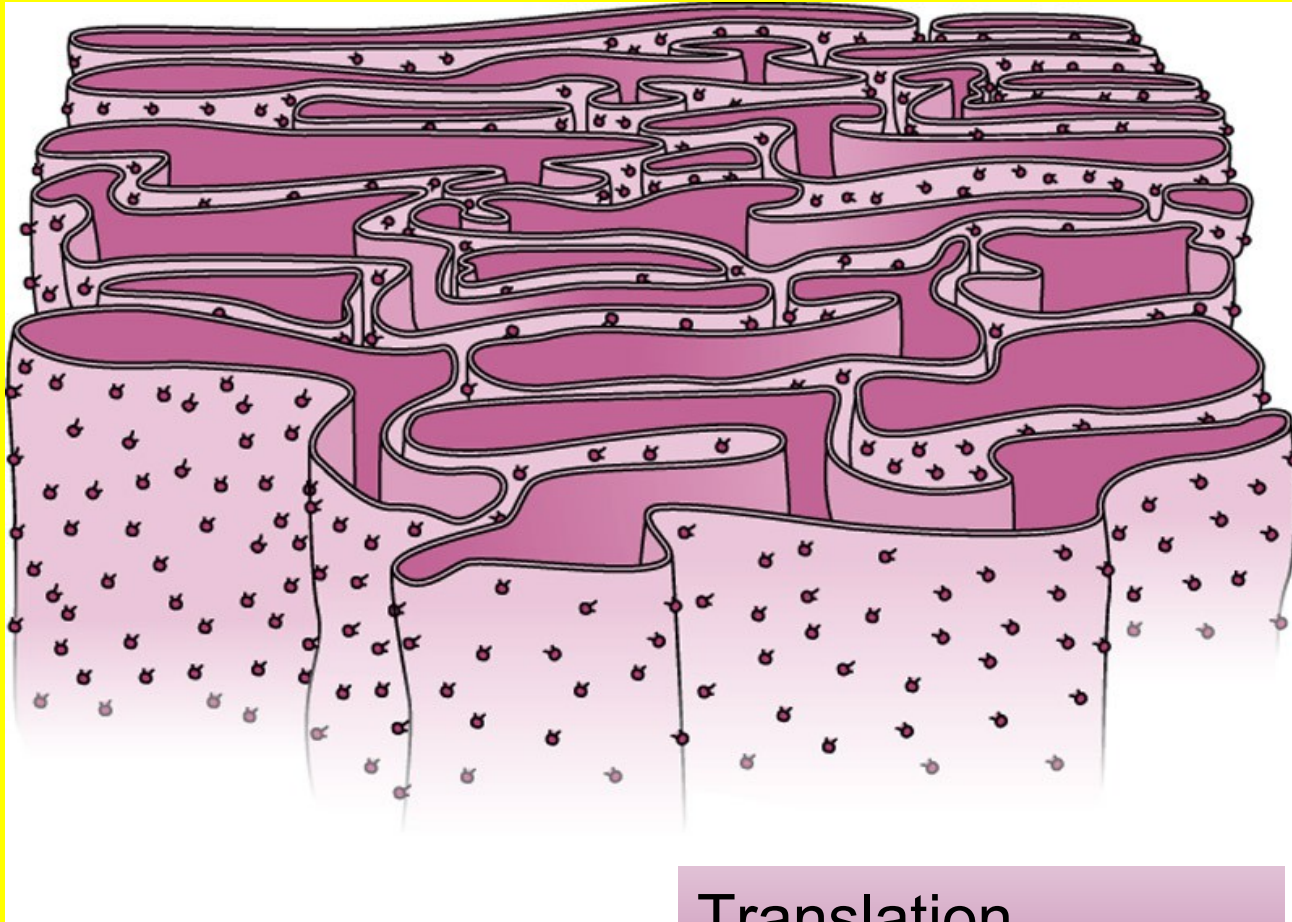
- **By electron microscopy, two types of ER are distinguished: the rough ER and the smooth ER**
- **The rough ER** which is formed by membrane cisternae covered with ribosomes, and is involved in the synthesis of secretory, lysosomal and membrane proteins, and the transport of newly assembled proteins to the Golgi apparatus.
- **Such compartmentalization facilitates protein synthesis- which occurs on the ribosomes.** It also separates DNA replication which occurs in the nucleus
- **The smooth ER is usually formed by vesicles and tubules and is devoid of ribosomes.**
- **The smooth endoplasmic reticulum does not contain ribosomes on the surface.**
 - **It mostly contains enzymes which functions in lipid and carbohydrate synthesis.**
 - **It also serves as a transitional area for vesicles that transport endoplasmic reticulum products to other destinations within the cell.**
 - **Regions of the ER also form and maintain low intracellular membrane-bound stores of Ca^{++} which can be mobilized for intracellular signalling (see Figs 7.8 and 7.9).**

Endoplasmic Reticulum



Rough Endoplasmic Reticulum –

RER occur usually as a parallel cisternae with attached ribosomes

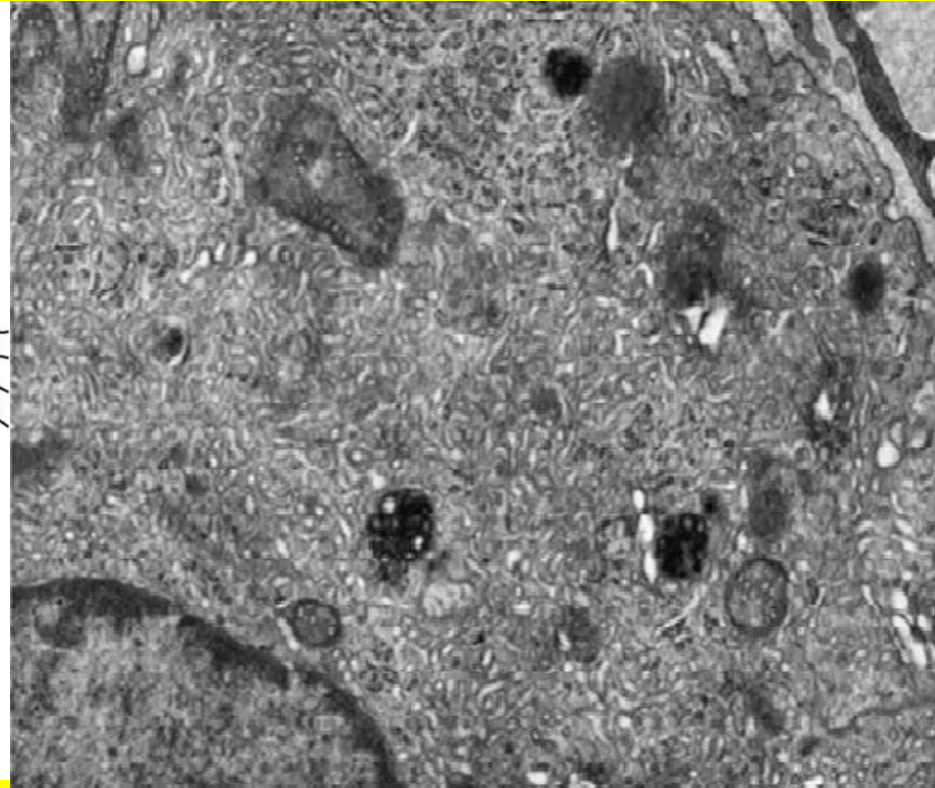
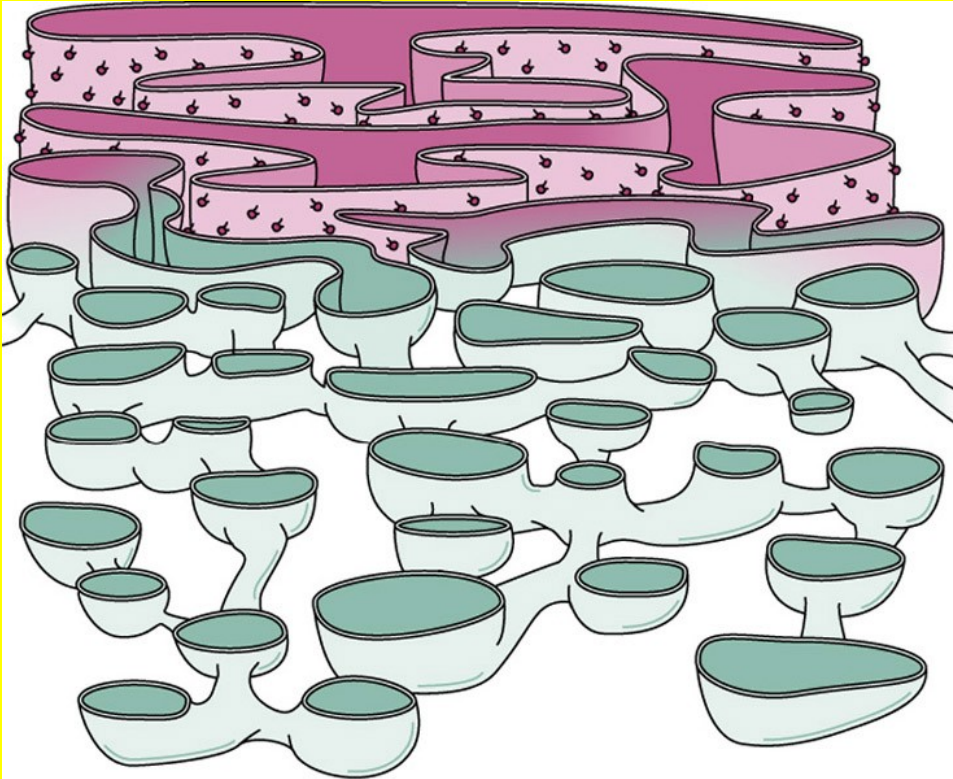


Functions

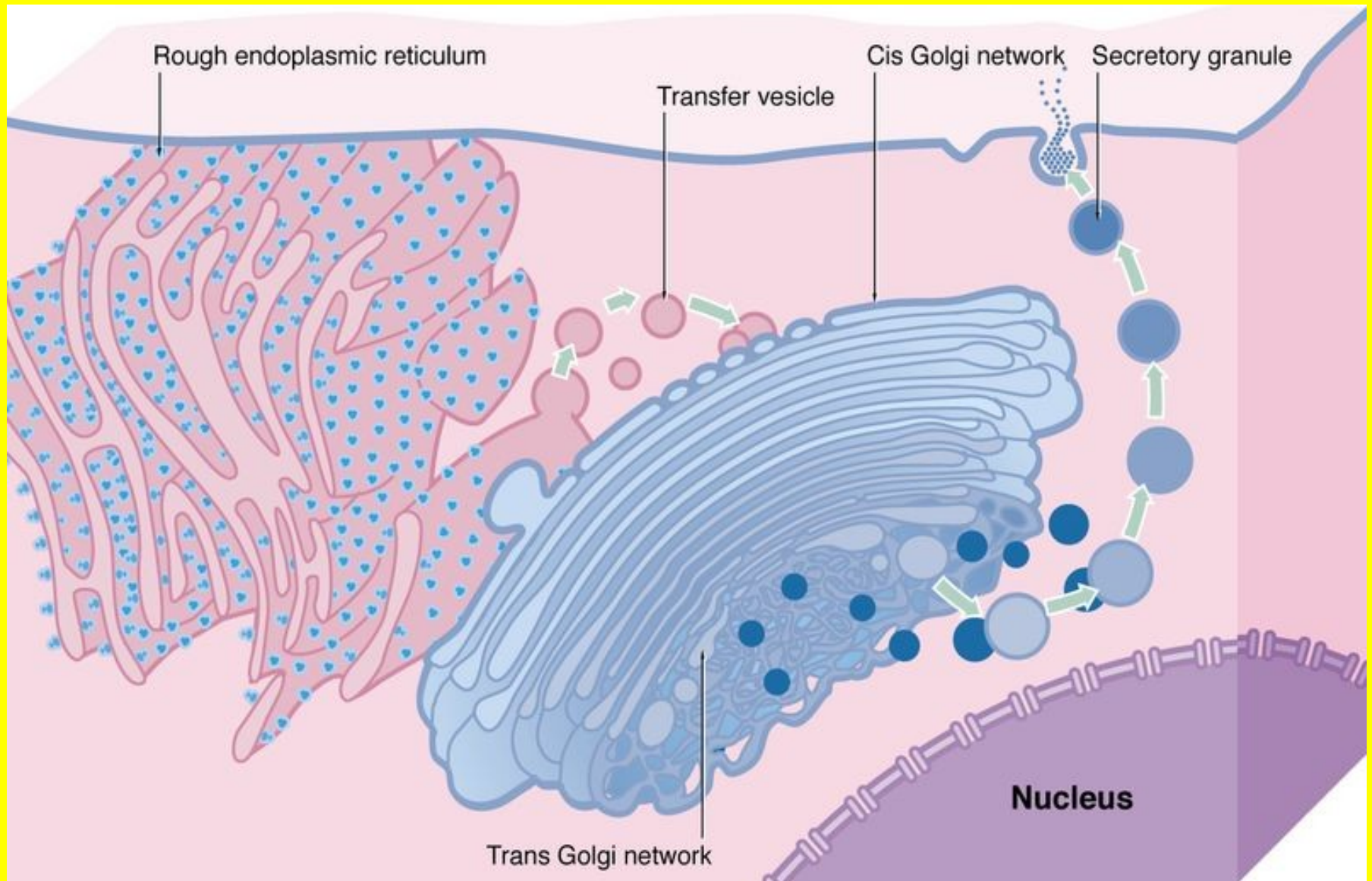
Translation
Segregation
Beginning of protein
Glycosylation

Smooth Endoplasmic Reticulum –

SER occur as a vesicles and tubules that show continuities with RER cisternae.



RER also shows continuities (at least indirectly) with Golgi apparatus by Transfer vesicles that interchange moieties between both organelles.



- **Lysosomes and peroxisomes**
- **Lysosomes may each contain more than 50 degradative hydrolytic enzymes that the cell uses to break down large food molecules (carbohydrate, lipids and proteins) and for digesting foreign bodies, and other toxins and whatever is necessary that enter the cell by phagocytosis.**
- **They thus function as the cellular digestive system.**
- Lysosomes digest waste materials and food within the cell, breaking down molecules into their base components with their strong digestive enzymes. These enzymes are only active within the acidic membrane of the lysosome so that in case of leakage or rupture, the enzymes will not be active within the cell.
- Each lysosome is surrounded by its own membrane due to the acidic conditions within it. **Sequestration of these activities in a distinct compartment protects cellular components in other regions from breakdown.**

- Of similar appearance to lysosomes are **peroxisomes**.
- These organelles contain enzymes that function in oxidative reactions involving molecular oxygen, resulting in the production of hydrogen peroxide (H_2O_2) and organic peroxides.
- For example, catalase is an abundant peroxisomal enzyme (up to 40% of peroxisomal protein) whose function is to convert toxic H_2O_2 to oxygen and water.
- **The compartmentalization of these reactions in peroxisomes protects the rest of the cell from oxidative damage. If either of these organelles were not compartmentalized, they would cause havoc in the cell and surrounding tissues!**
- **The function of lysosomes thus is a clear advantage of the compartmentalization of the eukaryotic cell, because the cell could not support such destructive enzymes if they were not contained in a membrane-bound lysosome.**

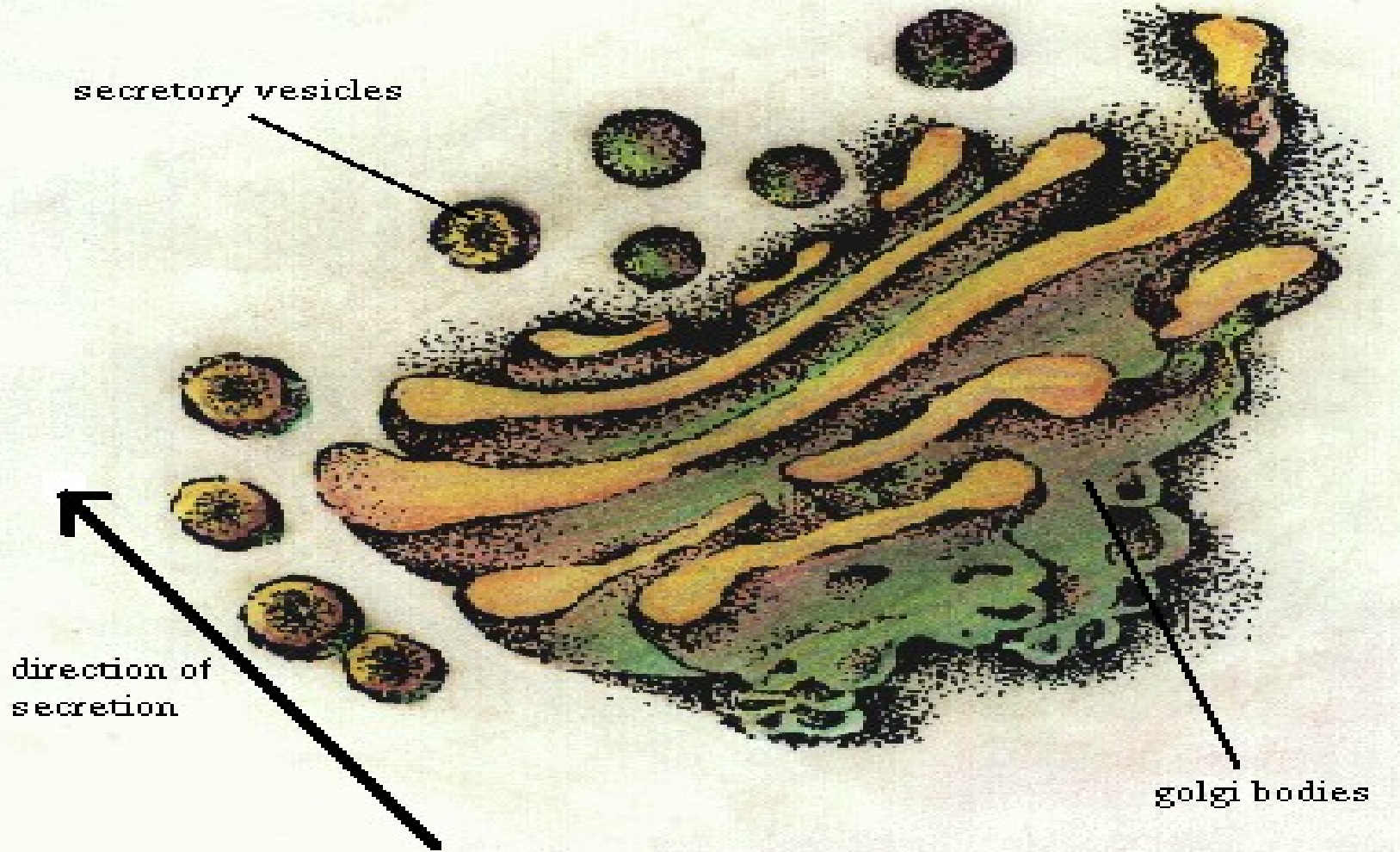
- **The function of lysosomes thus is a clear advantage of the compartmentalization of the eukaryotic cell, because the cell could not support such destructive enzymes if they were not contained in a membrane-bound lysosome.**

- **The Golgi apparatus.**
- The Golgi complex (Golgi apparatus) participates in many activities, particularly those associated with secretion.
- It has an essential role in coordinating membrane and vesicle traffic among organelles.
- The Golgi complex also has the following roles:
 - Polysaccharide synthesis
 - Modification of secretory products-it modifies proteins
 - Sorting of secretory products- it separates proteins according to their destinations.
 - Packaging of secretory products- it packages materials into vesicles which are exported out of the cell.
 - Concentration and storing of secretory products
- **This is a good example of compartmental organization of the cell.**
- **Again we see the role of confining cellular activity to specific areas of the cell by structured membranes!**

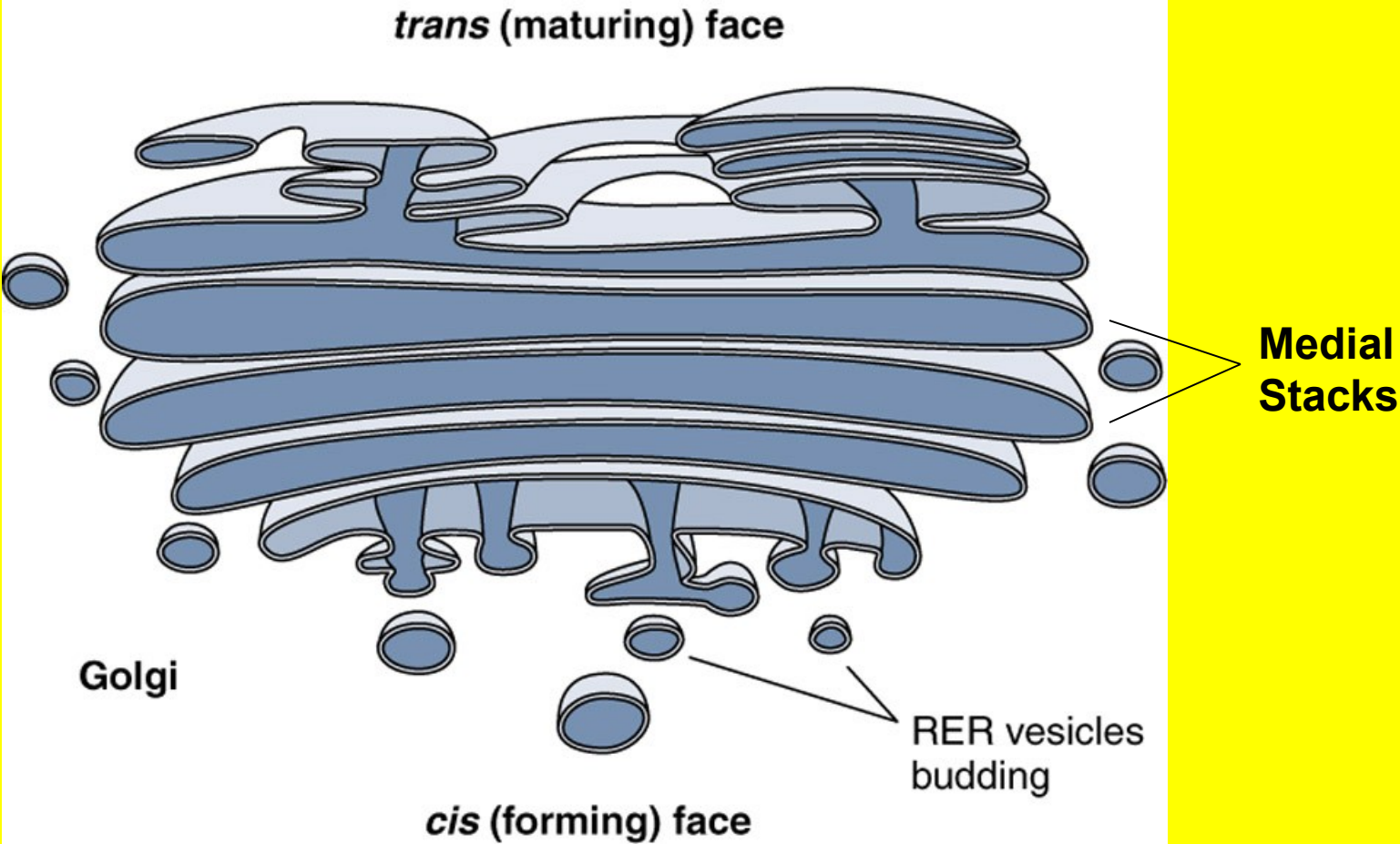
- **We have already noted that a prominent function of this organelle is the collection, packaging and distribution of molecules made in the cell and used elsewhere.**
- **This includes post-translational modification of secretory and membrane proteins by sequential glycosylation reactions.**
- **For this role the Golgi complex is organized such that it is continuous with the nucleus via the endoplasmic reticulum.**
- **The Golgi apparatus or complex consists of folded stacks of flattened smooth membrane sacs and vesicles.**
- **The folded stacks are called cisternae.**

- The Golgi complex is organized functionally into cis- median- and trans-Golgi such that distinct modifications are made in each region as a newly synthesized protein moves from the ER through the cis- (close to the ER) to trans-Golgi (close to the periphery of the cell).
- The sorting of newly synthesized membrane proteins to their cellular destination occurs in the trans-Golgi network, which is associated with the trans-Golgi.
- Unprocessed proteins enter the Golgi apparatus via the front end (cis), which faces the ER, are processed and exit at the back end (trans) which faces the cell membrane, for export.
- The sorting of newly synthesized membrane proteins to their cellular destination occurs in the trans-Golgi network, which is associated with the trans-Golgi. This is most certainly an ingenious example of compartmental organization of the cell.

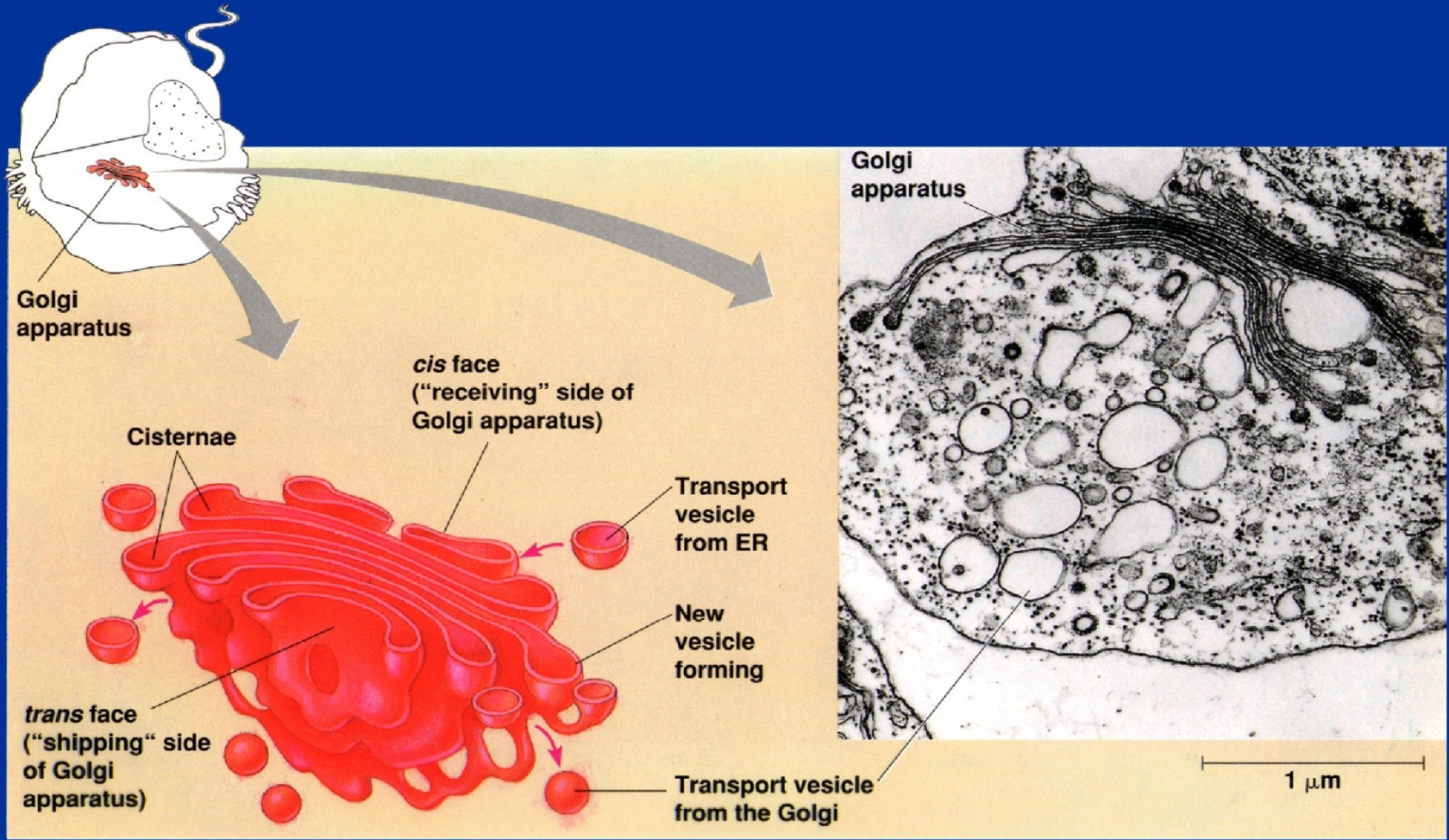
Golgi apparatus

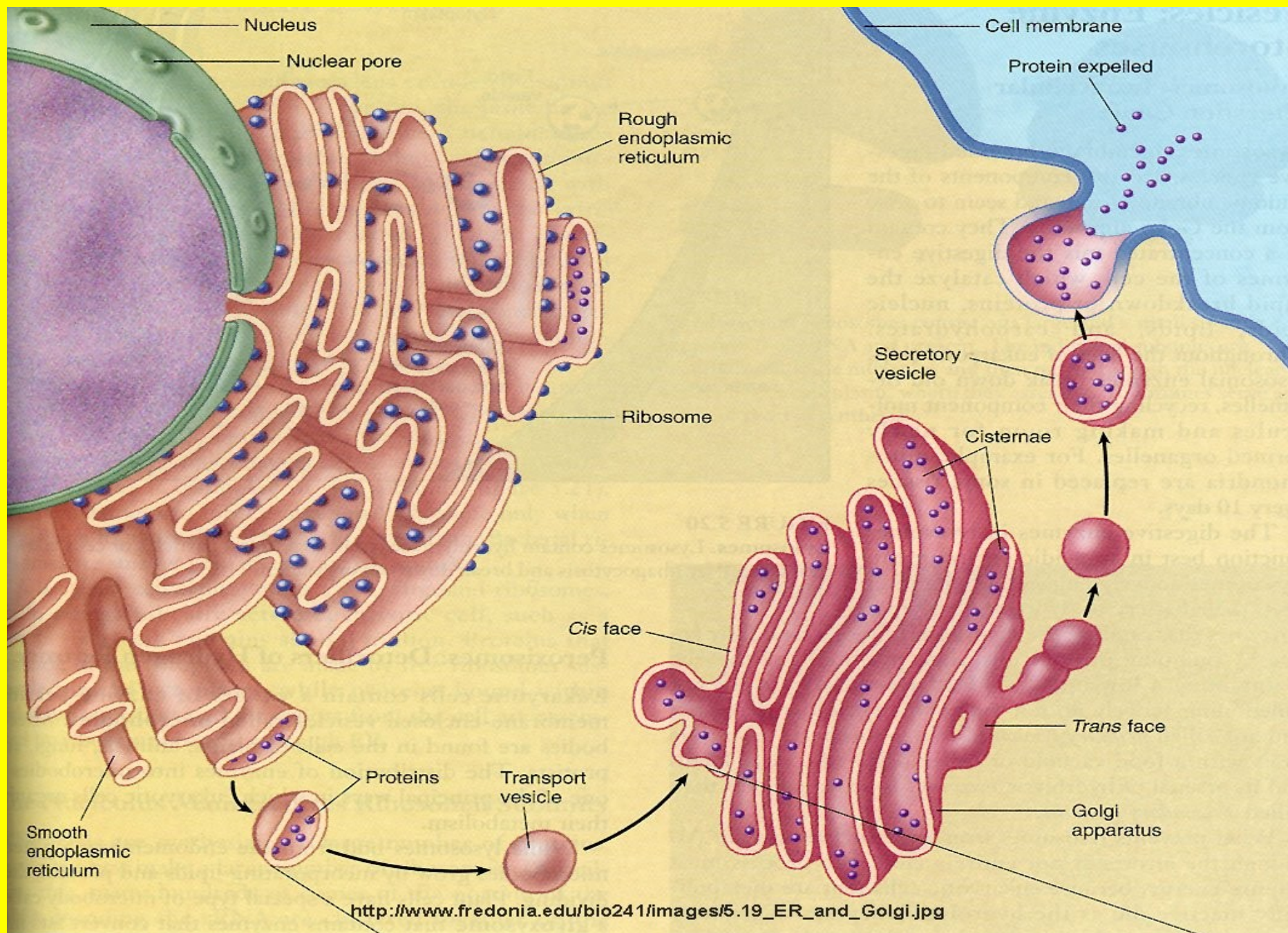


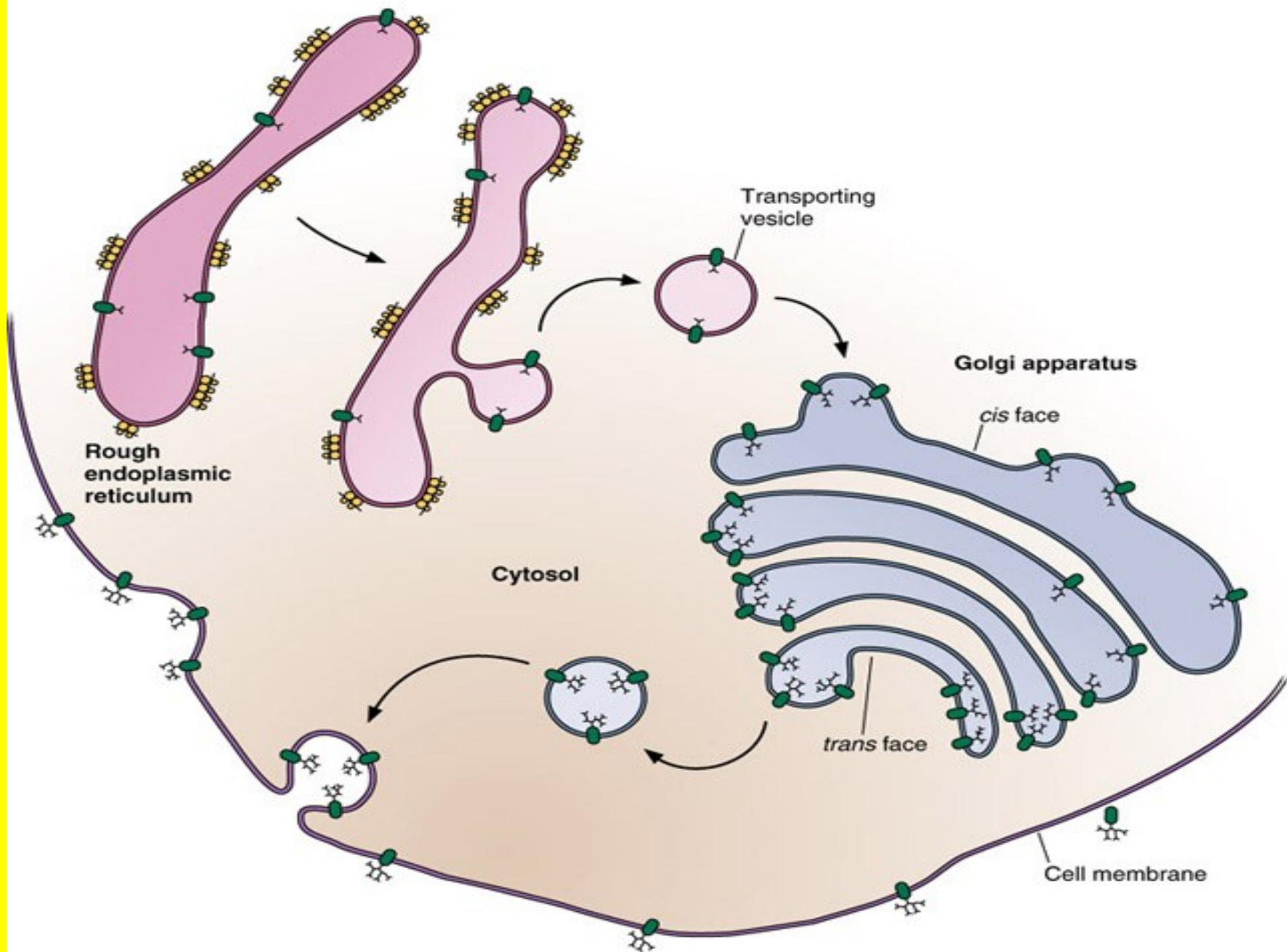
Golgi Apparatus



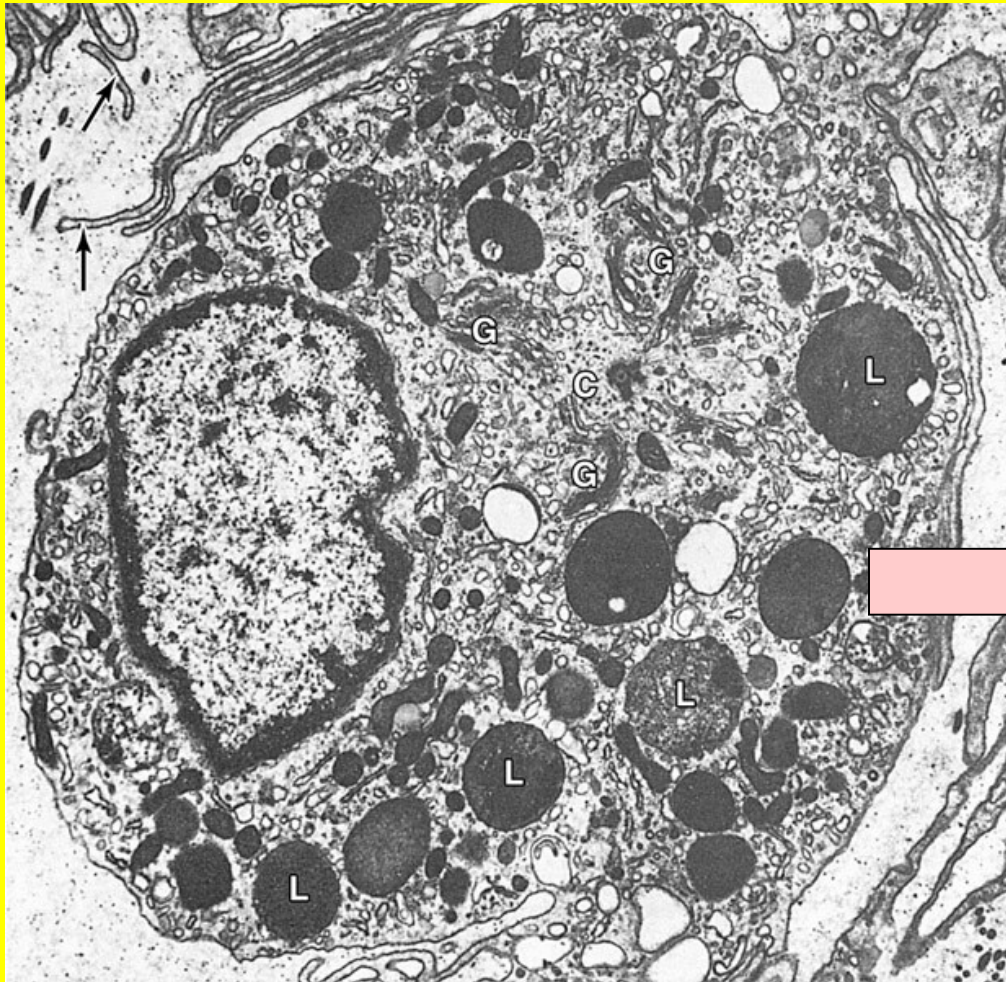
THE GOLGI APPARATUS







Lysosomes



Enzymes

Acid phosphatase

Lipases

Phospholipases

Proteases

Cathepsins

Ribonucleases

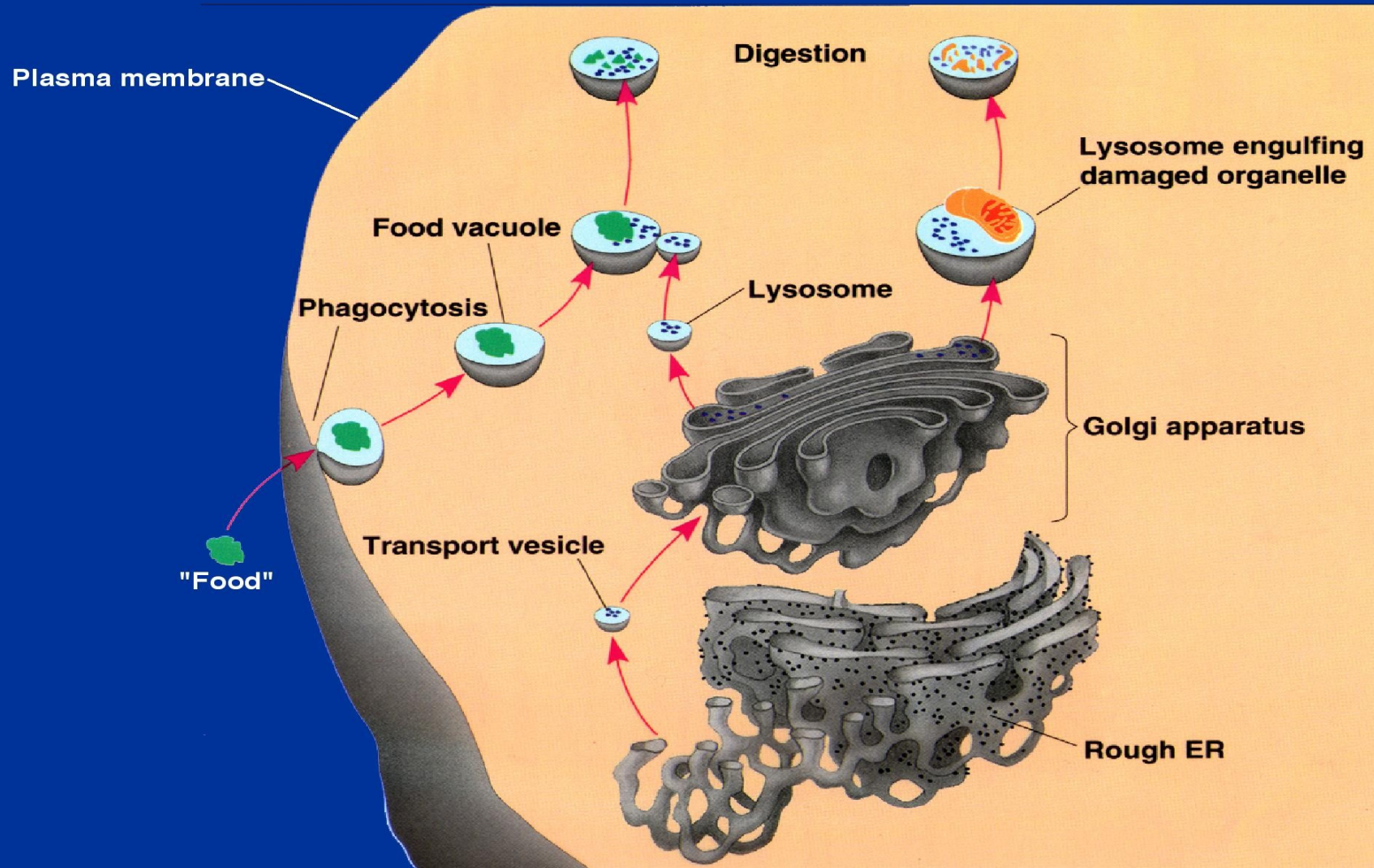
Deoxyribonucleases

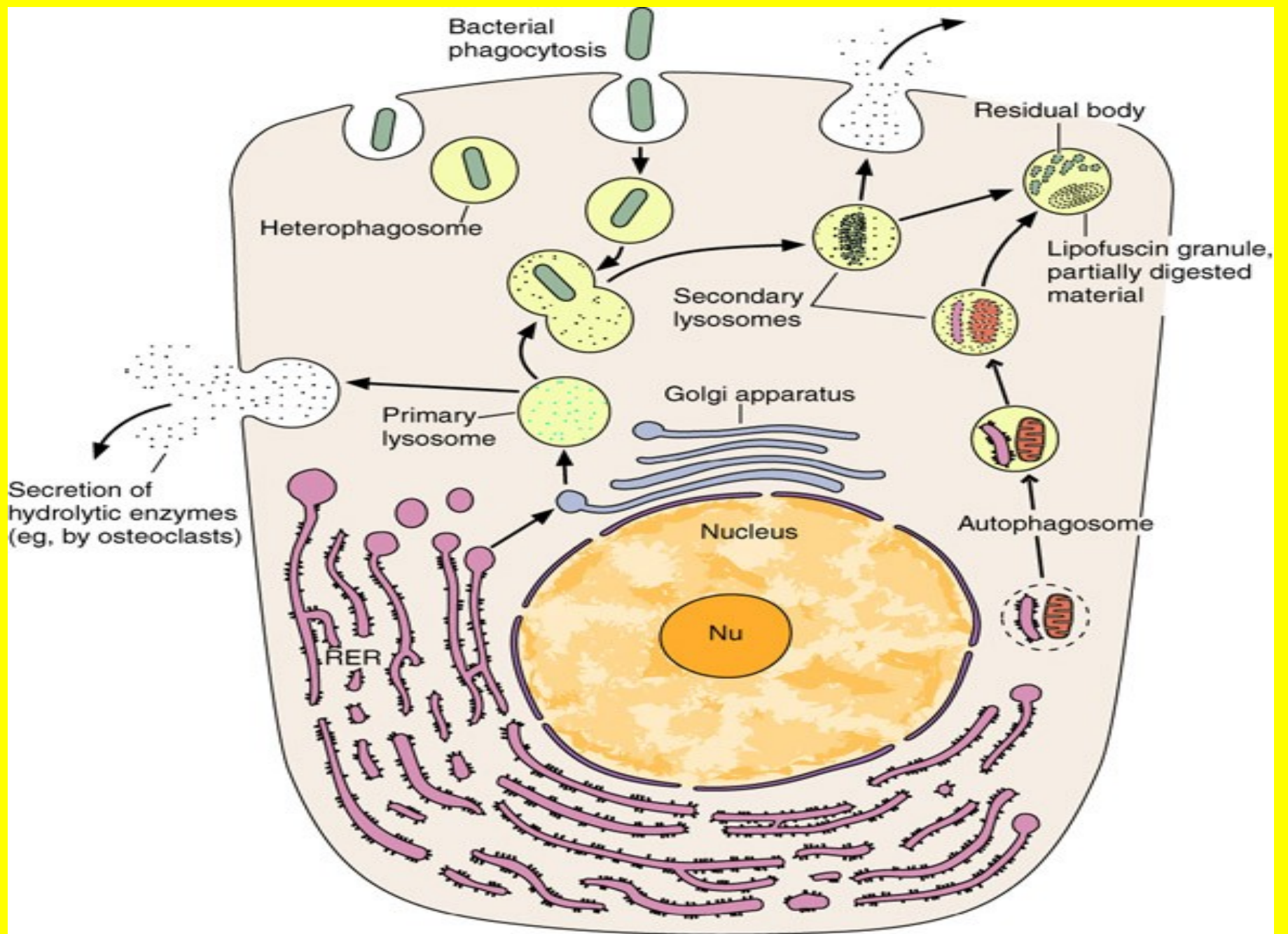
Glucosidases

Beta-Glucuronidase

This slide, and the next illustrate the importance of compartmentalization in the function of lysosomes

THE FORMATION AND FUNCTION OF LYSOSOMES





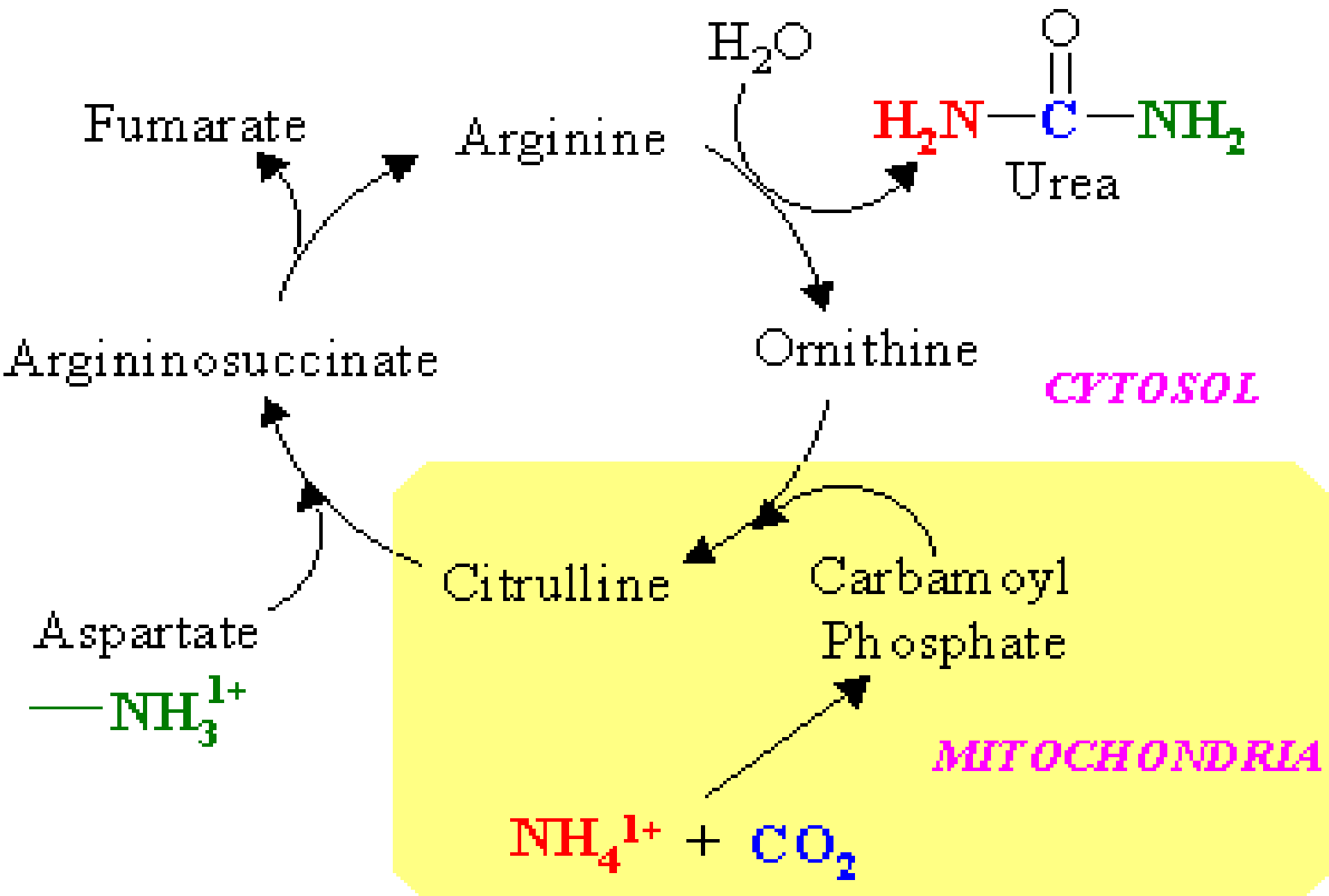
- **By now it should be clear to us that the ingenious use of membranes by the cell is an effective method of compartmentalization of the cell.**
- Let us now illustrate the role and importance of compartmental organization in cells by considering a few important biochemical processes.
- For example, **urea is produced by a five-step process called the urea cycle that occurs in liver cells.**
- **The next few slides will demonstrate that these reactions take place in both the mitochondria and the cytosol.**

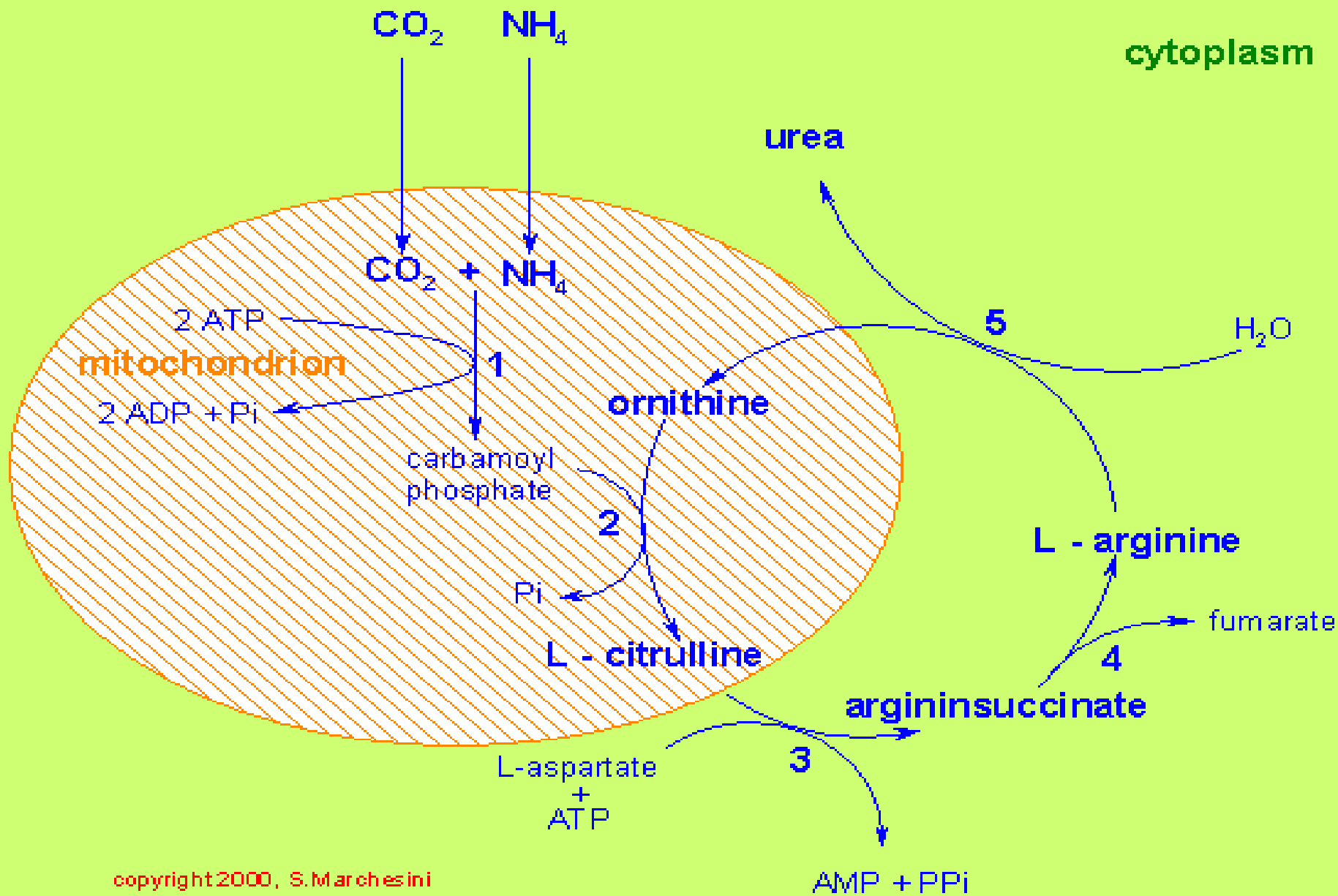
- **Note that.....the first 2 steps of the urea cycle occurs in the mitochondria.....**
- **ALL of the other steps** (all except the first 2 steps) of the urea cycle **occur in the cytosol.**



First 2 steps in mitochondria

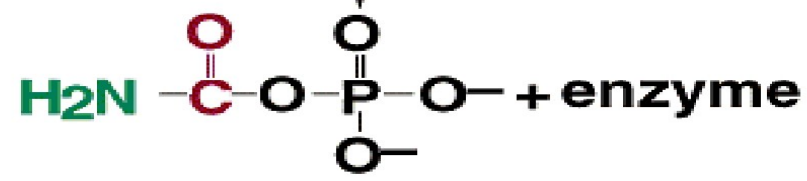
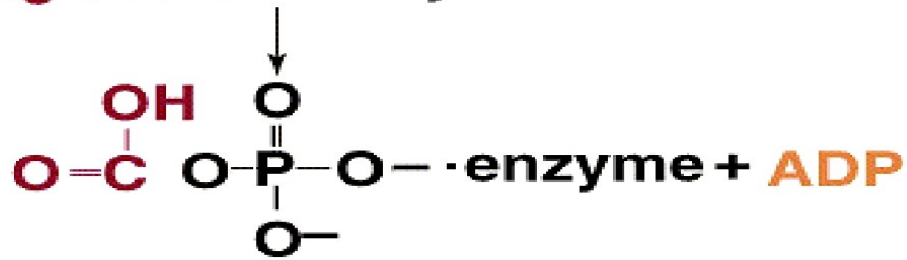
All of the other steps occur in the cytosol



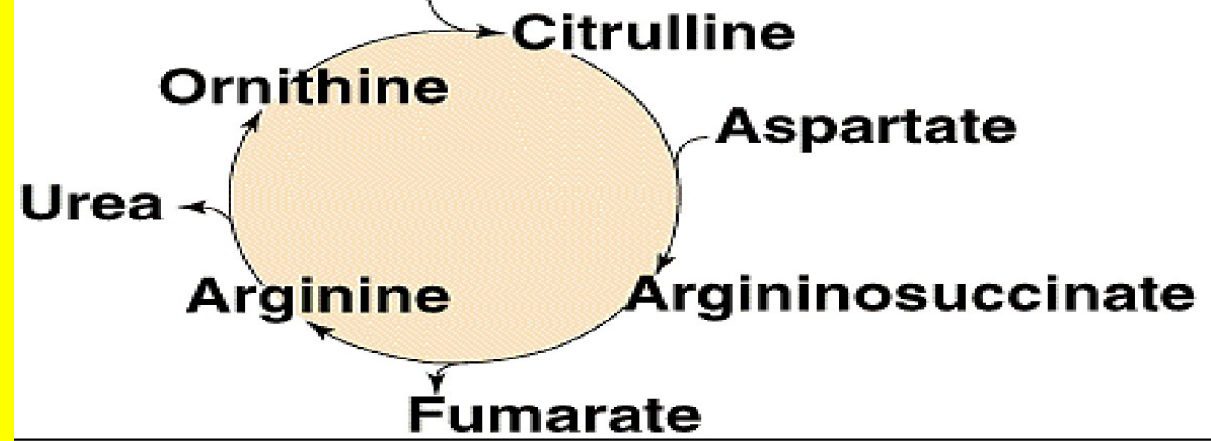


- It is thus clear that parts of the urea cycle occur in 2 different subcellular compartments, the cytosol and the mitochondria.
- In the first reaction of the urea cycle ammonium ions (NH_4^+), which are readily available from transamination reactions of protein degradation in energy generating reactions in the mitochondria of the liver, combine with carbon dioxide from energy generating reactions in the citric acid cycle which also occurs in the mitochondria, and two ATP also generated in the mitochondria to yield carbamoyl phosphate, in a reaction is catalyzed by carbamoyl phosphate synthase I (CPS I).
- This reaction occurs in the the mitochondrial matrix.
- And why not?
- After all-- the ammonium ions (NH_4^+), the carbon dioxide and the two ATP needed for this reaction are all generated in the mitochondria

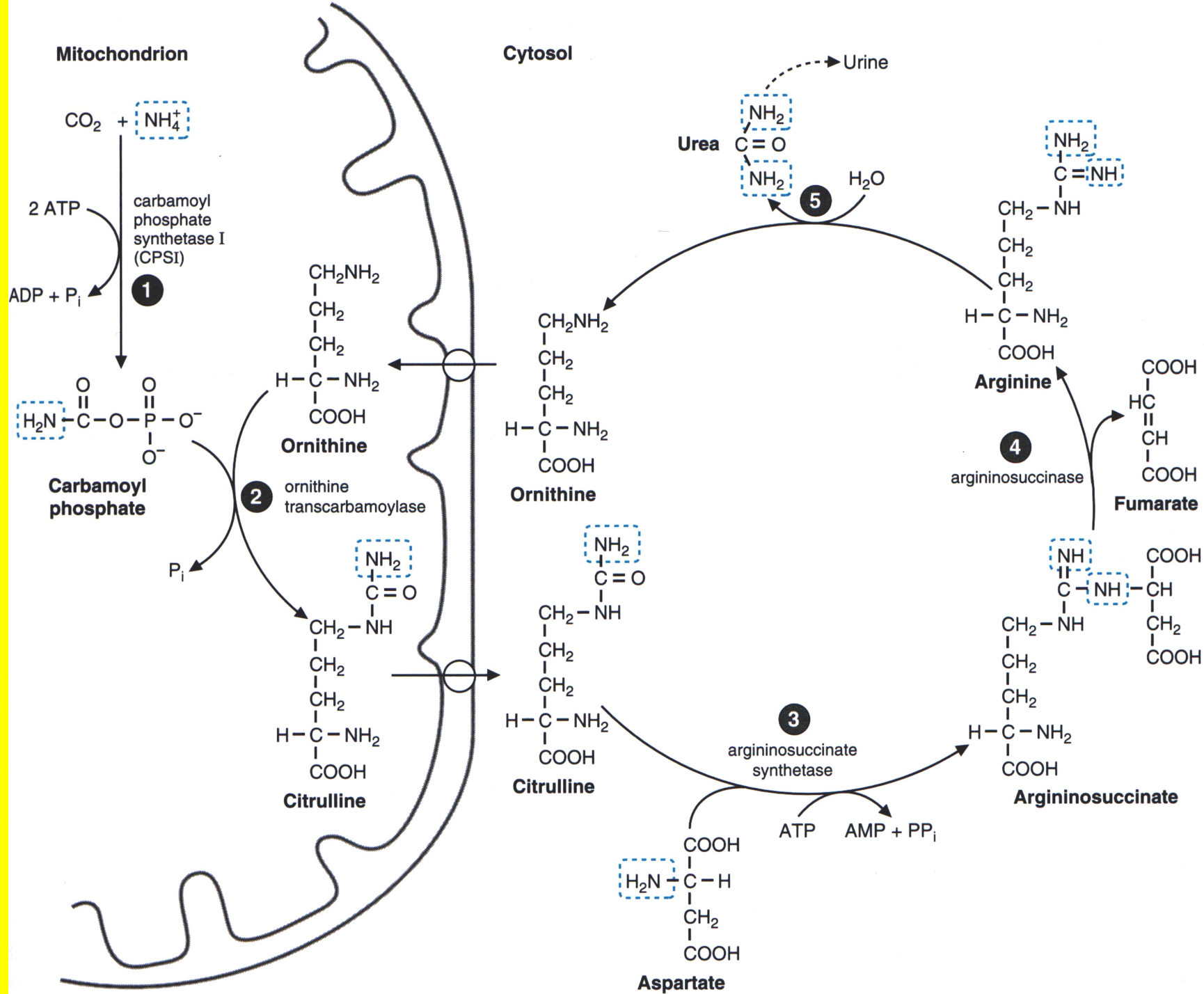
- **The second step of the urea cycle is the condensation of carbamoyl phosphate, formed as we just described, with ornithine, producing citrulline .**
- **This reaction also occurs in the mitochondrial matrix.**
- **Note that ornithine is made in the cytosol and must be transported to the mitochondrial matrix, where the second step of the urea cycle occurs.**
- **The product, citrulline, is then transported to the cytosol, where the remaining reactions of the cycle take place.**
- **Note that in step 2, ornithine is transported into the mitochondria and the citrulline that is formed is transported across the mitochondrial membrane out of the mitochondria into the cytosol.**
- **This transport is driven by an electrochemical gradient, which occurs partially because of compartmentalization.**
- **NOTE WELL- The remainder of the urea cycle occurs in the cytosol of the cell.**



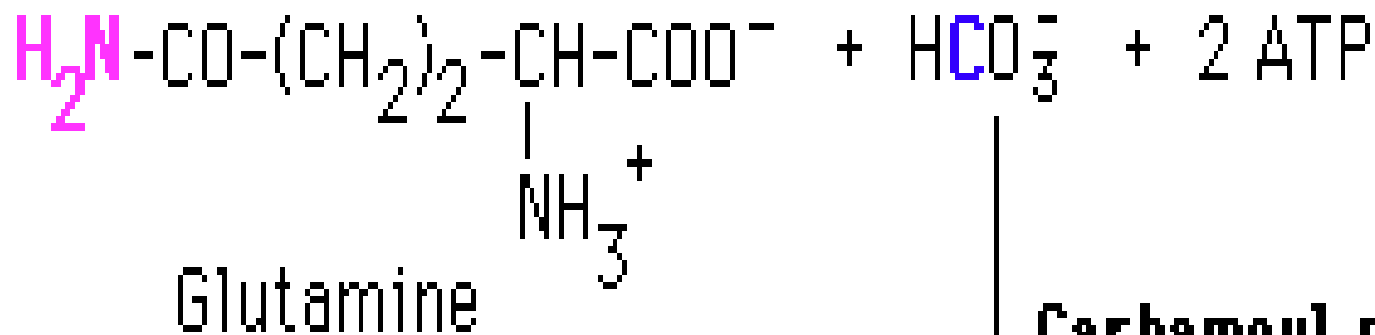
Carbamoyl phosphate



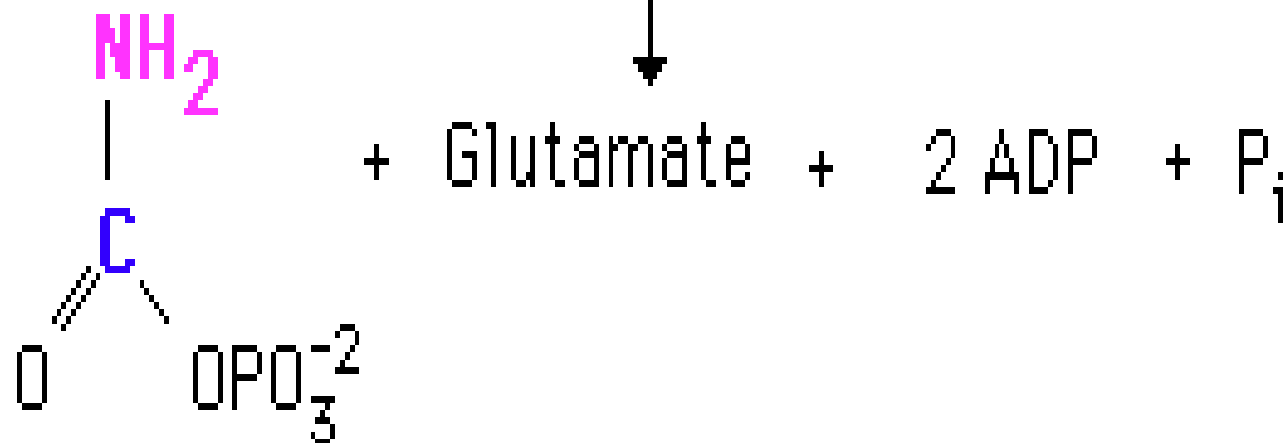
The cycle occurs partially in the mitochondria & in the cytoplasm



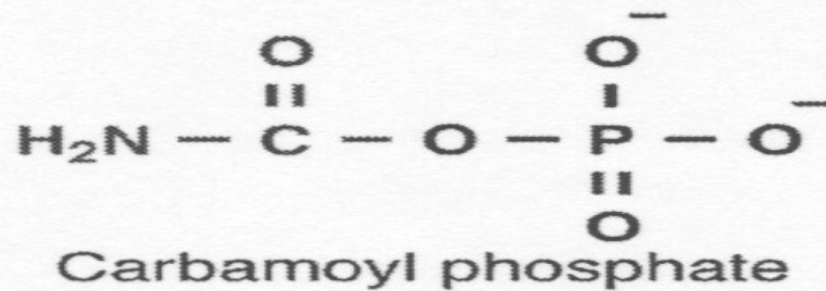
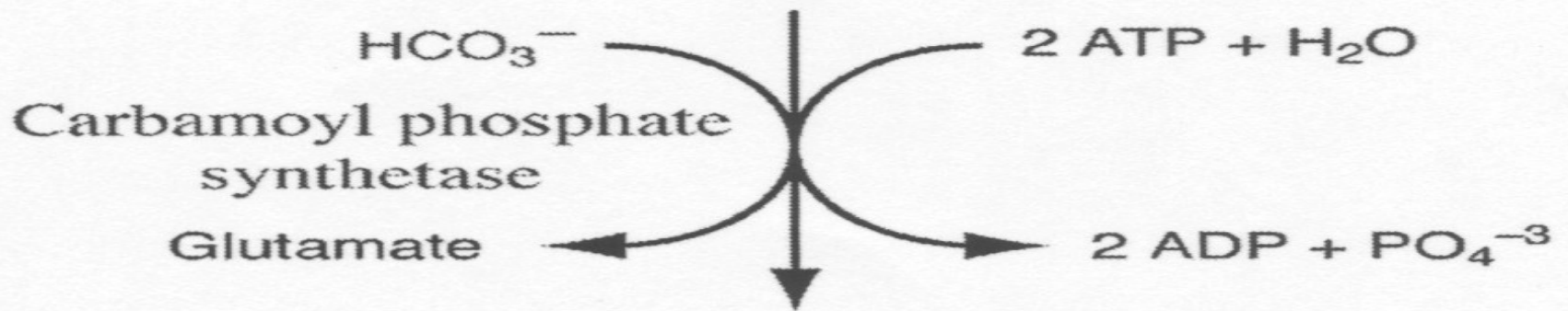
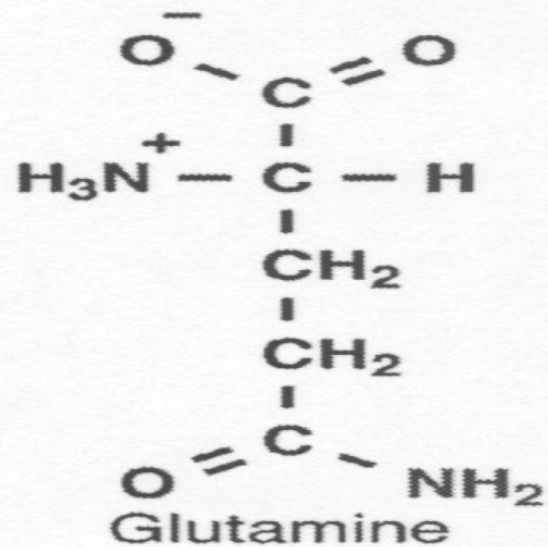
- The pathway for the formation of pyrimidine nucleotides also begins with **the formation of carbamoyl phosphate. This reaction, which is also the committed step of this pathway is catalyzed by carbamoyl phosphate synthetase II [CPS II].**
- This enzyme takes glutamine as the ammonia donor, HCO_3^- and 2 ATP and catalyzes the formation of carbamoyl phosphate.
- **NOTE that the formation of carbamoyl phosphate is formed in the body in two different ways, in two different pathways, for two different purposes, using two different enzymes in two different compartments of the cell!**
- The carbamoyl phosphate used for pyrimidine nucleotide synthesis is derived from glutamine and bicarbonate, within the cytosol, as opposed to the urea cycle carbamoyl phosphate derived from ammonia and bicarbonate in the mitochondrion.
- **Here we see that compartment organization of the cell allows to get rid of the toxic substance NH_3 derived from amino acid metabolism in one part of the cell, while synthesizing pyrimidine nucleotides for use in DNA and RNA synthesis etc from the amino acid precursor glutamine in another compartment of the cell!.**



**Carbamoyl phosphate
synthetase II**



Carbamoyl phosphate



- Remember we said earlier that the first reaction of the urea cycle is **catalyzed by carbamoyl phosphate synthase I (CPS I)**.
- Now we see that there are two CP synthetases: **a mitochondrial enzyme, CPS-I, which forms carbamoyl phosphate destined for inclusion in the urea cycle, and a cytosolic CP synthetase (CPS-II), which is involved in pyrimidine nucleotides biosynthesis.**
- **By having two specific isozymes, and by employing compartmentalization, the cell can engage in both amino acid degradation and pyrimidine nucleotides biosynthesis simultaneously.**

TABLE 29.1: SUMMARY OF THE DIFFERENCES BETWEEN CARBAMOYL PHOSPHATE SYNTHETASE (CPS) I AND II

	CPS I	CPS II
Cellular location	Mitochondria	Cytosol
Pathway involved	Urea cycle	Pyrimidine synthesis
Source of nitrogen	Ammonia	γ -Amide group of glutamine

• NOTE

- that carbamoyl phosphate is a precursor for both the urea cycle and the pyrimidine synthetic pathway
- However, in the pyrimidine synthetic pathway, carbamoyl phosphate is made in the cytosol, whereas in the urea cycle, carbamoyl phosphate is synthesized in the mitochondria by carbamoyl phosphate synthetase I (CPS I).
- 2- that Carbamoyl phosphate synthetase II (CPS II) is a different enzyme than the one involved in urea synthesis (CPS I) in that
- a) The urea cycle reaction is catalyzed by carbamoyl phosphate synthetase I (CPS-I) whereas the pyrimidine nucleotide precursor is synthesized by **CPS-II**.
- b) Carbamoyl phosphate synthetase II (CPS II) prefers glutamine to free ammonia and has no requirement for N-acetylglutamate.
- c) **Carbamoyl phosphate synthetase II is a cytosolic enzyme requiring glutamine as the ammonia donor, whereas carbamoyl phosphate Synthetase I is a mitochondrial enzyme that utilizes ammonia directly.**
- d) CPS I uses ammonia as the source of nitrogen, whereas CPS II uses the y amide group of glutamine

- **FATTY ACID SYNTHESIS**
- **Fatty acid synthesis involves the joining of acetyl CoA units together in the cytosol.**
- **But the high amounts of acetyl CoA needed for fatty acid synthesis is produced in the mitochondria.**
- **This means that the acetyl CoA needed to start fatty acid synthesis must be transferred from mitochondria to the cytosol for fatty acid synthesis to occur.**
- **However, since acetyl CoA cannot be transported into the cytosol from the mitochondrion it must be converted to citrate.**
- **This transportation of citrate occurs when ATP levels are high in the mitochondria. That is under the exact conditions when fatty acid synthesis is needed to occur for the purpose of storing any excess fats that the body might have taken in.**
- **Let us demonstrate how compartment organization comes into play in this process.**

- Acetyl-CoA units are transferred from the mitochondrion to the cytoplasm in the form of citrate via a transport system called the citrate shuttle as shown in the next few slides.
- 1- Since acetyl-CoA cannot cross the mitochondrial membranes because of its charge, it is converted to citrate in the mitochondrion matrix by the condensation of acetyl CoA and oxaloacetate.
- 2- Though the mitochondria are not readily permeable to acetyl CoA, it allows citrate to diffuse to the cytosol.
- Thus the barrier to acetyl CoA is bypassed by citrate, which carries acetyl groups across the inner mitochondrial membrane to the cytosol- the site of fatty acid synthesis.
- 3- In the cytoplasm, citrate is converted to oxaloacetate and acetyl-CoA
- $\text{Citrate} + \text{ATP} + \text{CoA} \longrightarrow \text{acetyl CoA} + \text{ADP} + \text{P}_i + \text{oxaloacetate}$
- Oxaloacetate formed in the transfer of acetyl groups to the cytosol is returned to the mitochondria.

- **To repeat**Acetyl-CoA enters the cytoplasm in the form of citrate via the transport system as shown in the next two slides.
- In the cytoplasm, citrate is converted to oxaloacetate and acetyl-CoA by the ATP driven *ATP-citrate lyase* reaction.
- This reaction is essentially the reverse of that catalyzed by the TCA enzyme *citrate synthase* except that it requires the energy of ATP hydrolysis to drive it forward.
- Oxaloacetate formed in the transfer of acetyl groups to the cytosol must now be returned to the mitochondria. But the inner mitochondrial membrane is impermeable to oxaloacetate.
- Hence, a series of bypass reactions are needed.
- Most important, **these reactions generate much of the NADPH needed for fatty acid synthesis.**
- First, oxaloacetate is reduced to malate by NADH in a reaction catalyzed by a *malate dehydrogenase* in the cytosol.
- Oxaloacetate + NADH + H⁺ < = > malate + NAD⁺

The acetyl CoA produced in the mitochondria must be ferried to the cytosol by the citrate shuttle.

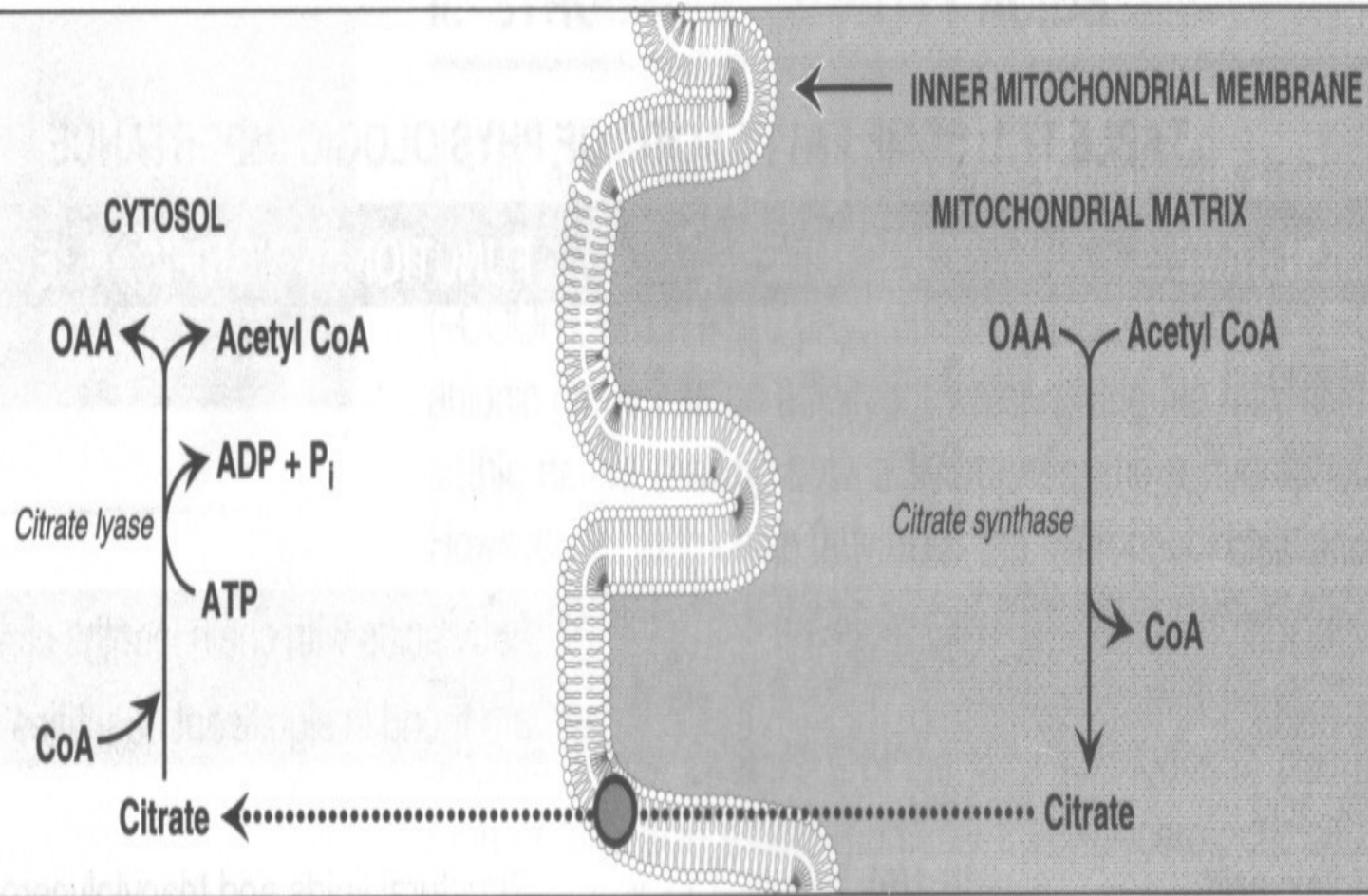
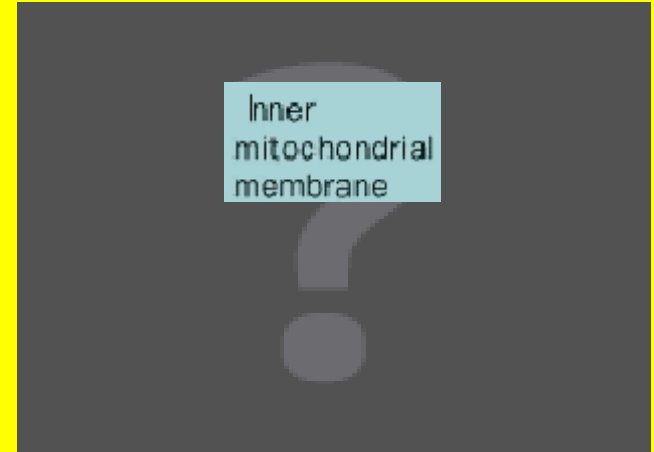
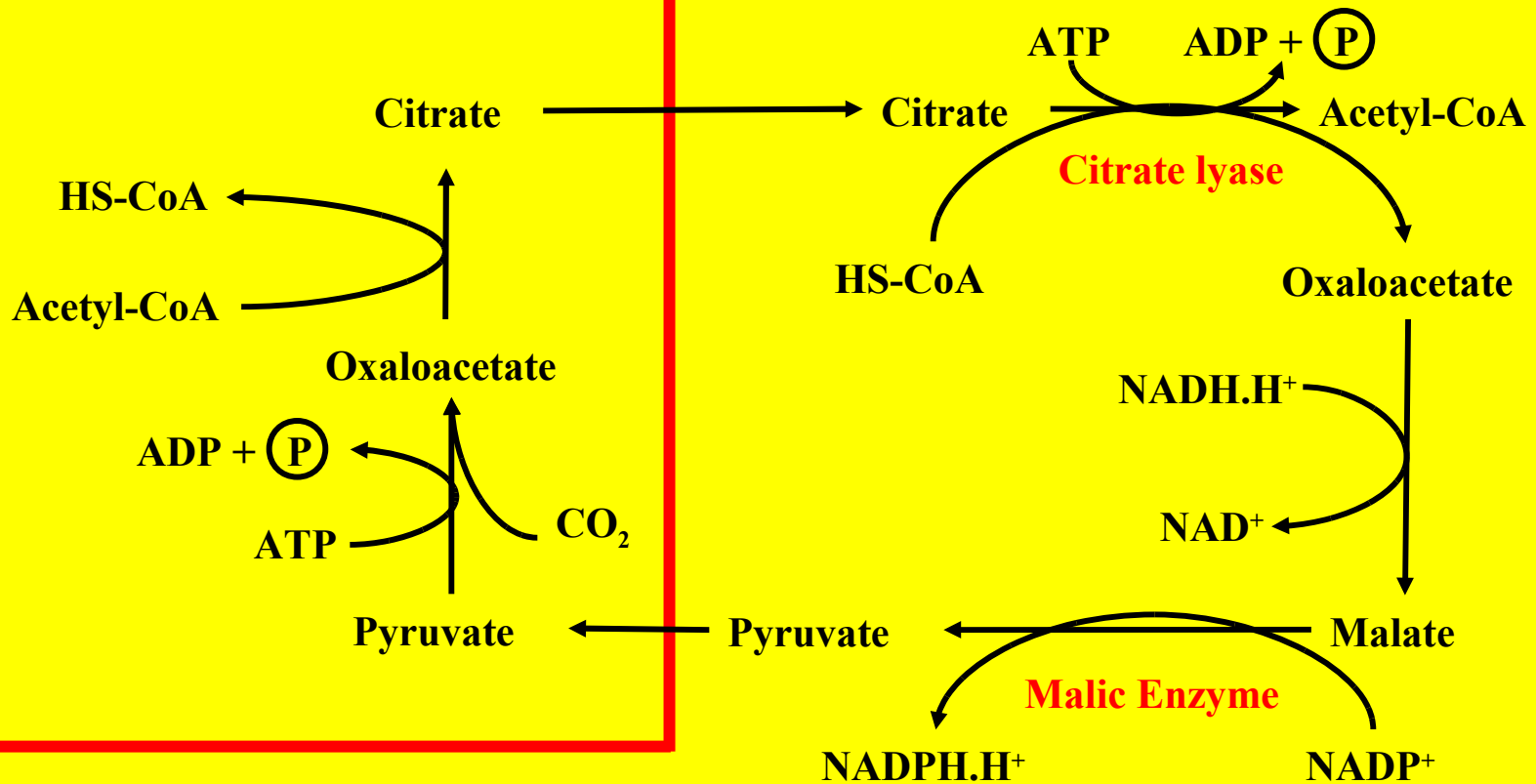


Figure 17.5
Production of cytosolic acetyl CoA.

CITRATE SHUTTLE



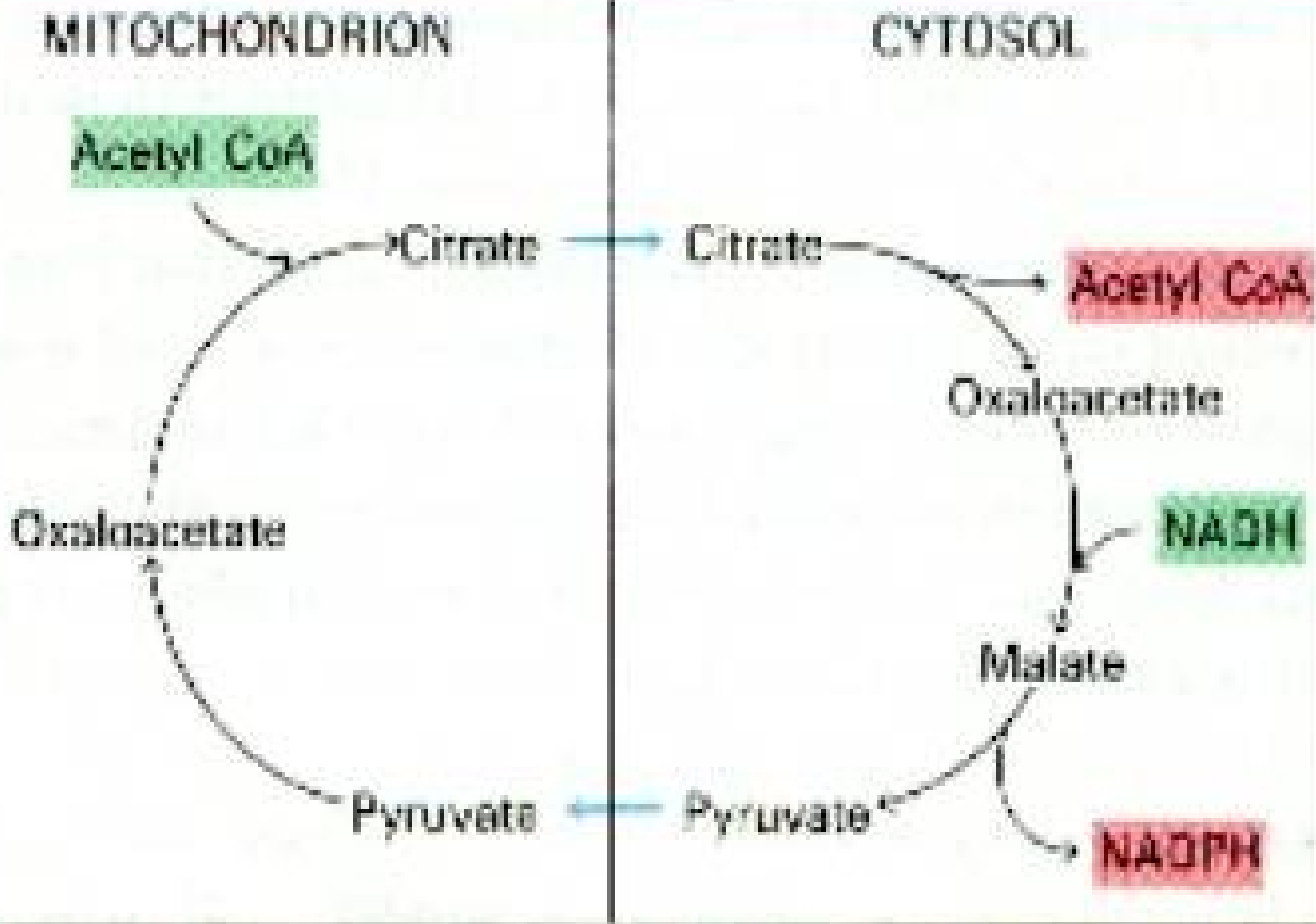
MITOCHONDRION



- **In addition to acetylCoA, NADPH is also required for fatty acid synthesis. The major source of NADPH for fatty acid synthesis is made in a metabolic pathway called the hexose monophosphate shunt or pentose phosphate pathway in the cytosol!**
- **The cytosolic conversion of malate to pyruvate and CO₂ by NADP⁺-dependent malate dehydrogenase (malic enzyme) is another source of cytosolic NADPH.**
- **Lets discuss how this happens**

- **As part of the citrate shuttle oxaloacetate is formed in the cytoplasm from citrate through the action of citrate cleavage enzyme.**
- **The oxaloacetate thus formed may then be reduced to malate by NAD⁺-dependent malate, and malate is oxidatively decarboxylated by an NADP⁺-linked malate enzyme to produce pyruvate and NADPH required for fatty acid synthesis on the spot in the cytosol!**
- **Malate + NADP⁺ ---→ pyruvate + CO₂ + NADPH**
- **The cell is essentially killing two birds with one stone, since acetyl CoA is transferred from mitochondria to the cytosol, and NADH is concomitantly converted into NADPH by the same series of reactions.**
- **In transferring the acetyl CoA from one compartment to another, the cell simultaneously generates a requirement for the same metabolic pathway it seeks to accomplish!!**

- The pyruvate formed in this reaction readily diffuses back into mitochondria, where it is carboxylated to oxaloacetate by **pyruvate carboxylase**.
- The sum of these three reactions is
- $NADP^+ + NADH + ATP + H_2O \longrightarrow NADPH + NAD^+ + ADP + P_i + H^+$
- **Thus, one NADPH is generated for each acetyl CoA that is transferred from the mitochondria to the cytosol.**
- **Note again that the reaction cycle based on the cleavage of citrate carries acetyl-CoA units from mitochondria to the cytosol and simultaneously generates some of the required NADPH for fatty acid synthesis in the cytosol!**
- **The advantage of this series of reactions for converting mitochondrial acetyl-CoA into cytoplasmic acetyl-CoA is that the NADPH produced by the *malic enzyme* reaction can be a major source of reducing co-factor for the *fatty acid synthase* activities.**
- This process is represented schematically by the two diagrams below.



- Acetyl CoA is transferred from mitochondria to the cytosol, **and NADH is concomitantly converted into NADPH by this series of reactions.**

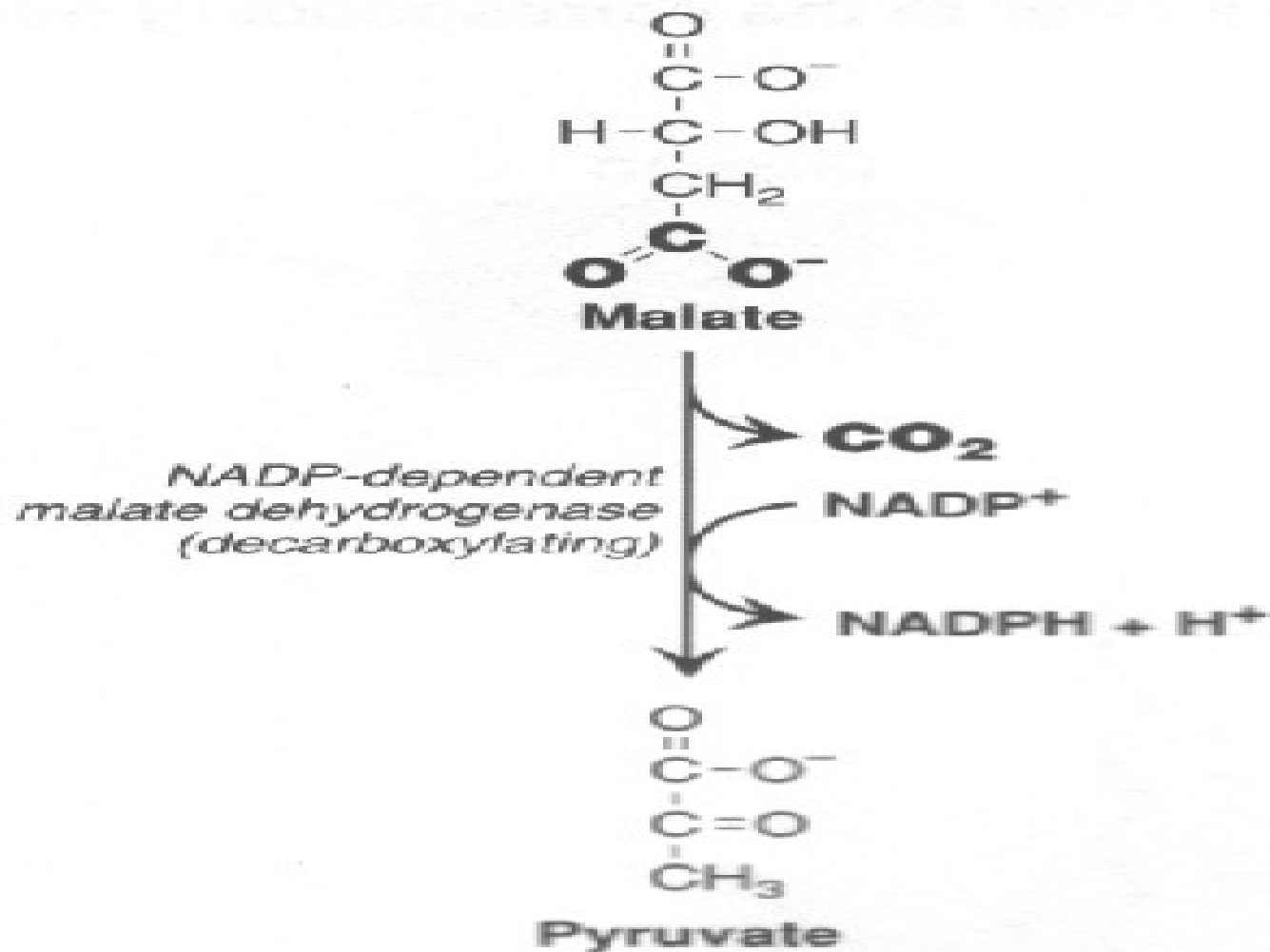
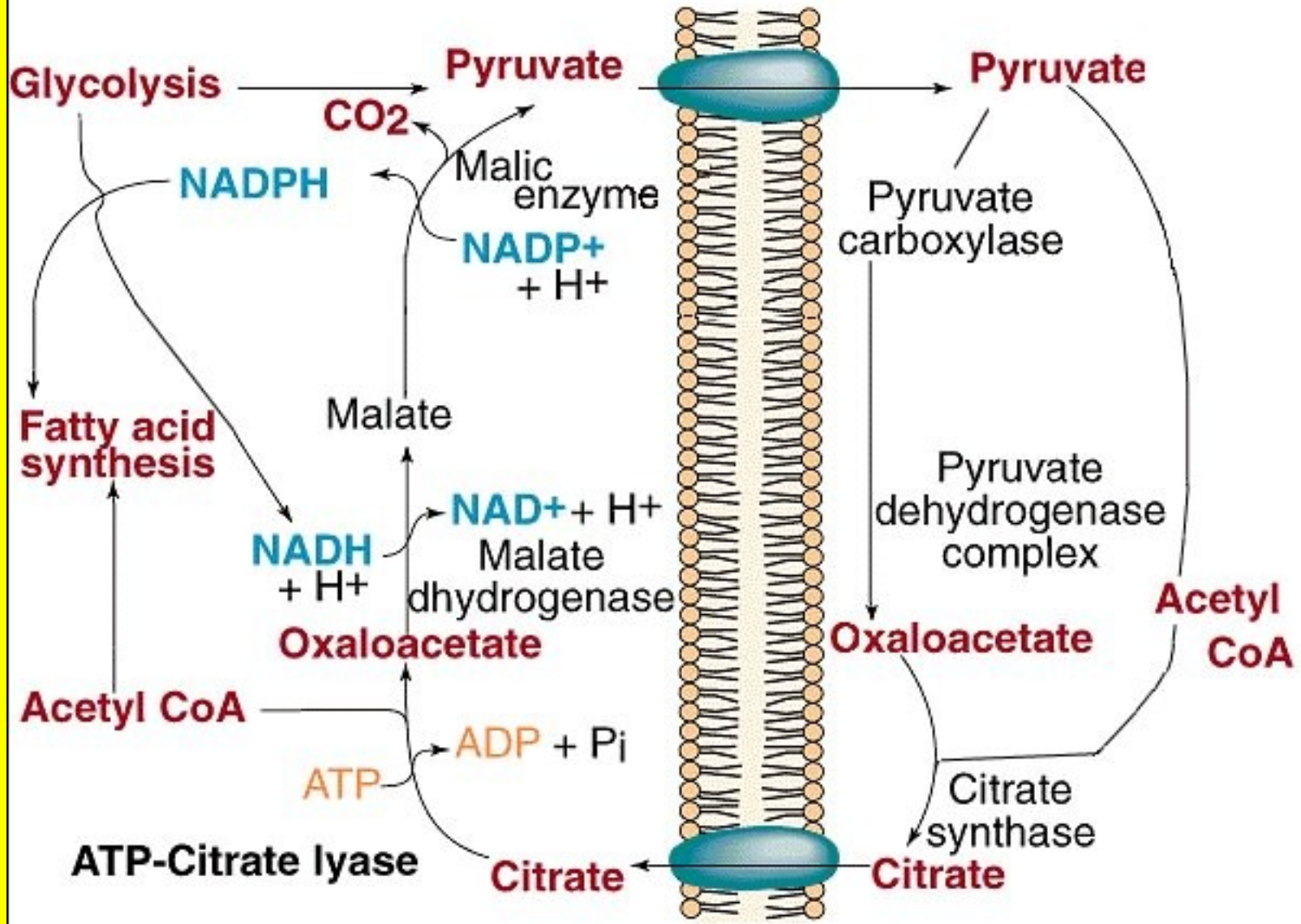


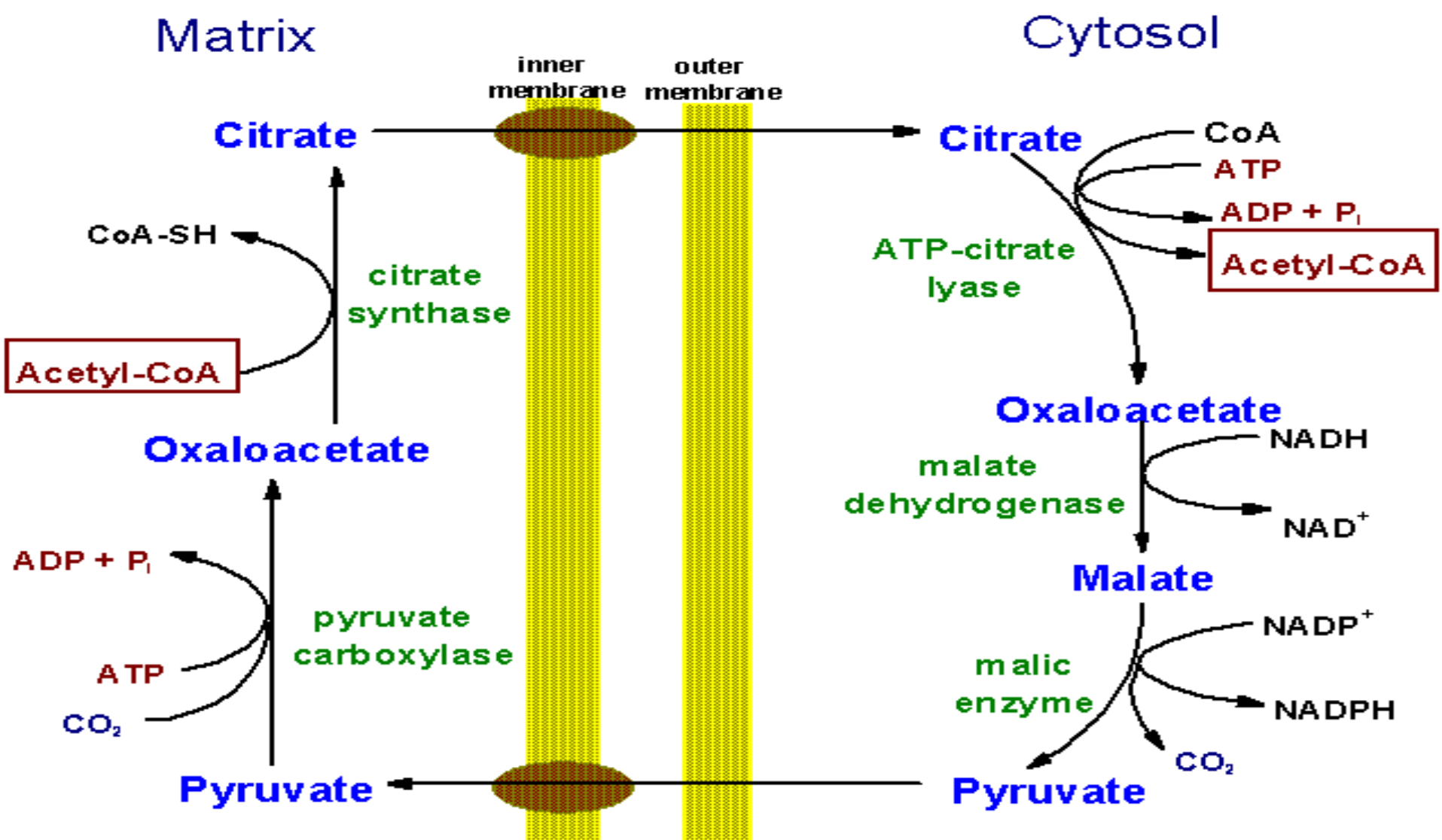
Figure 17.10

Cytosolic conversion of malate to pyruvate.

Cytosol

Mitochondrial Matrix





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- Pathway for the movement of acetyl-CoA units from within the mitochondrion to the cytoplasm for use in lipid and cholesterol biosynthesis. Note that **the cytoplasmic malic enzyme catalyzed reaction generates NADPH which can be used for reductive biosynthetic reactions such as those of fatty acid and cholesterol**

WHY GO TO ALL THIS TROUBLE?

- When one analyzes the steps in the synthesis of fatty acids they appear to be similar to those that occur in beta oxidation, and to be merely the reverse of the reactions in fatty acid oxidation.
- **Yet fatty acid synthesis does not occur by a reversal of the degradative pathway, but rather consists of a new set of reactions, which exemplifies the principle that synthetic and degradative pathways in biological systems are usually distinct.**
- **The reason for this is that the pathways are separated within different cellular compartments to facilitate distinct regulation of the two pathways.**
- **It would be futile to try to breakdown fatty acids by beta oxidation, and try to synthesize fatty acids at the same time.**
- **The body ensures that this cannot happen by compartmentalizing these two pathways.**
- **Consequently beta oxidation occurs in the mitochondria, and fatty acid synthesis occurs in the cytosol.**
- **Similarly glycolysis and the PPP occurs in the cytosol, and gluconeogenesis in the mitochondria.**

- **The shift from fatty acid oxidation and glycolytic oxidation occurs when the need for energy diminishes.**
- **This results in reduced oxidation of acetyl-CoA in the **TCA cycle** and the **oxidative phosphorylation pathway**.**
- **Under these conditions the mitochondrial acetyl units can be stored as fat for future energy demands.**

CONCLUSION

- **ASSIGNMENT!! (NOT FOR THIS COURSE!)**
- Write a short summary of the concept of **COMPARTMENT ORGANIZATION** that would make a fitting conclusion to this lecture!

THE END OF LECTURE

