UNIVERSITY OF BASRAH

AL-ZAHRAA MEDICAL COLLEGE

Ministry of higher Education and Scientific Researches

MGD module

Session 8

Lecture 14

Duration 1hr

Proteolytic Processing within the secretory pathway , Collagen synthesis.

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Marks' Basic Medical Biochemistry Chapters 8, 15, 26, 49
Medical Biochemistry Chapters 21, 28, 33
Lippincott's Illustrated Reviews: Biochemistry Chapters 4, 23, 31
Lippincott's Illustrated Reviews: Cell and Molecular Biology Chapter 11

Learning outcomes:

- Outline the formation of the mature insulin molecule. (LO6)
- Describe the structure of the triple-stranded collagen helix and provide an overview of collagen biosynthesis. (LO7)
- Outline the mechanisms involved in targeting proteins to several different cellular compartments. (LO8)
- Describe the structure and mode of action of selected antibiotics and growth inhibitors. (LO9)
- Provide an overview of the general mechanisms by which cells can become resistant to an antibiotic or drug. (LO10)

The formation of the mature insulin molecule. (LO6)

Insulin is synthesized in beta cells in the pancreas

Insulin mRNA is translated as a single chain precursor (preproinsulin)

Removal of its signal peptide during insertion into the ER generate proinsulin

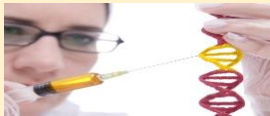
The formation of the mature insulin molecule. (LO6)

Insulin

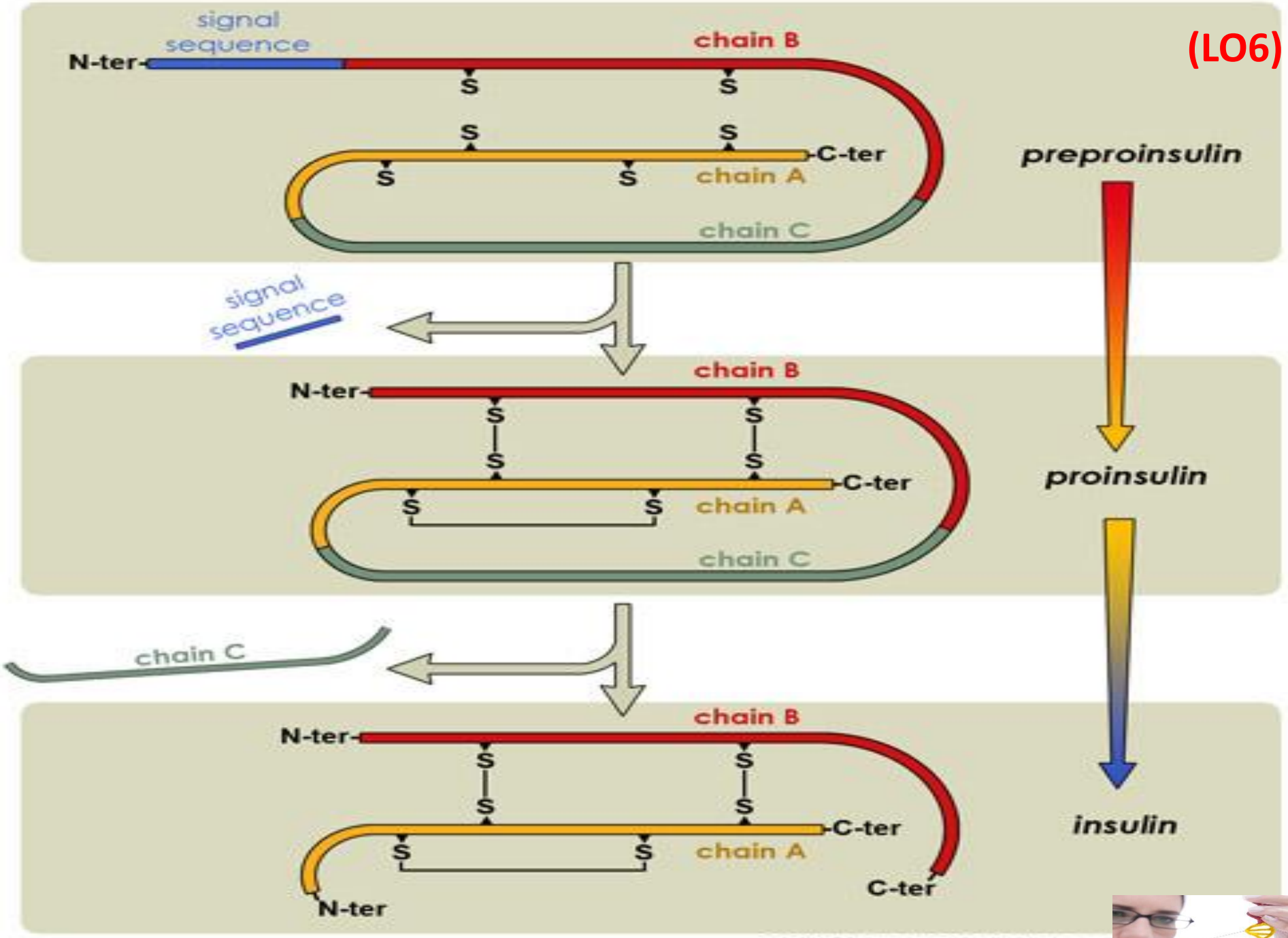
* an example of a secretory protein that undergoes proteolytic cleavage to generate the final active molecule.

*It is initially synthesized as an inactive single polypeptide chain (preproinsulin). The signal sequence is removed and 3 disulphide bonds are formed (proinsulin).

*Then further modification by proteases. The mature insulin molecule is composed of the A and B chains which are held together by disulphide bonds. The C-peptide is released.

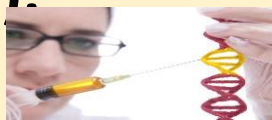


(LO6)



Collagen biosynthesis : (LO 7)

- Collagen is a fibrous protein that serve structural functions in the body.
- It is making up about 25% of the total protein in the body.
- Composed of 3 chains which are wound together to form a triple helix.
- There are many different types of collagen (28 type) particularly in connective tissue.
- Collagen has an unusual amino acid composition. Approximately one-third of the amino acids are glycine (Gly), about 10 % proline (Pro), and 10% hydroxyproline (Hyp).



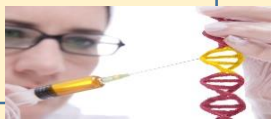
Collagen biosynthesis (organization) (LO7)

1-As agel to give support to the structure e.g. extracellular matrix of the vitreous humor of the eye

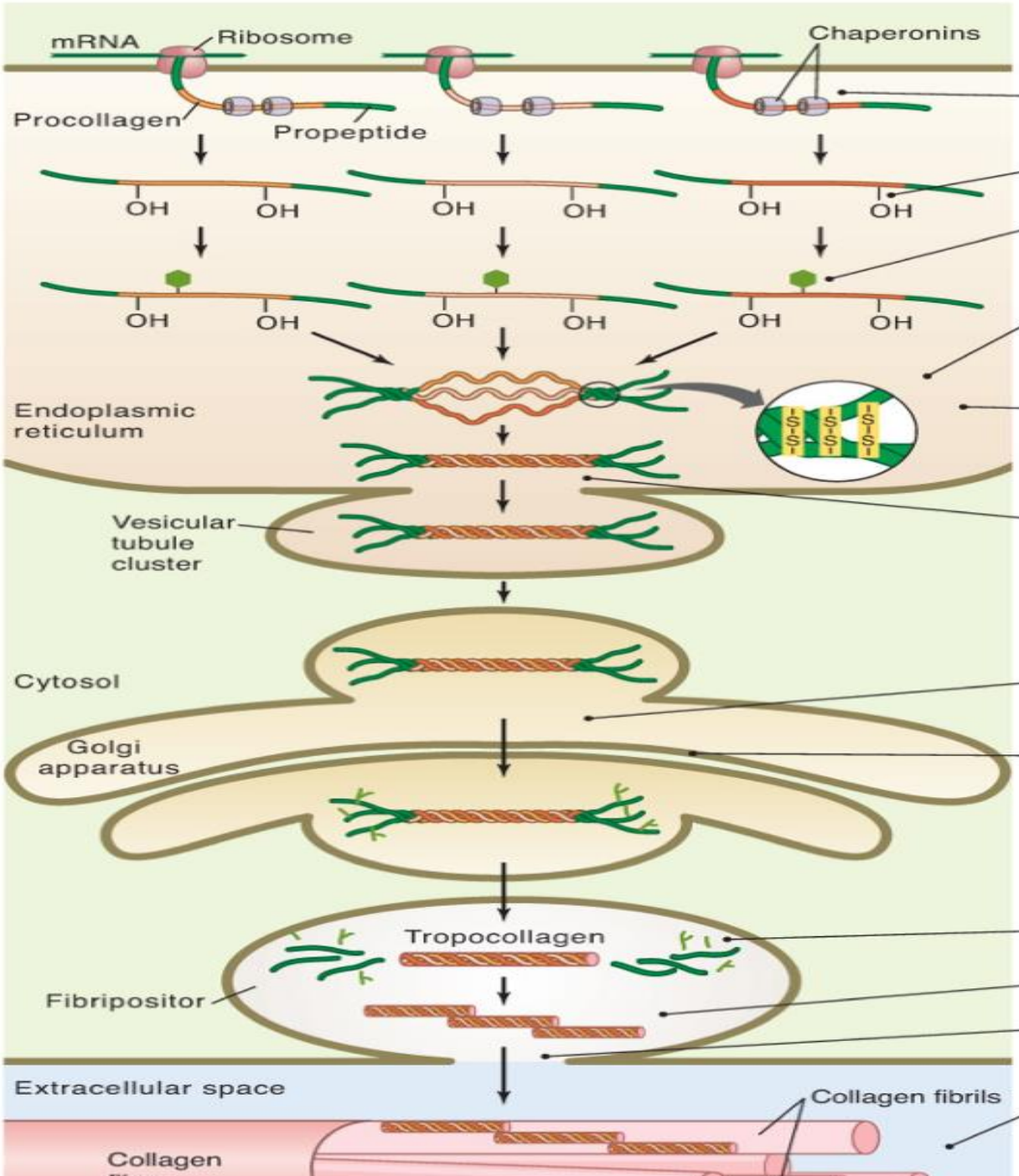
2- bundled in a tight, parallel fibers for great strength e.g. tendon

3-stacked so as to transmit light with a minimum of scattering e.g. cornea of the eye

4-as fibers arranged at an angle to each other to resist a mechanical shear from any direction e.g. bone

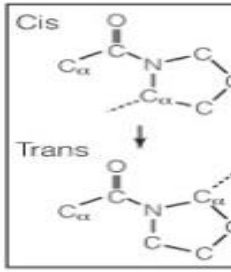


(LO 7)



Procollagen assembly

1. Chaperonins assist folding.
2. Hydroxylation.
3. N-linked glycosylation.
4. Self-assembly, formation of disulfide bonds.
5. Conversion of proline peptide bonds from cis to trans configuration.
6. Formation of a triple helix.



7. Transport through Golgi apparatus.
8. Modification of N- and O-linked sugars.

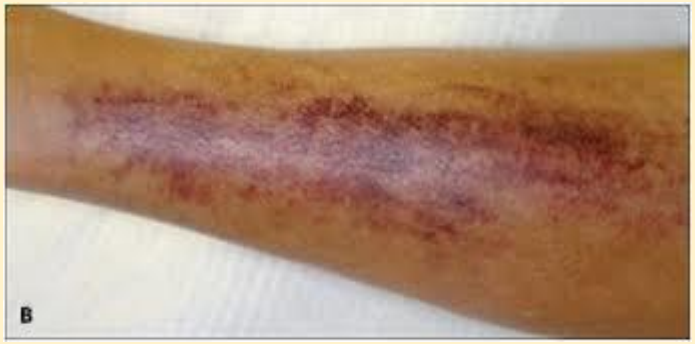
Fibril/fiber assembly

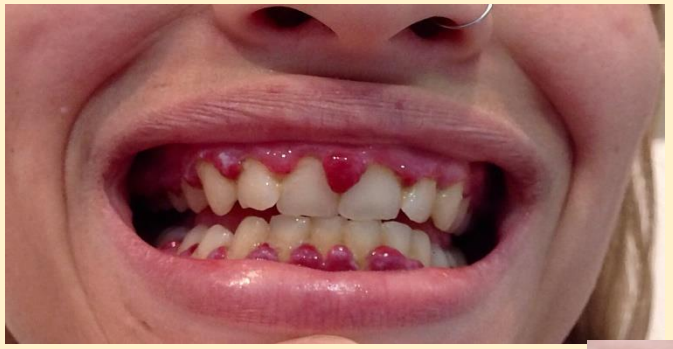
9. Cleavage of propeptides.
10. Self-assembly of fibril.
11. Secretion.
12. Fiber assembly.

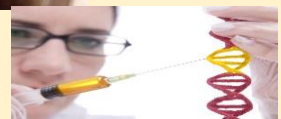
Vitamin C Deficiency and Scurvy (LO7)

* general weakness, anaemia.
* gingivitis (gum disease).
* skin haemorrhages.

caused by a prolonged deficiency of vitamin C (ascorbic acid) in the diet. Vitamin C plays a crucial role in the formation of collagen, a major component of connective tissue.







scurvy (LO7)

- Ascorbate is required for the hydroxylation of proline residues in collagen.

- Hydroxyproline stabilize the collagen triple helix by forming inter strand hydrogen bonds.
- The abnormal fibers formed by insufficiently hydroxylated collagen contribute to the skin lesions and blood vessels fragility.

The mechanisms involved in targeting proteins to several different cellular compartment (LO8)

- **Protein targeting or protein sorting:**

is the biological mechanism by which proteins are transported to their appropriate destinations in the cell or outside it.

protein can be targeted to inner space of an organelle, intracellular membranes, plasma membrane, or to exterior of the cell via secretion.

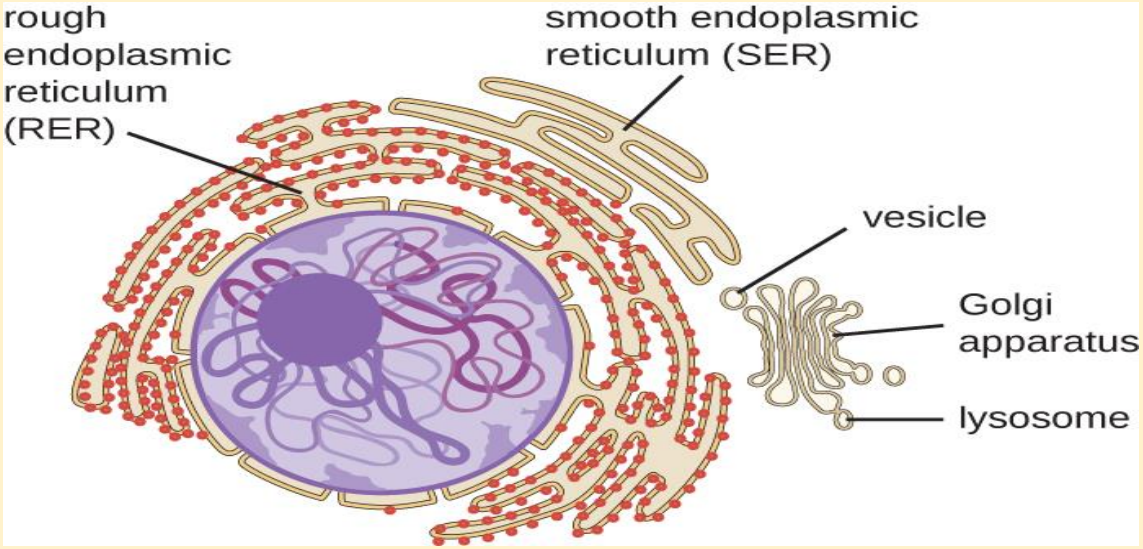
- **This process is based on information contained in the protein itself.**
- **Correct sorting is crucial for the cell; errors can lead to diseases.**

The mechanisms involved in targeting proteins to several different cellular compartment (LO 8)

- Targeting signals: are the pieces of information that enable the cellular transport machinery to correctly position a protein inside or outside the cell. This information is contained in the polypeptide chain or in the folded protein.
- The continuous stretch of amino acid residues in the chain that enables targeting are called signal peptides or targeting peptides. Which are 2 type:
- the presequences
- the internal targeting peptides.

The mechanisms involved in targeting proteins to several different cellular compartment (LO8)

- **Where does protein sorting occur?**
- **The rough endoplasmic reticulum (RER) which is continuous with the outer membrane of the nuclear envelope plays an important role in the sorting of proteins .**



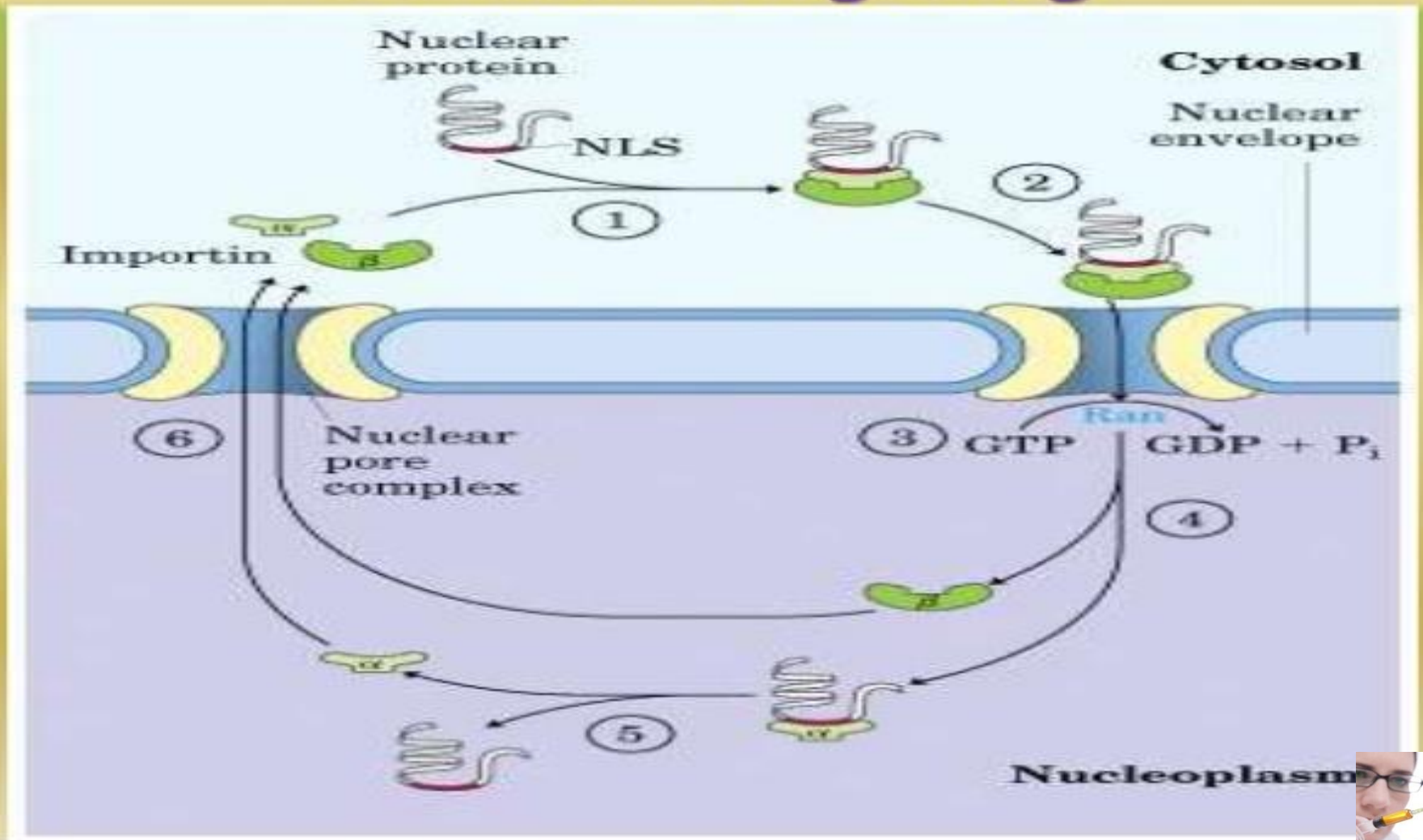
The mechanisms involved in targeting proteins to several different cellular compartments: (LO 8)

Nuclear proteins:

The nuclear import of proteins into the cell nucleus involves the recognition of a nuclear localization signal sequence(NLS) borne by the protein to be transported, by complex molecules called importins, that will subsequently mediate the crossing over of the nuclear envelope

(LO 8)

Nuclear Targeting.



The mechanisms involved in targeting proteins to several different cellular compartments. (LO8)

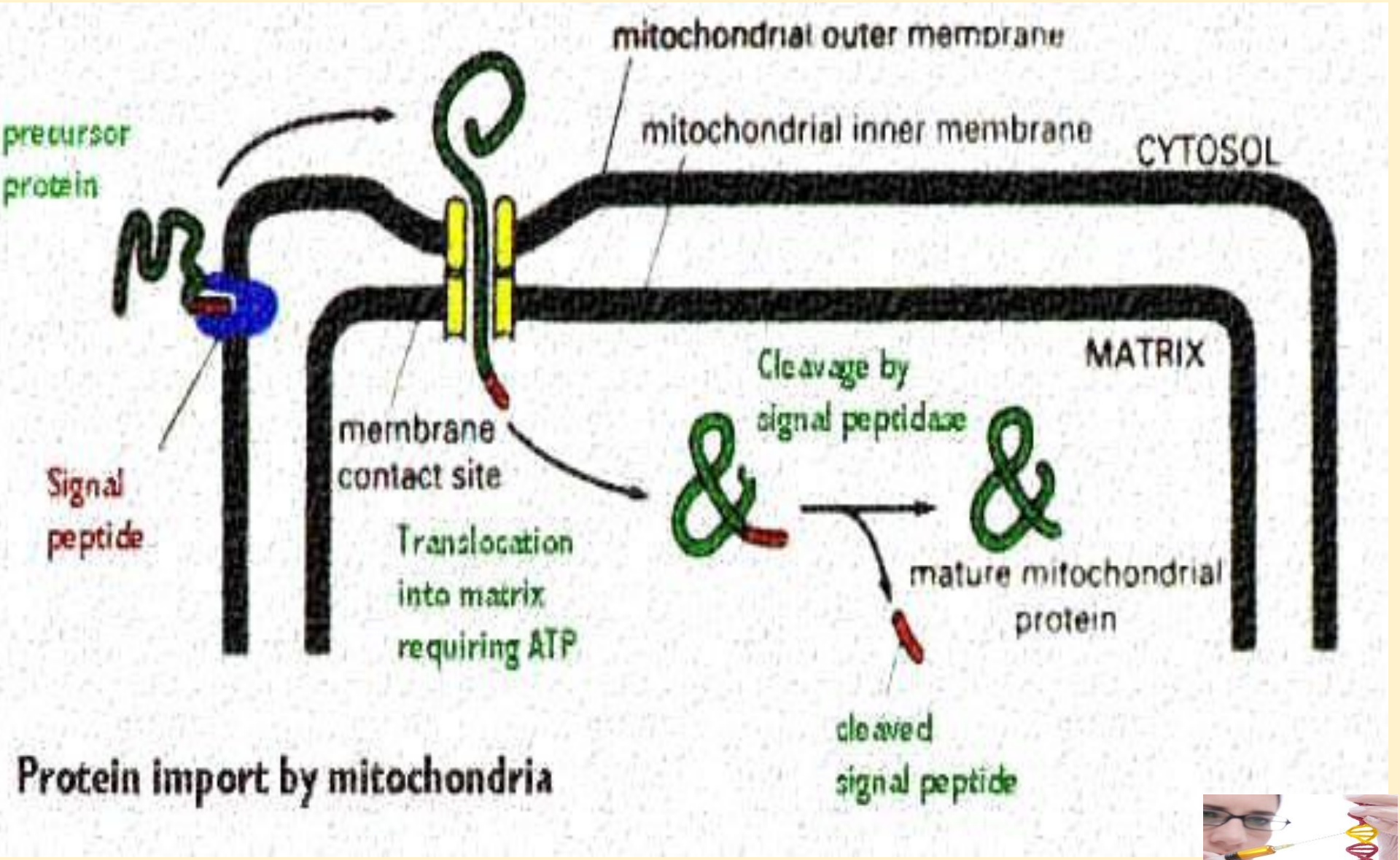
- **Mitochondrial protein:**

Most mitochondrial proteins are synthesized as cytosolic precursors containing uptake peptide signals.

Cytosolic chaperones deliver preproteins to channel linked receptors in the mitochondrial membrane. ... Binds to internal targeting peptides and acts as a docking point for cytosolic chaperones.

Mitochondrial protein, contain N-terminal mitochondrial import sequence.

LO8



Protein import by mitochondria

The mechanisms involved in targeting proteins to several different cellular compartments. (LO8)

- **Peroxisomal protein:**
- **Protein needed in the peroxisome have a specific sequence of a.as called <u>peroxisomal targeting signal</u>.**
- **The classic signal consist of 3 a.as serine-lysine-leucin, found at C-terminus of protein.**
- **This a.as recognized by a <u>helper protein</u> in the cytosol which bring the protein to the peroxisome.**

The mechanisms involved in targeting proteins to several different cellular compartments. (LO8)

- A congenital defect in this protein signal result in a disease called Zellweger syndrome is a rare congenital disorder characterized by the reduction or absence of functional peroxisomes in the cells of an individual.
- The affected individual do not survive beyond 6 month in sever condition.

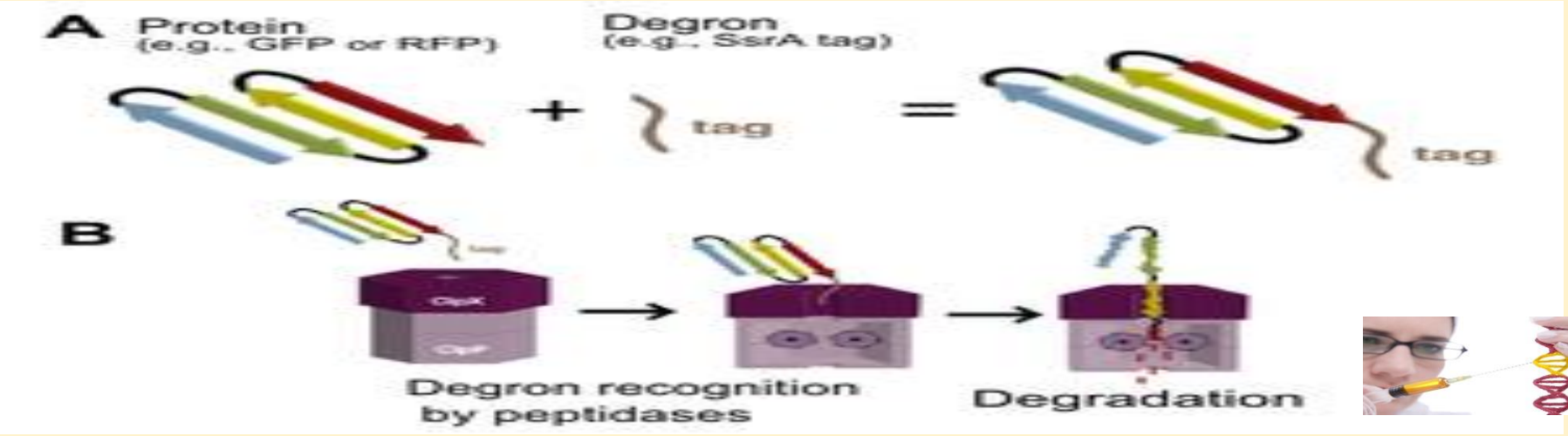
The mechanisms involved in targeting proteins to several different cellular compartments. (LO8)

Lysosomal storage disease (I-cell disease):

- It is inherited as an autosomal recessive trait, result from defect in the phosphorylation of mannose, so the hydrolases will be mislocated to the outside the cell, while nonfunctional macromolecules will not hydrolyzed in lysosome and sever psychomotor retardation and skeletal deformities result.

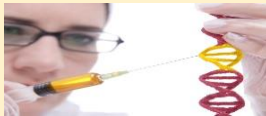
Protein Degradation: (LO8)

- In order to keep a cell working it needs to remove:
  - Incorrectly synthesized proteins (with errors in amino acid sequence).
  - Damaged proteins (i.e. oxidative damage).
  - Other signaling proteins which are no longer necessary.



Protein degradation (LO8)

- What causes protein degradation?
- Proteins are marked for rapid degradation by the covalent attachment of several molecules of ubiquitin.
- Ubiquitin is first activated by the enzyme E1. Activated ubiquitin is then transferred to one of several different (more...) ... Most cells contain a single E1, but have many E2s and multiple families of E3 enzymes.



Protein degradation (LO8)

The role of ubiquitin in protein degradation:

- Ubiquitination affects cellular process by:
- regulating the degradation of proteins (via the proteasome and lysosome),
- coordinating the cellular localization of proteins.
- activating and inactivating proteins

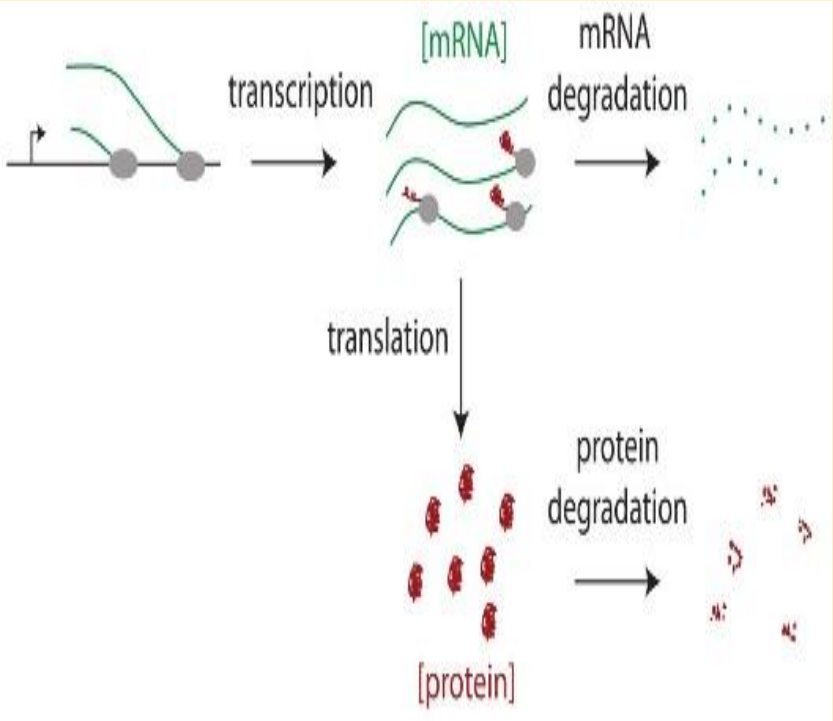
Protein degradation: (LO8)

- All proteins are degraded eventually using the cell's proteolytic system.

The two systems are:

1- the lysosomal protein degradation system.

2- the ATP dependent proteosomal degradation pathway.



Protein Degradation: (LO8)

1- Lysosomal Protein Degradation

- Lysosomes are acidic vesicles that contain about 50 different enzymes involved in degradation:

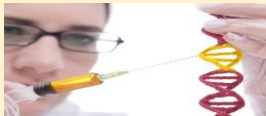
1-Proteases: cleave peptide bonds.

2-Phosphatases: remove covalently bound phosphates.

3-Nucleases: cleave DNA/RNA.

4-Lipases: cleave lipid molecules.

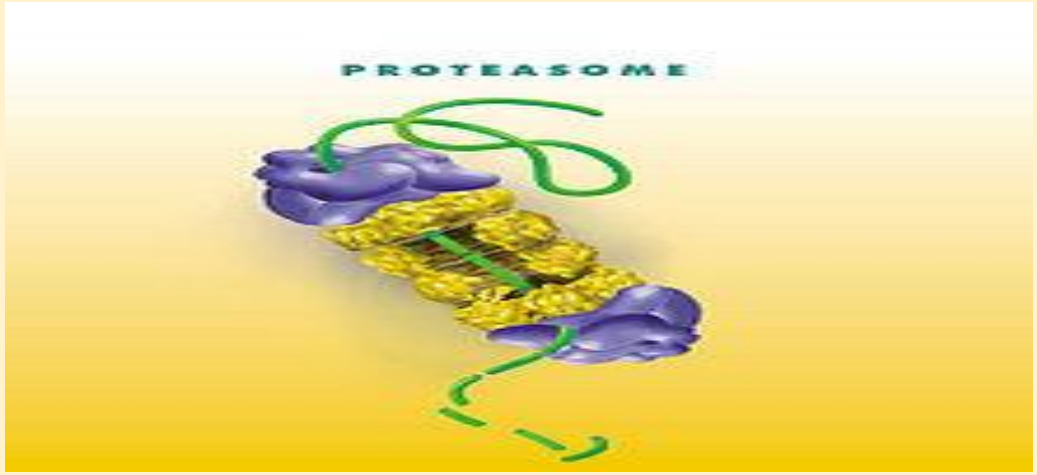
5-Carbohydrate-cleaving enzymes: remove covalently bound sugars from glycoproteins.

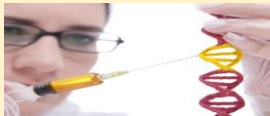


2-ATP dependent proteasomal degradation:

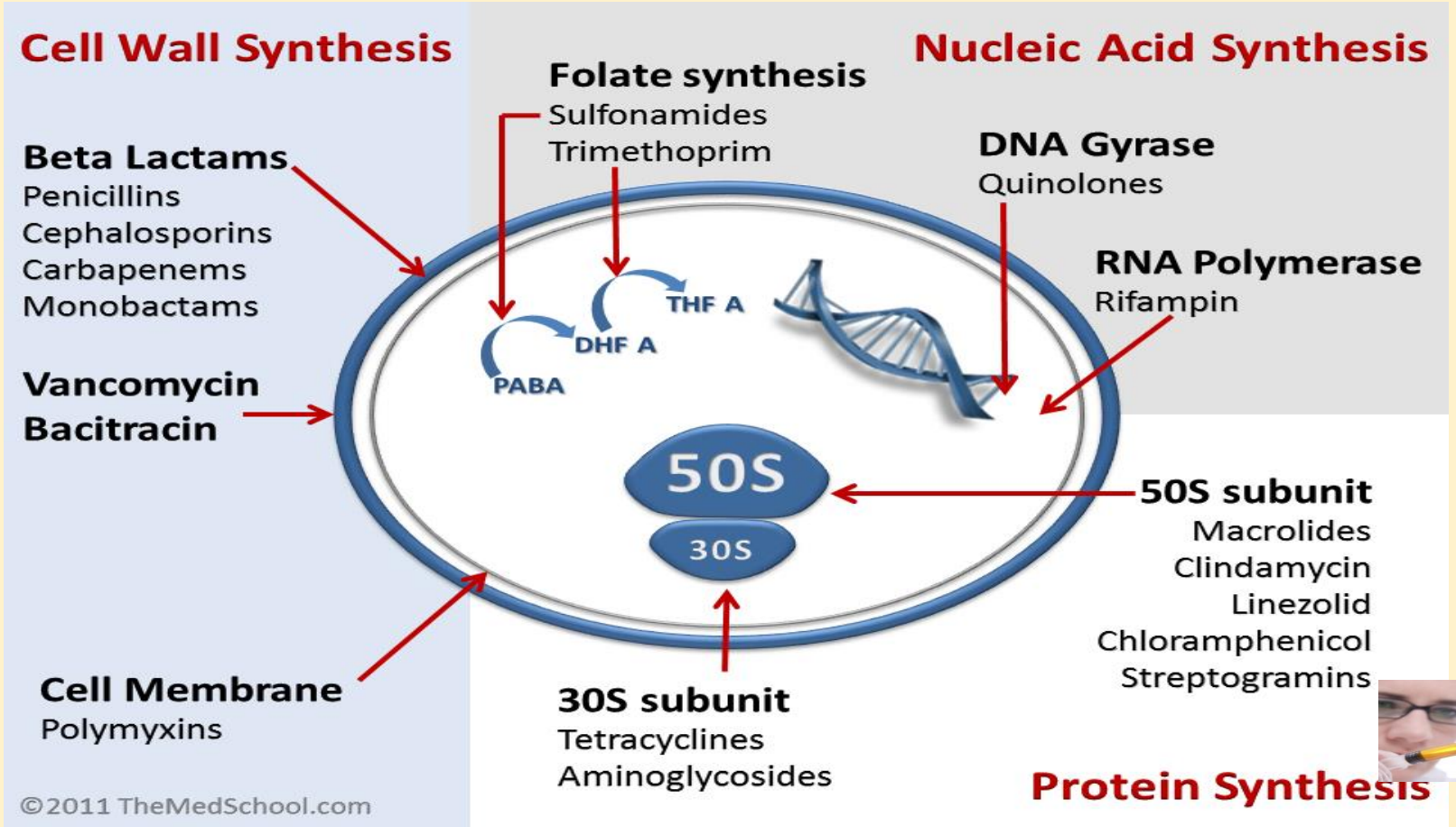
LO8

Proteasomes are protein complexes which degrade unneeded or damaged proteins by proteolysis, a chemical reaction that breaks peptide bonds, by proteases enzyme.



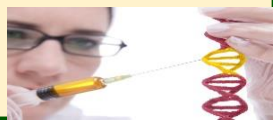


The structure and mode of action of selected antibiotics and growth inhibitors. (LO9)



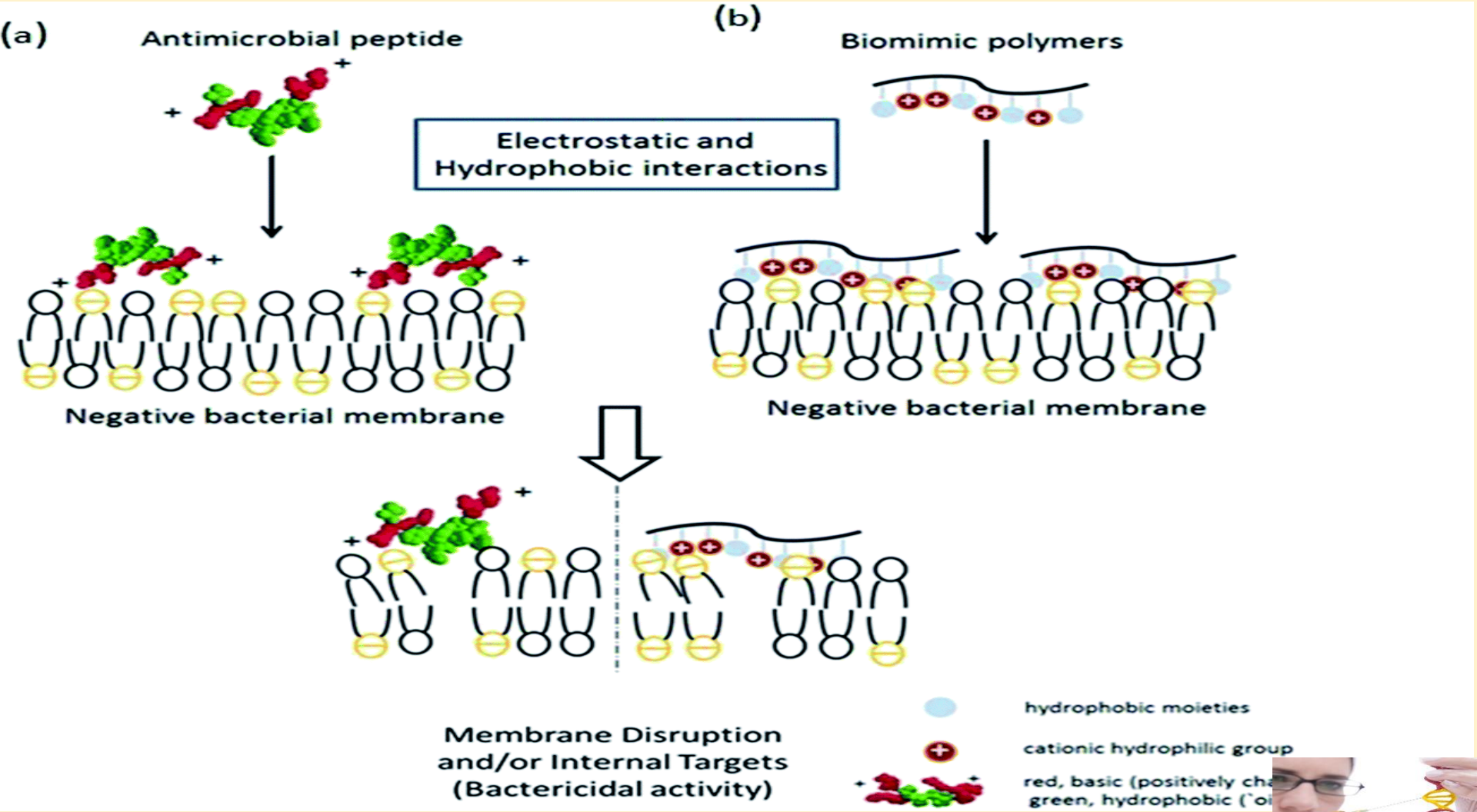
The structure and mode of action of selected antibiotics and growth inhibitors. (LO9)

Drug	Target	Mechanism of action
Penicillin	Bacterial cell wall	The penicillins interfere with the last step of bacterial cell wall synthesis (transpeptidation or cross-linkage), resulting in exposure of the osmotically less stable membrane. Cell lysis can then occur, either through osmotic pressure or through the activation of autolysins.
Tetracycline	Bacterial protein synthesis	The drug binds reversibly to the 30S or50s subunit of the bacterial ribosome, and inhibit protein synthesis by many mechanism.



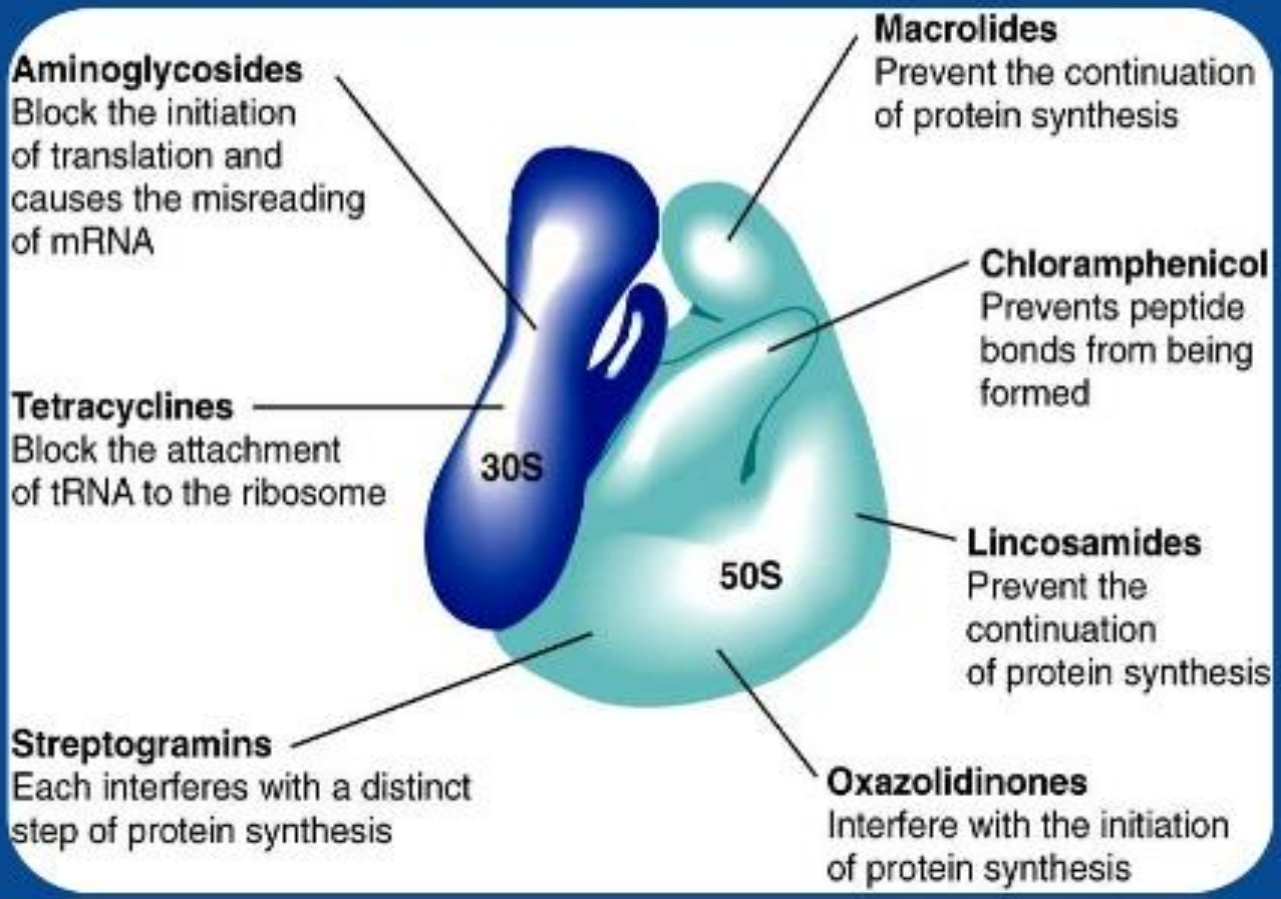
Cell wall inhibition

(LO9)



(LO9)

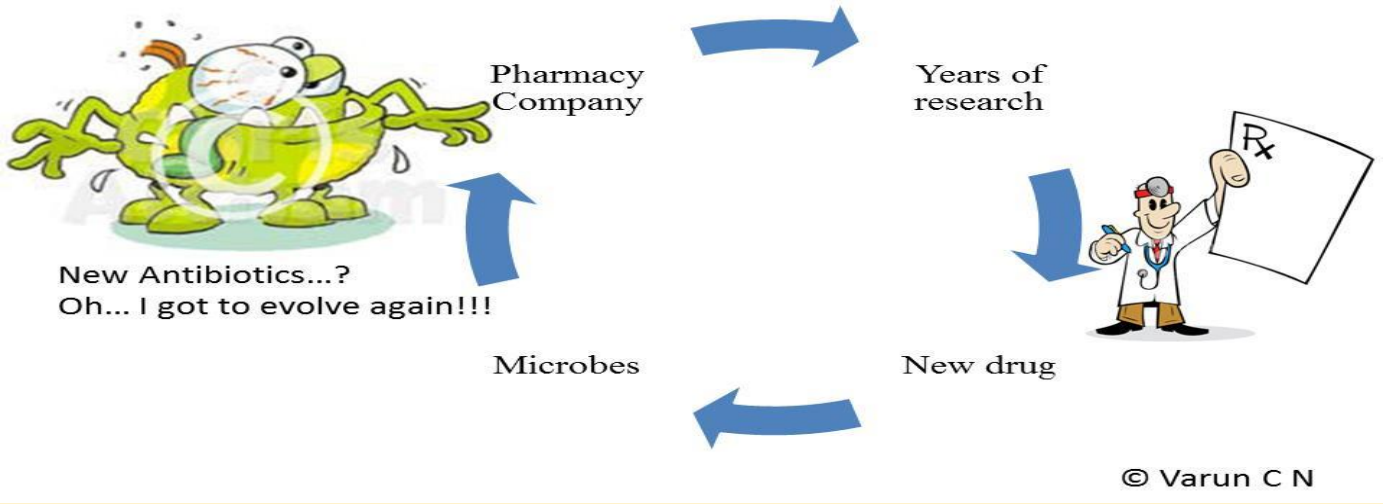
Inhibition of Protein Synthesis



Drug	Target	Mechanism of action
Rifampicin	Bacterial transcription	Blocks transcription by interacting with the β subunit of bacterial, DNA dependent RNA polymerase. It inhibits mRNA synthesis by suppressing the initiation step.
Methotrexate (growth inhibitor)	Antifolates in chemotherapy	MTX effectively inhibits dihydrofolate reductase (DHFR), which is the enzyme that converts folic acid to its active, coenzyme form, tetrahydrofolic acid. This inhibition deprives the cell of folate coenzymes and leads to decreased production of compounds that depend on these coenzymes for their biosynthesis. These molecules include the nucleotides adenine, guanine, and thymidine and the amino acids methionine and serine. This leads to depressed DNA, RNA, and protein synthesis and, ultimately, to cell death.

The general mechanisms by which cells can become resistant to an antibiotic or drug. (LO 10)

- **Antibiotic resistance** occurs when bacteria change in some way that reduces or eliminates the effectiveness of drugs, chemicals, or other agents designed to cure or prevent infections. The bacteria survive and continue to multiply causing more harm.



(LO 10)

- Bacteria are said to be <u>resistant</u> to an antibiotic if the maximal level of that antibiotic that can be tolerated by the host does not halt their growth.

1- Genetic alterations leading to drug resistance.

2- Altered expression of proteins in drug-resistant organisms

(LO 10)

The five mechanisms of antimicrobial resistance:

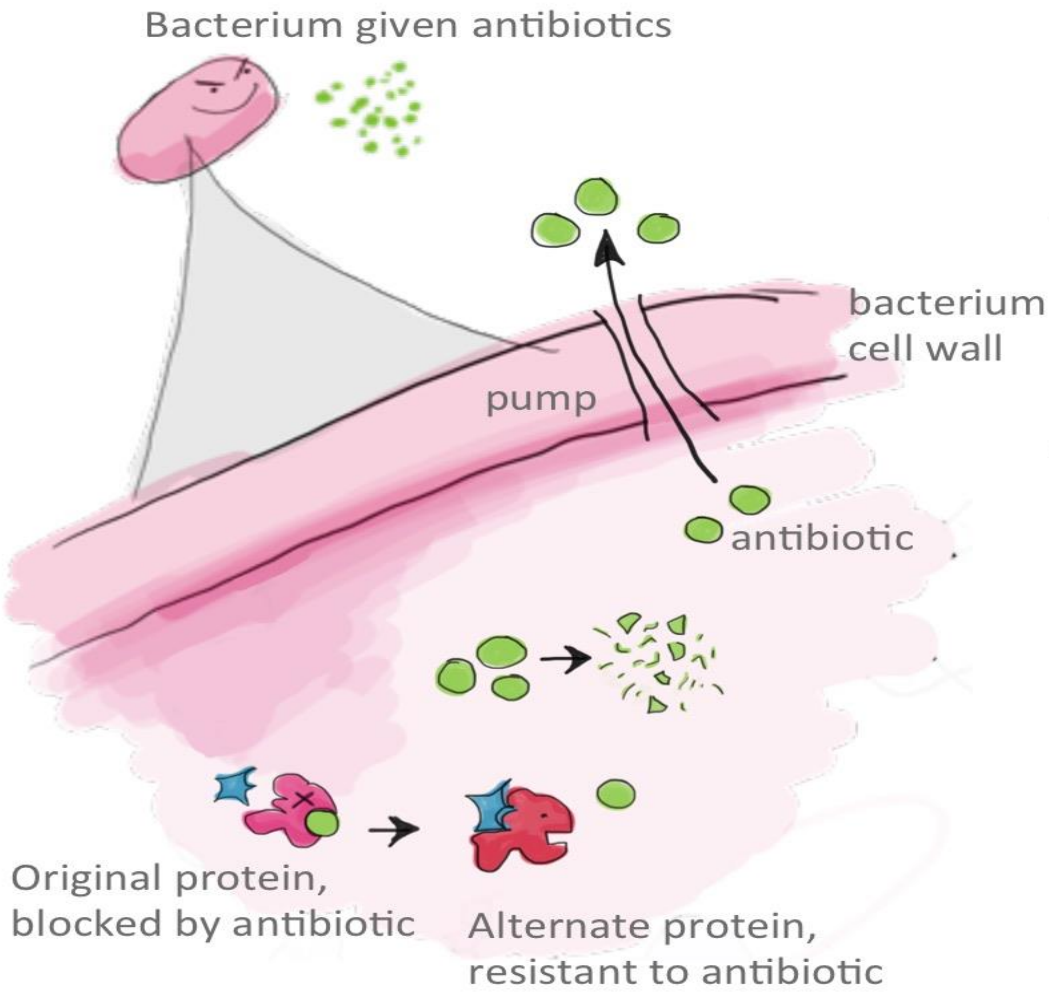
1- production of drug-inactivating enzymes.

2- modification of an existing target.

3- Development of a target by-pass system.

4- reduced cell permeability and drug removal from the cell.

(LO10)



Mechanisms of resistance to antibiotics:

Remove the antibiotic:
Antibiotics can be pumped out of the cell

Destroy the antibiotic:
Enzymes can cut up or deactivate the antibiotic

Bypass the inhibited protein:
Bacteria can produce an alternate protein to bypass the inhibited one

LO10

Drug	Mechanism of resistance
Pencillins and chloramphenicol	β lactamase cleavage of the βlactam ring
Aminoglycosides	Modification by phosphorylating,adenylating and acetylating enzymes
Chloramphenicol	Modification by acetylation
Erythromycin	Change in receptors by methylation of rRNA
Tetracycline	Reduced uptake/ increased export
Sulfonamides	Active export out of the cell & reduced affinity of enzymes


Thank
You