MGD module
Session 8
Lecture 13
Duration 1hr

Protein Processing in cells, the secretory pathway

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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 :

- Contrast the constitutive and regulated secretory pathways. (LO 8.1)
- Provide an overview of the secretory pathway in mammalian cells. (LO 8.2)
- List protein modifications which occur in the ER and Golgi complex. (LO 8.3)
- Distinguish between N-linked and O-linked glycosylation of proteins. (LO 8.4)
- Describe the role that proteolytic processing plays in the formation of important secreted proteins. (LO 8.5)

Contrast the constitutive and regulated secretory pathways (LO 8.1)

Protein that are destined for insertion into the plasma membrane, lysosomes, Golgi/ER or secretion

→ Are synthesized on ribosomes associated with the endoplasmic reticulum(rough ER)

Protein that will function in the nucleus, mitochondria or peroxisomes

→ Are synthesized on free ribosomes

Types of secretion:

(LO 8.1)

1. Constitutive secretion :

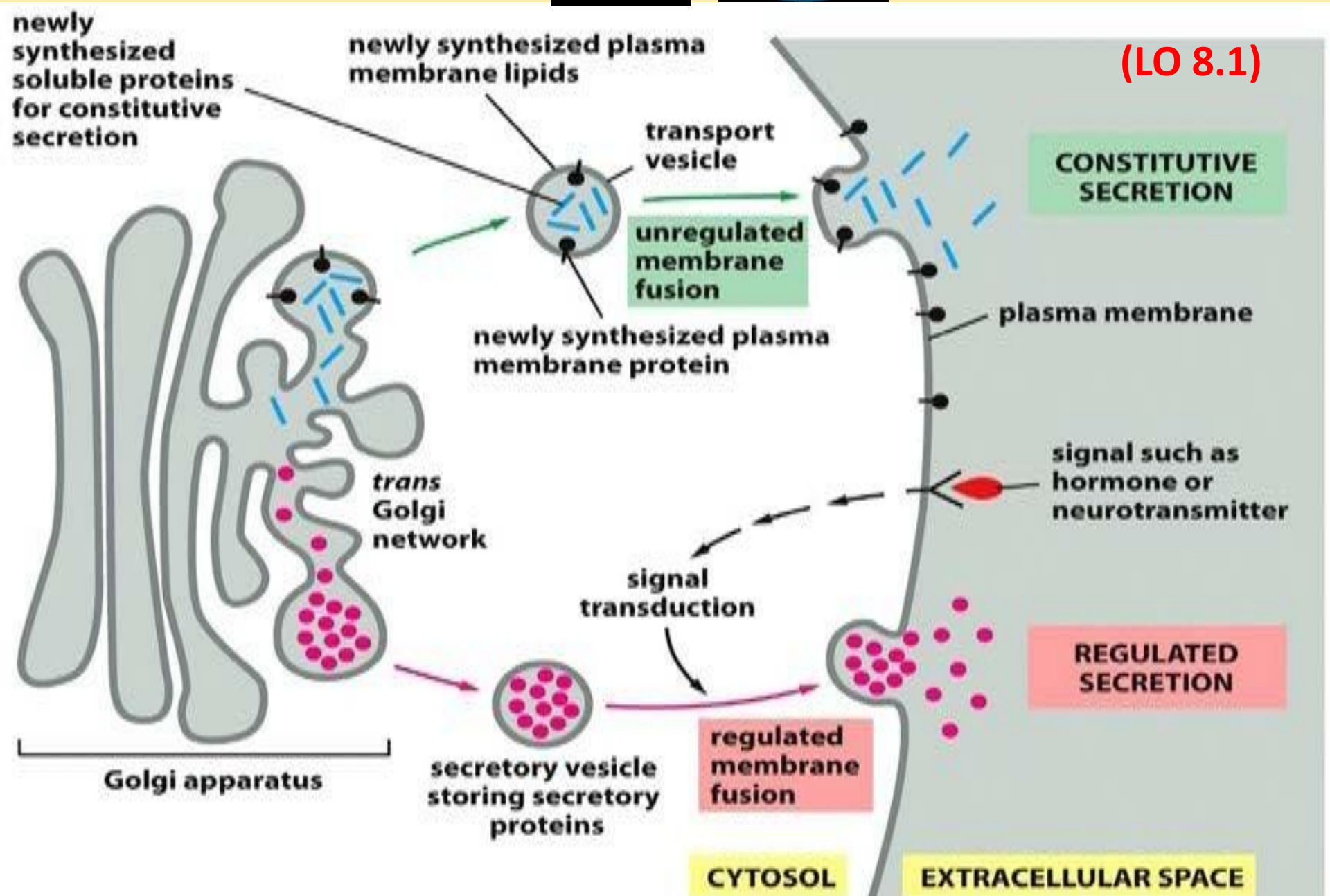
- proteins packaged into vesicles and release continuously by exocytosis

e.g. serum albumin, collagen.

2. Regulated secretion :

- Proteins released in response to a signal e.g. hormone

-proteins packaged into vesicles but not released until stimulus received e.g. insulin


(LO 8.1)
newly synthesized soluble proteins for constitutive secretion
newly synthesized plasma membrane lipids
transport vesicle
CONSTITUTIVE SECRETION
unregulated membrane fusion
plasma membrane
newly synthesized plasma membrane protein
signal such as hormone or neurotransmitter
trans Golgi network
signal transduction
REGULATED SECRETION
regulated membrane fusion
secretory vesicle storing secretory proteins
Golgi apparatus
CYTOSOL
EXTRACELLULAR SPACE

Signal sequences

(LO8.1)

*Structural features within the protein being produced are recognized by the organelles,facilitating the movement of the protein.

These structural features:

- are called signal sequences (sometimes called a leader sequence) and direct the protein to locations where it can be modified properly in order to become functional.

Secreted proteins have a signal sequence at the N-terminus that targets them to the ER.

Signal sequences vary in length from 13-36 amino acids, typically contain: (LO 8.1)

*A stretch of 10-15 hydrophobic residues

*A few polar amino acids within the C-terminal region

*1 or more +ve charge residues near the N- terminus before the hydrophobic sequences.

*Asmall,neutral side chain on the amino terminal side of the cleavage site. Alanine is most common

(LO 8.1)

A signal sequence on the growing polypeptide chain directs the ribosome to the ER membrane.

Signal sequences are absent from normally secreted proteins, because they are cleaved by signal peptidase on the luminal side of the ER membrane.

The sequences act as "address labels" routing the new proteins to their proper destinations

New proteins (nascent polypeptide) that leave the TGN (Trans Golgi Network) and not destined to function in lysosomes or to insert into plasma membrane will be secreted from the cell.

(LO 8.2)

The protein secretion pathway

1. Protein synthesis initiated on free ribosomes.
2. N-terminal signal sequence produced.
3. Signal sequence of newly formed protein is recognized by the signal recognition particle (SRP).
4. GTP-bound SRP directs the ribosome synthesizing the secretory protein to SRP receptors on the cytosolic face of the ER.

5. SRP dissociates. (LO 8.2)

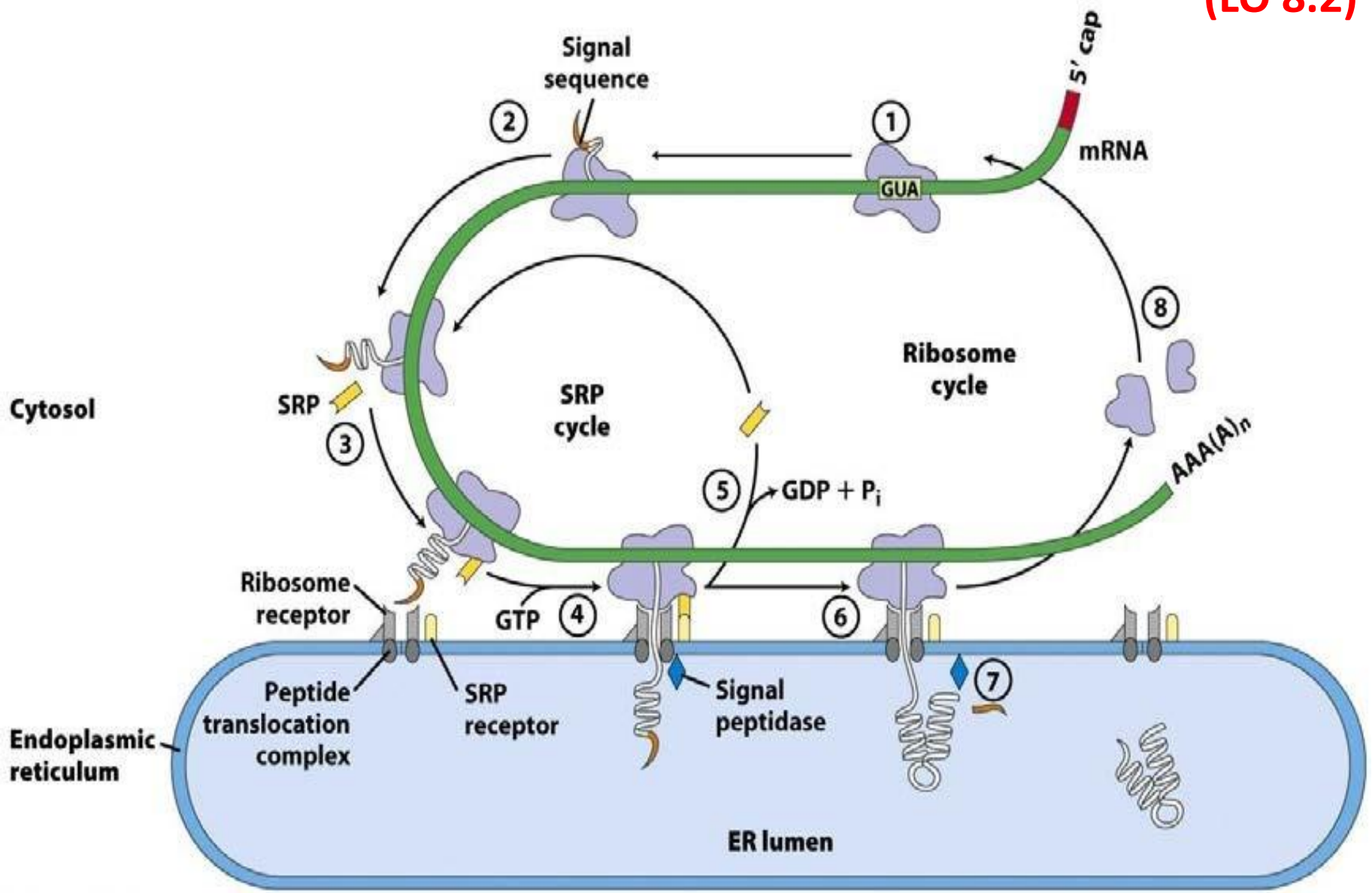
6. Protein synthesis continues and the newly formed polypeptide is fed into the ER via a pore in the membrane (peptide translocation complex).

7. Signal sequence is removed by a signal peptidase once the entire protein has been synthesized.

8. The ribosome dissociates and is recycled.

In all cases, if the appropriate signals are not incorporated within the new proteins, then the proteins will follow a default pathway. The default is to remain in the cytosol.

(LO 8.2)


Signal sequence
5′ cap
mRNA
GUA
Ribosome cycle
SRP cycle
Cytosol
SRP
GDP + Pi
AAA(A)n
Ribosome receptor
GTP
Peptide translocation complex
SRP receptor
Signal peptidase
Endoplasmic reticulum
ER lumen

(LO 8.2)

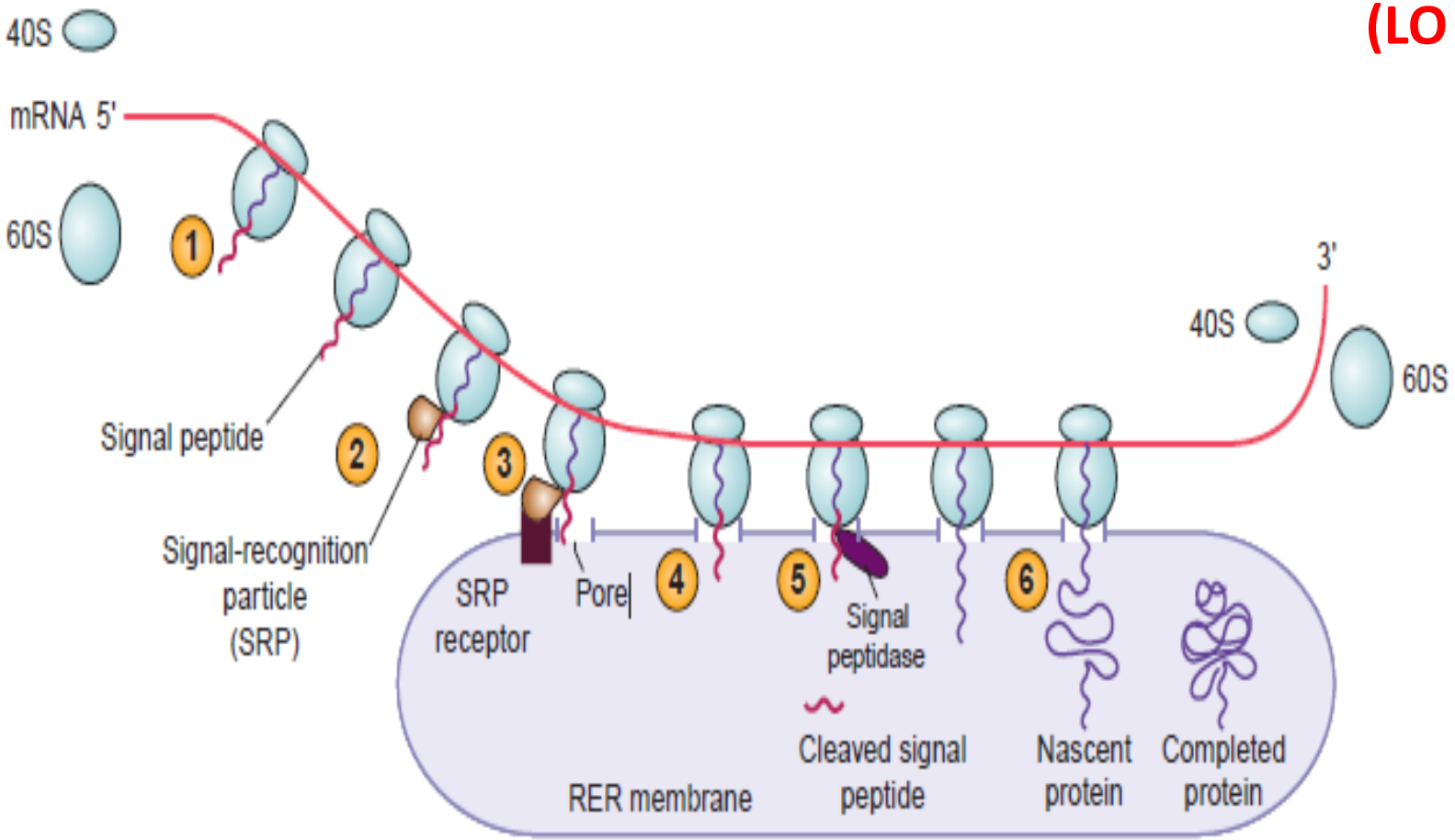
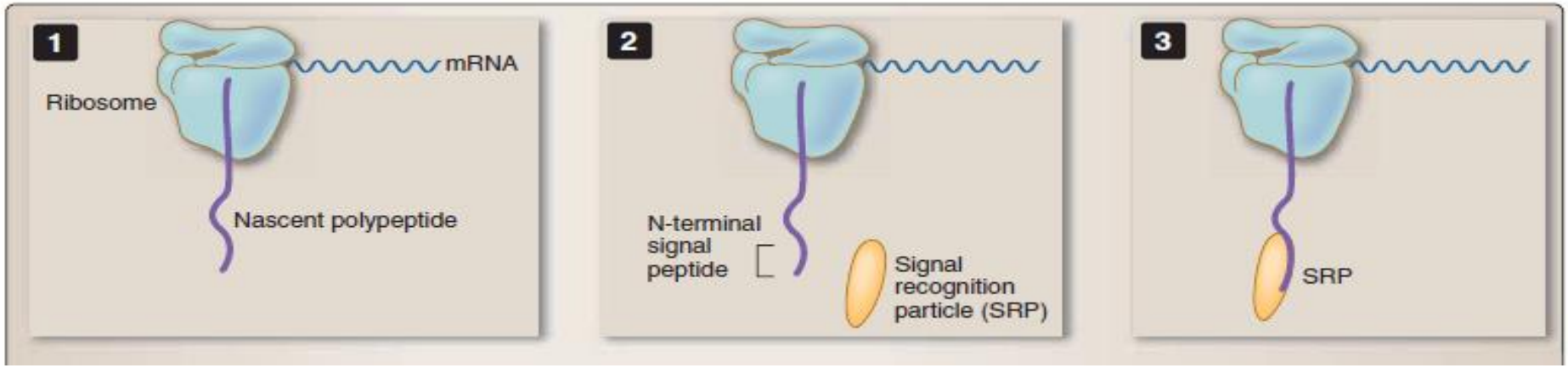
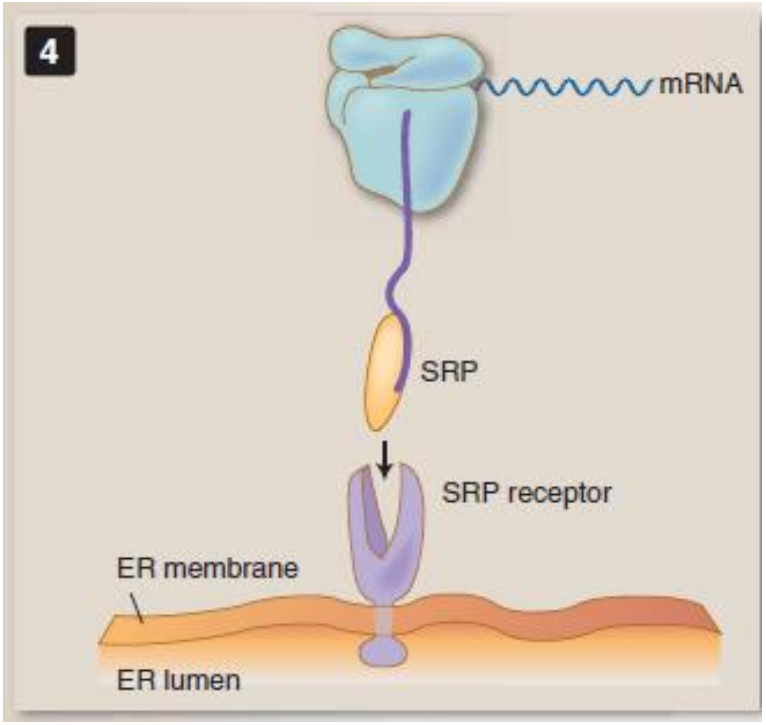
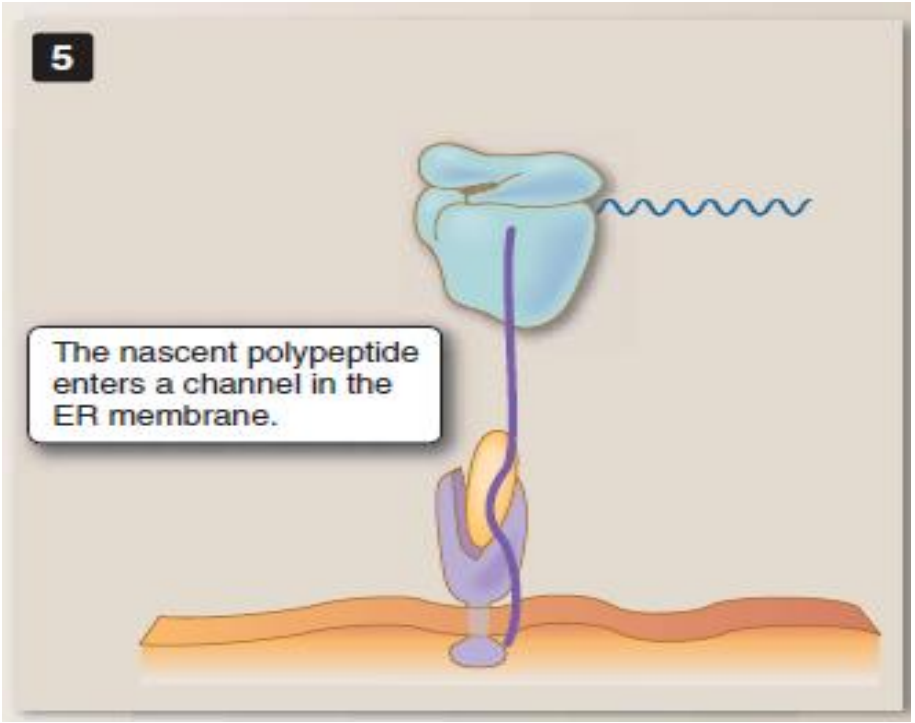


FIG. 15.13. Synthesis of proteins on the RER. *(1)* Translation of the protein begins in the cytosol. *(2)* As the signal peptide emerges from the ribosome, an SRP binds to it and to the ribosome and inhibits further synthesis of the protein. *(3)* The SRP binds to the SRP receptor in the RER membrane, docking the ribosome on the RER. *(4)* The SRP is released and protein synthesis resumes. *(5)* As the signal peptide moves through a pore into the RER, a signal peptidase removes the signal peptide. *(6)* Synthesis of the nascent protein continues, and the completed protein is released into the lumen of the RER.

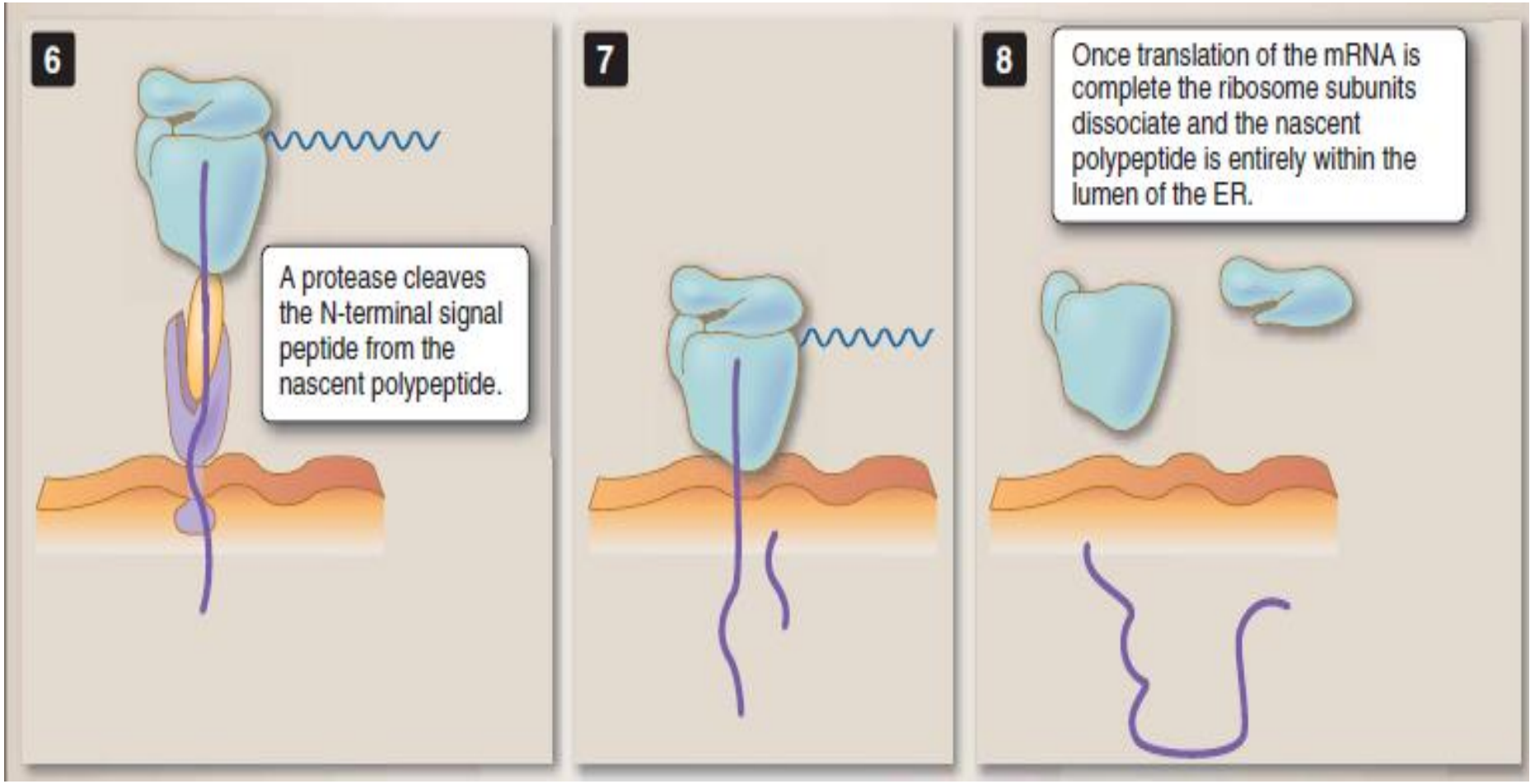
(LO 8.2)


1
Ribosome
mRNA
Nascent polypeptide
2
N-terminal signal peptide
Signal recognition particle (SRP)
3
SRP


4
mRNA
SRP
SRP receptor
ER membrane
ER lumen


5
The nascent polypeptide enters a channel in the ER membrane.

(LO 8.2)

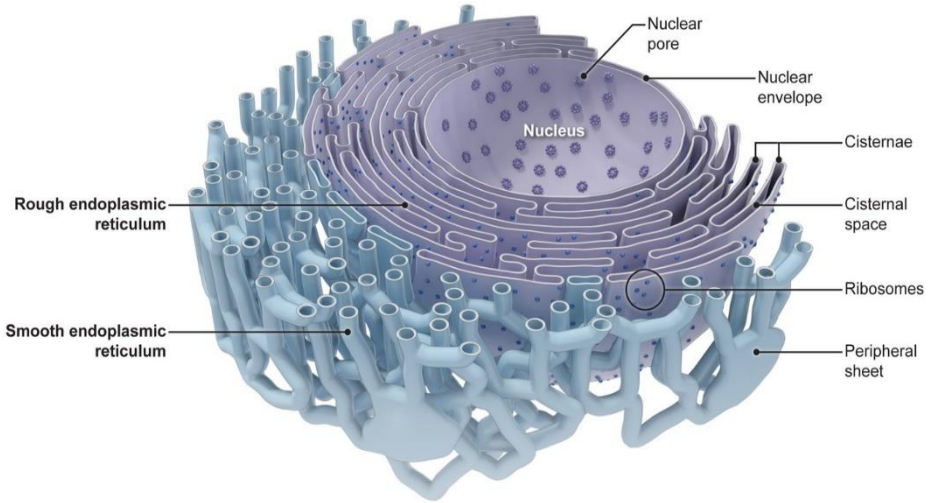

6
A protease cleaves the N-terminal signal peptide from the nascent polypeptide.
7
8
Once translation of the mRNA is complete the ribosome subunits dissociate and the nascent polypeptide is entirely within the lumen of the ER.

Protein modifications which occur in the ER and Golgi complex (LO 8.3)

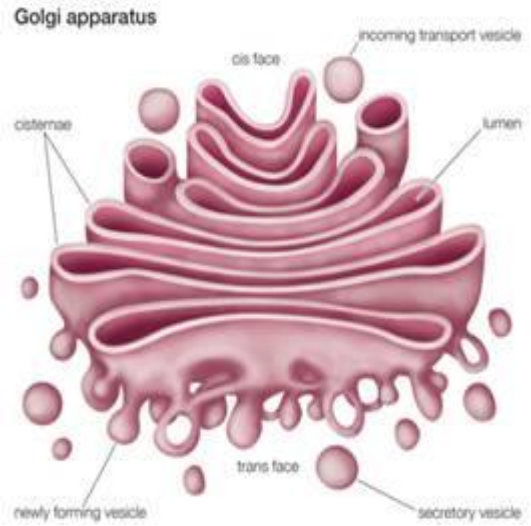
In the ER:

* **N-linked glycosylation.**

***Removal** of the **signal sequence** by the action of proteases, upon entry of the signal sequence into the lumen of the ER.



Protein modifications which occur in the ER and Golgi complex (LO 8.3)



- **In the Golgi:**

- **O-linked glycosylation.**
  - **Sulfation: addition of sulfur.**
  - **Phosphorylation: addition of phosphate.**
  - **Proteolysis: cleavage of peptide bonds (by action of enzymes).**

Distinguish between N-linked and O-linked glycosylation of proteins. (LO 8.4)

Glycosylation: the attachment of carbohydrate groups to proteins via glycosidic linkages.

It is an important and highly regulated mechanism of secondary protein processing within cells.

It play a critical role in determining protein structure, function and stability

Some times is used to target proteins to specific organelles e.g. lysosomal enzymes (mannose-6-phosphate)

Distinguish between N-linked and O-linked glycosylation of proteins. (LO 8.4)

N-linked glycosylation	O-linked glycosylation:
Occurs in ER.	Occurs mainly in Golgi.
Carbohydrate added at the amide group of asparagine .	Carbohydrate added to OH group of serine or threonine.
It is important for the correct folding, secretion and function of protein.	It is important for changing protein stability and regulating its activity.

The role that proteolytic processing plays in the formation of important secreted proteins (LO 8.5)

For some secretory proteins (e.g., growth hormone), removal of the N-terminal signal sequence from the nascent chain is the only known proteolytic cleavage required to convert the polypeptide to the mature, active species.

(LO 8.5)

However, some plasma-membrane and most secretory proteins initially are synthesized as relatively long-lived, inactive precursors, termed *proproteins*, which require further proteolytic processing to generate the mature, active proteins.

Examples

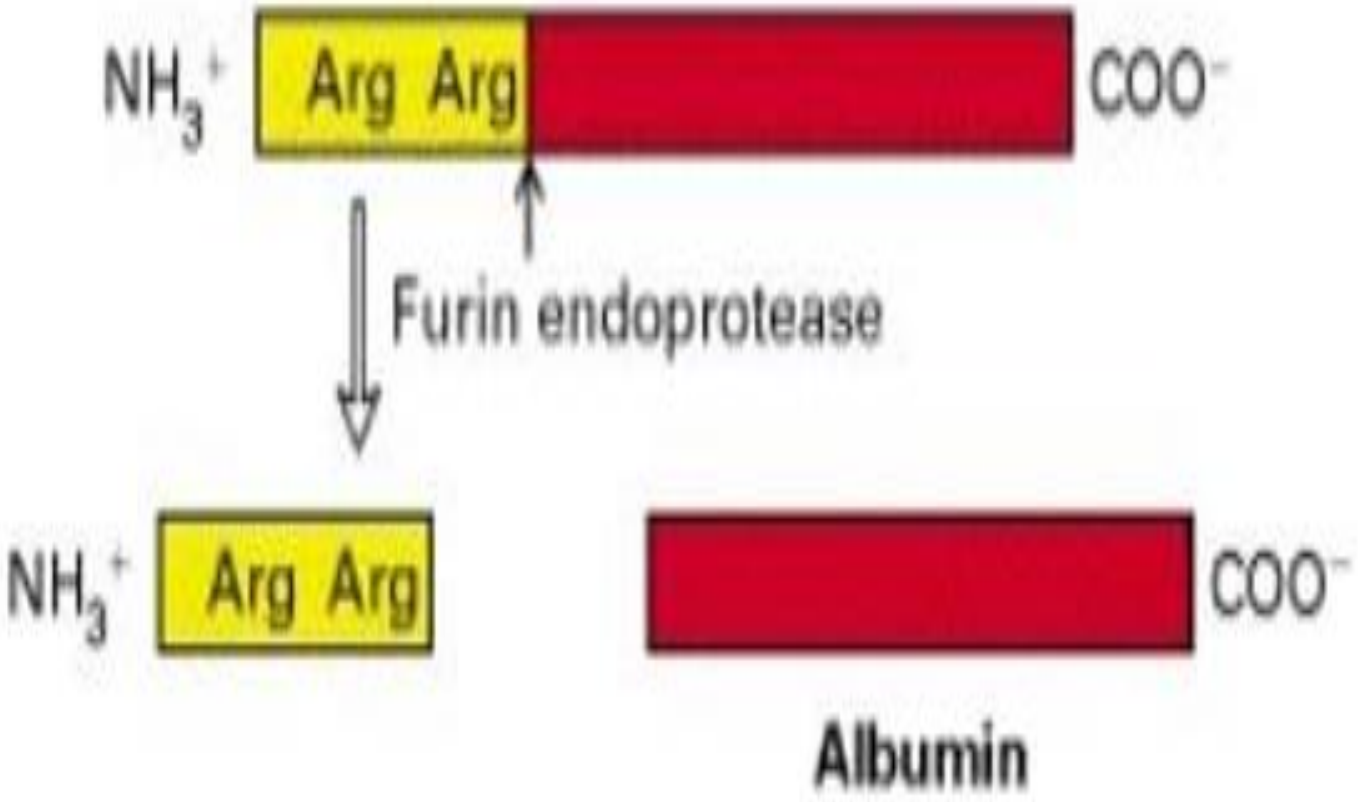
serum albumin, insulin and glucagon,

all of which are secretory proteins.

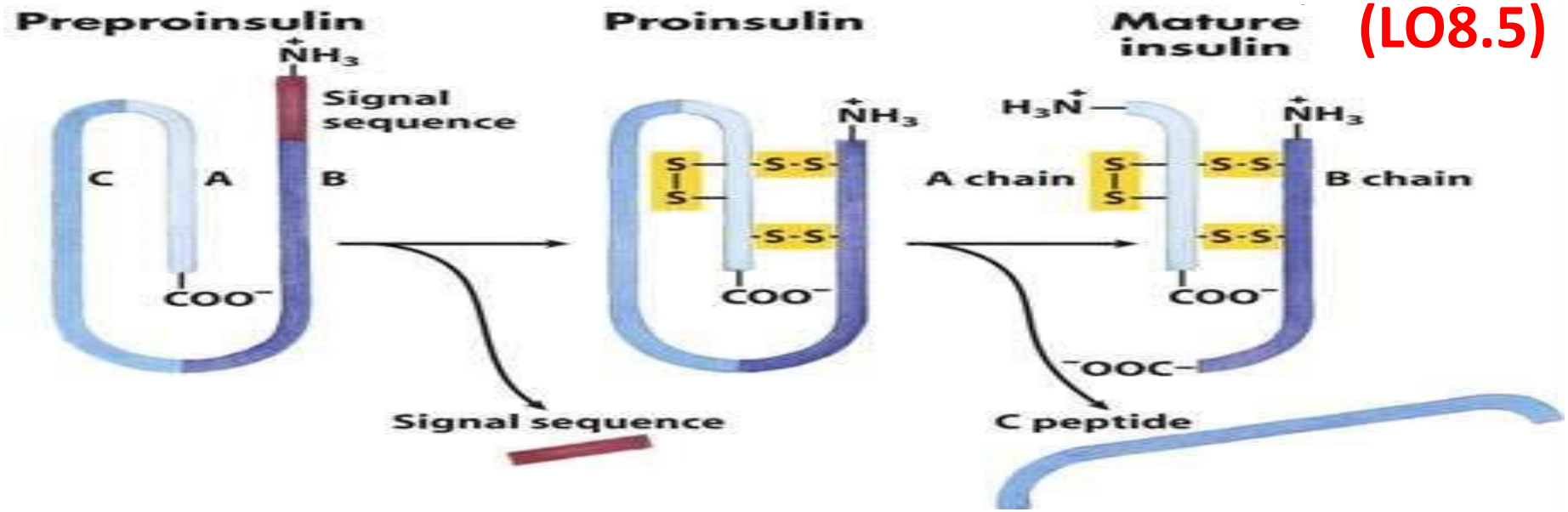
(LO8.5)

Constitutive secretory proteins

Proalbumin



(LO8.5)



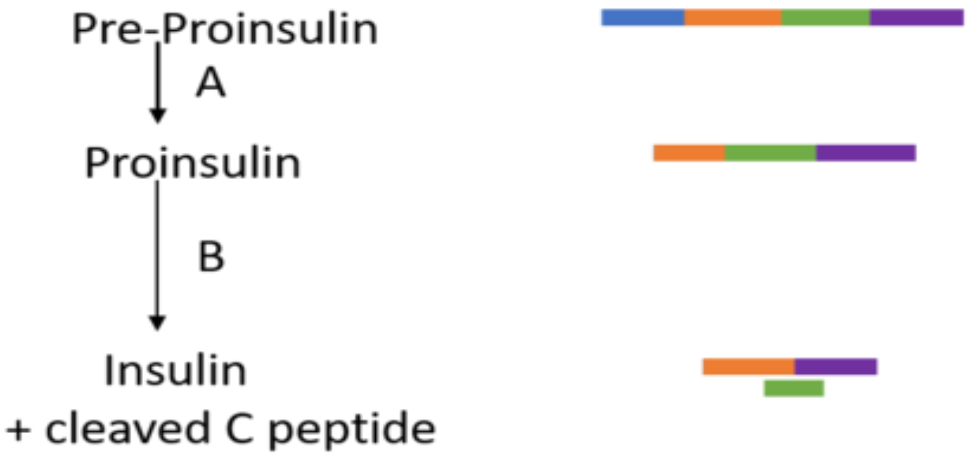


Figure 1: Progression of Insulin-like structures. A. The signal peptide of pre-proinsulin is cleaved, forming proinsulin. B) Proinsulin is folded in the ER, then transported to the Golgi apparatus where the C-peptide is cleaved using type I and type II endoproteases to form free C peptide and mature insulin.

signal peptide
B chain
C peptide
A chain

(LO8.5)

The proteolytic conversion of a proprotein to the corresponding mature protein occurs in secretory vesicles as they move away from the trans- Golgi.

Some proproteins, including proalbumin, are cut once at a site C-terminal to a dibasic recognition sequence such as Arg-Arg or Lys-Arg.

In other proproteins, additional amino acids are cleaved at the N-terminus or at both ends of the proproteins e.g. proinsulin.

The formation of mature insulin molecule. (LO8.6)

⇓

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

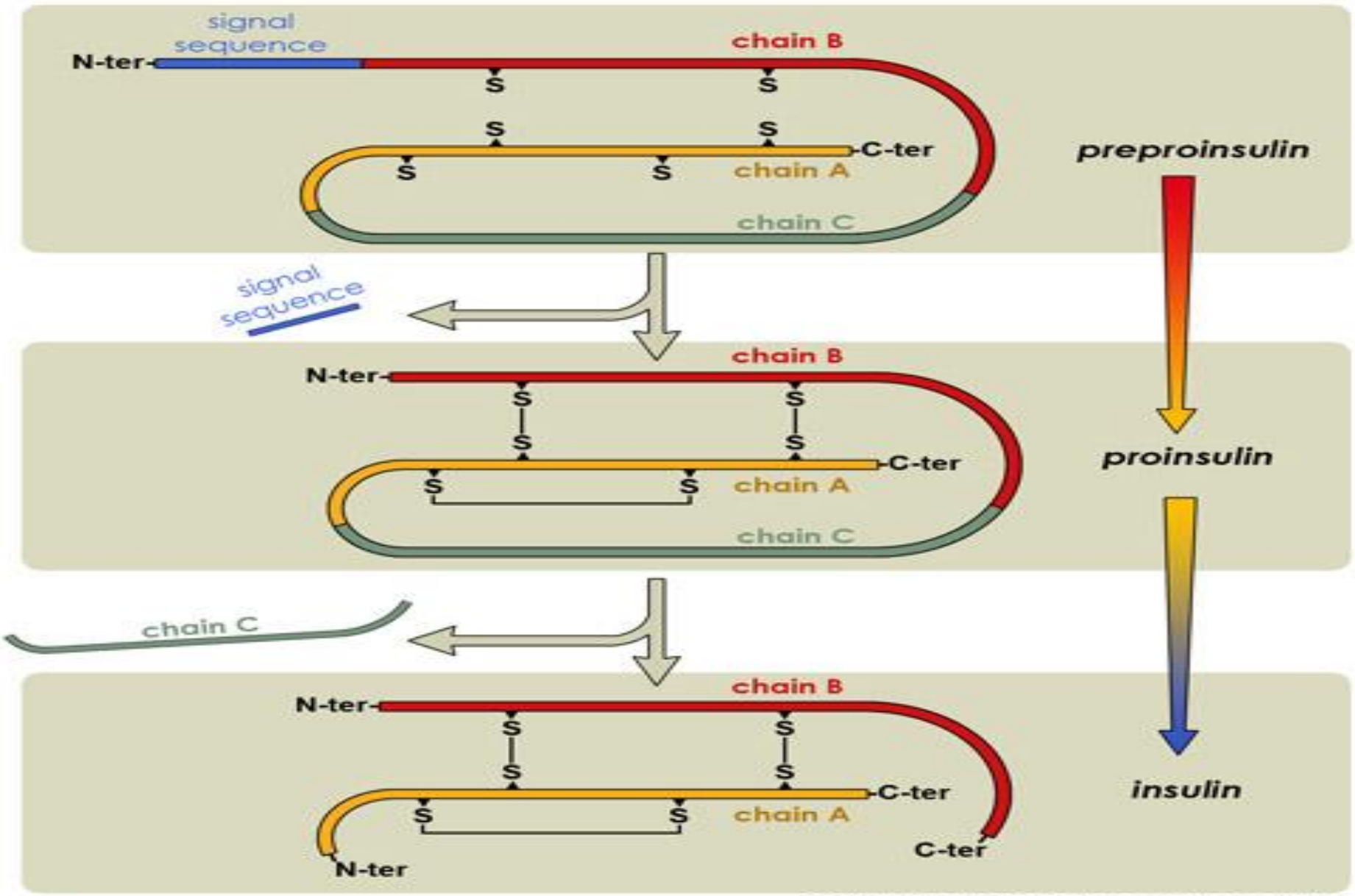
The formation of mature insulin molecule. (LO8.6)

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.


signal sequence
N-ter
chain B
S
C-ter
chain A
chain C
preproinsulin
proinsulin
insulin
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- What are the clinical significant of measurement of C-peptide ?

Thank You