

The Synthetic Machinery of the Cell

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Objectives

- State the characteristics of messenger, ribosomal and transfer RNA
- Distinguish between transcription and translation
- Describe the composition and structure of the nucleolus
- Explain what constitutes the nucleolus organizer region
- Outline the role of ribosomes in protein synthesis
- Discuss how antibiotics inhibit the growth of bacteria and have different sites of action
- List the main sites of protein synthesis
- Identify RER, SER and Golgi apparatus in electron micrographs
- Explain the roles of the RER and Golgi apparatus in protein glycosylation and sorting
- Name the functions of the SER in different cells

Recommended Reading

The World of the Cell: Becker WM, Kleinsmith LJ, Hardin J. 4th Edition

- Chapter 12 Intracellular compartments ...
 - The endoplasmic reticulum
 - The Golgi complex
 - Roles of ER and Golgi complex in protein glycosylation
 - Roles of ER and Golgi complex in protein sorting
- Chapter 19 Gene Expression: I. The Genetic Code and Transcription
- Chapter 20 Gene Expression: II. Protein Synthesis and Sorting

The nucleolus and ribosomes form part of the protein synthesising machinery

- The nucleolus is the site where most of the ribosomal RNA (r-RNA) is transcribed
- Ribosomes are composed of r-RNA and proteins
- The ribosomes are the sites where protein synthesis occurs
- Synthesis of specific proteins (translation) also requires the action of other RNAs:
 - m-RNA as a template
 - t-RNA for the assembly process

The cellular machinery for protein synthesis involves RNA.

RNA (ribose nucleic acid) is a single-stranded nucleic acid composed of four different types of nucleotides adenine(A), uracil (U), Cytosine (C) and guanine (G).

The Cell Has 3 Types of RNA

■ Messenger RNA (m-RNA)

- Is transcribed from DNA in the nucleus
- Carries a coded message to cytoplasm
- There are about 10^5 varieties in humans (corresponding to the number of genes)

■ Ribosomal r-RNA (r-RNA)

- There are 4 varieties
- Correspond to the 4 RNA constituents of ribosomes

■ Transfer t-RNA (t-RNA)

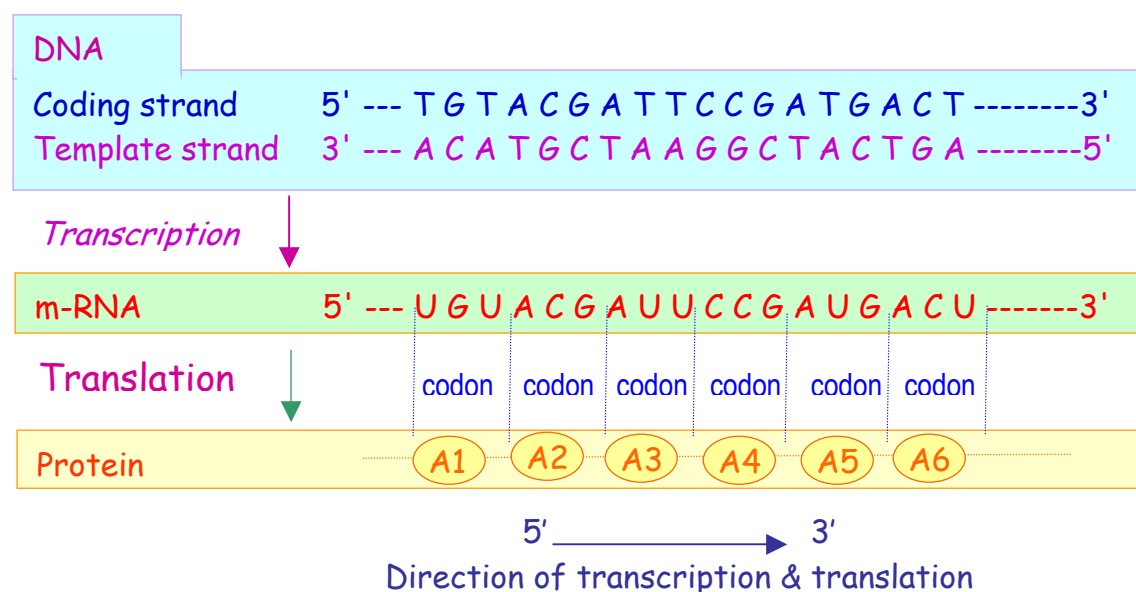
- There are 20 varieties
- Each corresponds to an amino-acid
- Transfers the appropriate amino acid to polypeptide chains

M-RNA is the transcript of a gene

DNA has two strands.

- The **template strand** carries the message, and is transcribed to m-RNA.
- The **coding strand** carries a sequence that is:
 - Complementary to that on the template strand, and
 - Coincides with the sequence on m-RNA, except that T in DNA is replaced by U in RNA.

The following diagram summarizes the process of transcription and translation.



Transcription

- Is the process of **synthesis of m-RNA** using the transcribed strand of DNA as template
- Is the process whereby the genetic message for a specific protein is transcribed on to m-RNA
- Occurs in the nucleus of eukaryotic cells
- Occurs in the 5' to 3' direction (i.e. nucleotides are always added at the 3' end)

Translation

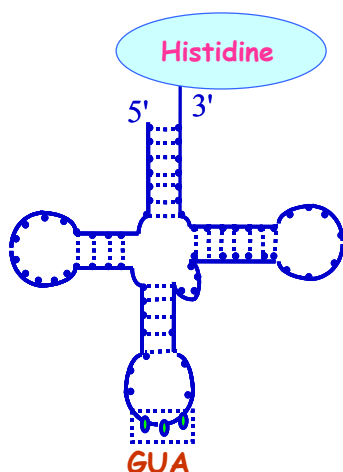
- Is the process of **synthesis of a specific protein** using m-RNA as template.
- Occurs in the ribosomes in the cytoplasm
- The message is read in codons (triplets of nucleotides) in the 5' to 3' direction

- Each codon is a triplet of bases on m-RNA corresponding to an amino acid according to the genetic code

THE GENETIC CODE

	U		C		A		G	
U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys
	UUC	Phe	UCC	Ser	UAC	Tyr	UGC	Cys
	UUA	Phe	UCA	Ser	UAA	Stop	UGA	Stop
	UUG	Leu	UCG	Ser	UAG	Stop	UGG	Trp
C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg
	CUC	Leu	CCC	Pro	CAC	His	CGC	Arg
	CUA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
	CUG	Leu	CCG	Pro	CAG	Gln	CGG	Arg
A	AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser
	AUC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
	AUA	Ile	ACA	Thr	AAA	Lys	AGA	Arg
	AUG	Met Start	ACG	Thr	AAG	Lys	AGG	Arg
G	GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly
	GUC	Val	GCC	Ala	GAC	Asp	GGC	Gly
	GUA	Val	GCA	Ala	GAA	Glu	GGA	Gly
	GUG	Val	GCG	Ala	GAG	Glu	GGG	Gly
	U		C		A		G	

- The genetic code specifies the possible codons and the amino acids corresponding to each codon.
- By convention, the genetic code is interpreted with reference to the sequence of bases on m-RNA.
- In the genetic code there are four bases - A, U, C, G; 64 possible codons ($=4^4$); and 20 amino acids
- The genetic code is **degenerate** i.e. One amino acid may be represented by more than one codon
- Codon AUG codes for methionine but at the beginning of a gene also serves as a start signal for translation.
- Codons UAA, UAG and UGA are "stop" signals for translation.



Transfer RNA

- t- RNA consists of RNA folded in the form of a cross
- It has an anti-codon at one end (a triplet complementary to a codon sequence)
- It has the corresponding amino-acid attached to the opposite end
- The example in the adjacent figure is of t- RNA for histidine, which is attached to one end, and has the anticodon GUA

(complementary to the codon CAU) at the other end.

Ribosomal RNA (r-RNA)

- Is involved, together with proteins, in the assembly of ribosomes,
- There are 4 types of r-RNA referred to as **5S, 5.8S, 18S, 28S** (indicating the sedimentation coefficient in Svedberg units)
- Is transcribed from **multiple copies** of DNA (unlike m-RNA transcribed from a unique gene)
- Is synthesised in the nucleolus

The Nucleolus

Consists of two parts:

- A fibrillar part consisting of chromatin - DNA transcribing r-RNA
- A granular part consists of ribonucleoprotein particles (r-RNA + proteins)

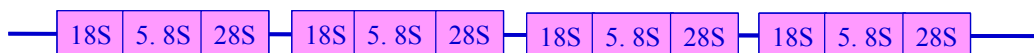
The nucleolar DNA

- The genes for 5.8S, 18S and 28S r-RNA form a gene cluster
- They are all transcribed together forming a r-RNA complex of 45S
- These genes are present on the **nucleolus organiser regions** on the satellites of the acrocentric chromosomes 13, 14, 15, 21 and 22

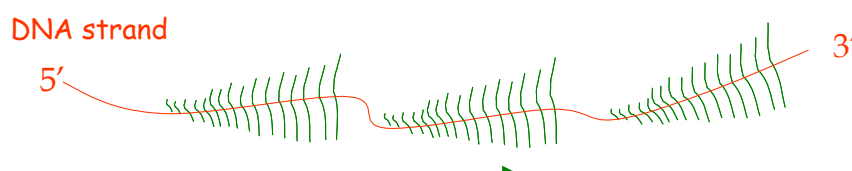


The gene for 5S r-RNA is located on chromosome 1 and is transcribed separately.

The nucleolus organiser regions contain many repeated copies of the r-RNA gene cluster. This is an example of gene amplification.



The multiple copies of r-RNA are transcribed simultaneously. As they are transcribed from the 5' moving towards the 3' end, the growing chain of the r-RNA complex gives rise to form a "feather" arrangement.



Ribosome structure

Ribosome structure has been extensively studied using isolated ribosomes. The ribosomes are separated from the RER by using mild detergents and isolated using differential centrifugation.

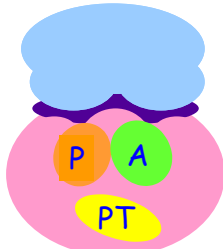
Eukaryotic ribosomes have a sedimentation coefficient of 80 S. Further treatment of the ribosomes with a salt solution containing a low concentration of $[Mg^{++}]$ ions splits them into two subunits with the following composition:

- 18S r-RNA
33 proteins

5S, 5.8S, 28S
r-RNA
49 proteins

1. The **small subunit** has a sedimentation coefficient of 40S and consists of 18S r-RNA together with 33 different proteins.
 2. The **large subunit** has a sedimentation coefficient of 60S consists of 5S, 5.8S and 28S r-RNA together with about 49 different proteins.

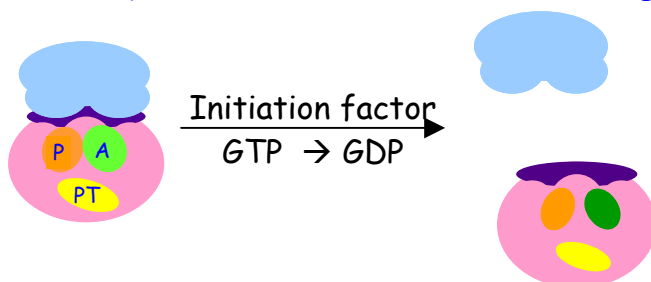
Ribosomes have **four binding sites**, important for their function in protein synthesis:

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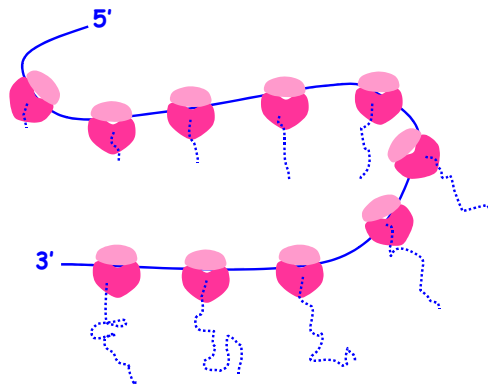
1. Groove for binding of m-RNA
 2. Amino-acyl site (A) - for binding to t- RNA
 3. Peptidyl transferase site (PT) - for binding a newly added amino acid by peptide bonds
 4. Peptidyl site (P) - for the growing polypeptide chain

Steps In Protein Synthesis

1. Initiation The ribosome dissociates into its small and large subunits. This requires an initiation factor and energy derived from GTP



Polyribosomes.



Frequently, one m-RNA runs successively through several ribosomes producing multiple copies of a protein as shown in this diagram. The longest protein chains are close to the 3' end of the m-RNA

Clinical Applications

Many Antibiotics Inhibit Protein Synthesis in Bacteria

Prokaryotic (bacterial) ribosomes are similar in general structure and function to eukaryotic ribosomes, but have a sedimentation coefficient of 70S and consist of 30S and 50S subunits.

The following are some examples of antibiotics that inhibit protein synthesis in bacteria. They could be bacteriostatic or bactericidal.

- **Streptomycin** binds to 30S subunit and inhibits binding of t-RNA
- **Tetracycline** inhibits binding of t-RNA to A-site
- **Erythromycin** binds to 50S subunit and prevents ribosome assembly

Some antibiotics act at different stages of protein synthesis

- Fusidic acid inhibits the attachment of t-RNA to A-site
- Puromycin is a molecular analogue of t-RNA and causes premature chain termination
- Chloramphenicol and Lincomycin inhibit peptidyl transferase
- Thioseptrin prevents translocation from A to P site

Why do antibiotics inhibit protein synthesis in bacteria but not in humans?

- Many antibiotics are specific for prokaryotic (bacterial) ribosomes (70S).
- Prokaryotic ribosomes (70S) have a different protein composition from eukaryotic (80S) ribosomes.
- The bacterial cell wall is permeable to some antibiotics while the plasma membrane of eukaryotic cells is not

Some protein synthesis inhibitors also act on eukaryotic cells

- Antibiotics that inhibit protein synthesis in human cells are useful as cytostatic agents e.g. cycloheximide
- They are lethal to cells that are actively dividing

The Cell's Export Industry

Ribosomes attached to RER produce proteins for use outside the cell

- Secretion into the extracellular space e.g. collagen

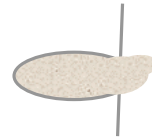


- Secretion into the bloodstream

e.g. plasma proteins, immunoglobulins, hormones



- Secretion into a secretory duct - exocrine secretions



- Secretion into lysosomes - acid hydrolase



Free ribosomes produce proteins for use within the cell

- In dividing cells
- In haemopoietic tissue
- In gamete formation - oocyte
- In the zygote
- In embryonic cells

Rough Endoplasmic Reticulum is usually present as stacks of flattened lamellae.



Distinguish between the cytoplasmic and cisternal faces of the RER
Ribosomes indicate the cytoplasmic face
Cisternae appear as clear spaces containing proteins

Rough endoplasmic reticulum is found in abundance in cells that actively secrete proteins for export outside the cell. The following are some examples:

- Protein secreting glands - pancreas, parotid gland etc.
- Antibody secreting cells - plasma cells
- Fibroblasts - collagen synthesis
- Neurons - for production of neurotransmitter and membrane synthesis

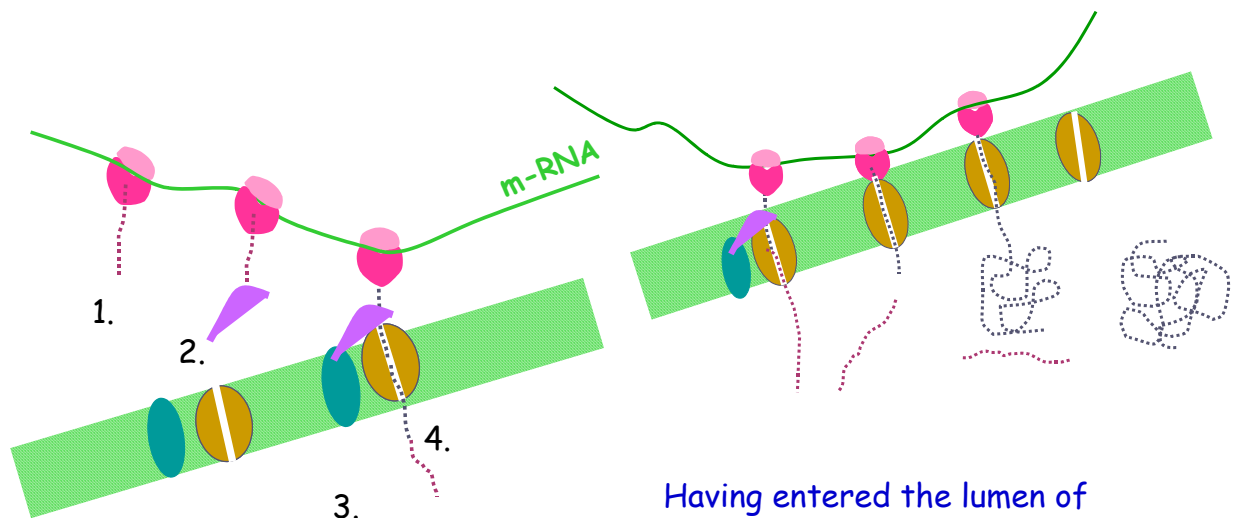
Proteins for export are synthesized on ribosomes attached to the RER

Synthesis of secretory proteins begins in free ribosomes, which then become attached to the RER.

Proteins destined for export have a signal protein that guides the ribosome to the RER.

Attachment of ribosomes to the RER involves a number of intermediate molecules:

1. A **signal peptide** - the first part of the protein serves as a signal for the ribosome to attach to the RER
2. A **signal recognition particle** (SRP) present in the cytosol combines with the signal peptide.
3. A **docking protein** present in the membrane attaches to the SRP and brings it close to the membrane of the RER
4. **Ribophorin** is a membrane protein channel for passage of the protein. The SRP guides the signal protein to the ribophorin.



Having entered the lumen of the RER, the signal protein is cleaved off and protein synthesis continues.

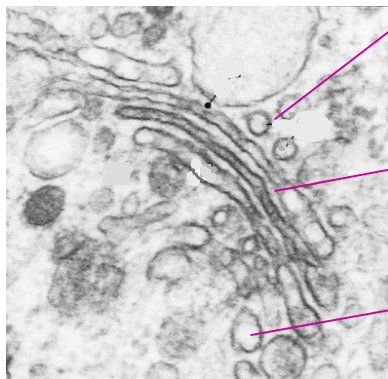
Functions of the Golgi Apparatus

- Packaging of secretory materials
- Processing of proteins - **glycosylation, phosphorylation, sulphation**
- Synthesis of polysaccharides and glycolipids
- Sorting of proteins for several destinations
- Formation of new membranes e.g. plasma membrane

The Golgi complex consists of:

- **Cisternae** - stacks of 3 to 8 flat, disc-shaped, membrane-bound sacs
- Vesicles of variable size surrounding the cisternae

The Golgi Complex has two faces and a middle compartment:



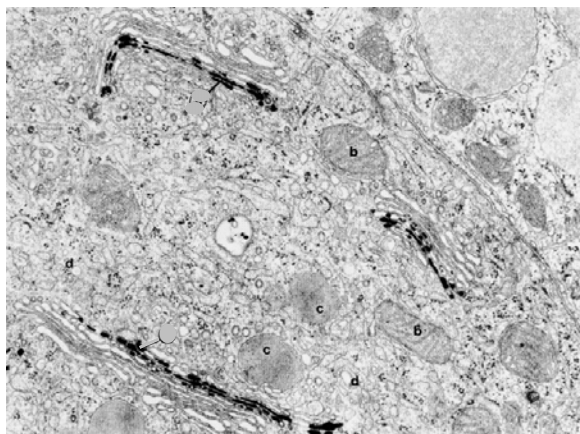
Cis face - (forming face) where vesicles from RER and SER merge into Golgi apparatus

Medial cisternae - the transition between the cis and trans faces, where most of the processing of proteins occurs

Trans face - (maturing face) where vesicles bud out from the Golgi apparatus to their various destinations

The vesicles are transport vesicles transporting contents from:

- The RER and SER to the Golgi
- One Golgi cisterna to the next
- The trans face to their various destinations.



The Golgi complex contains different enzymes in different compartments. Some of these enzymes can be stained cytochemically or used as identification markers. In this electron micrograph the **trans face** has been stained for thiamine pyrophosphatase.

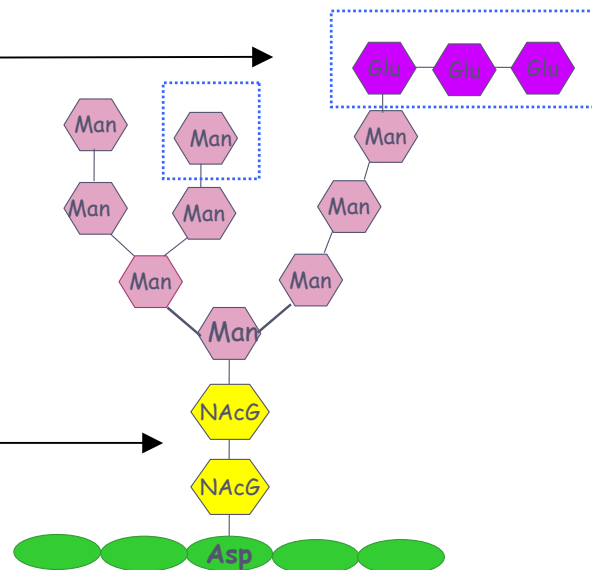
Proteins undergo post-transcriptional modification in the RER and Golgi complex.

Most of the post-transcriptional modification involves glycosylation, the addition of carbohydrate side-chains, forming glycoprotein. This occurs in two steps:

1. **Core glycosylation** is the initial glycosylation is the same in all proteins. It involves the addition of branched oligosaccharide chains of 14 sugar units consisting of:

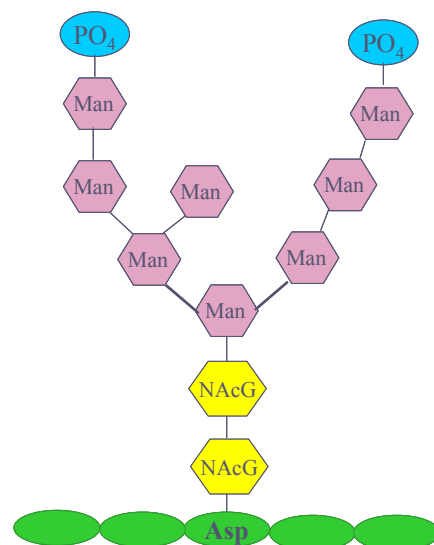
- 3 terminal glucose units
 - 9 mannose units
 - 2 N-acetylglucosamine attached to the asparagine residues of the protein.
- This process is also termed **N- glycosylation**, and occurs in the lumen of the RER.

This is followed by pruning of the oligosaccharide chain involving the removal of the 3 glucose and one mannose units (shown boxed in the diagram) by the enzymes **glucosidase** and **mannosidase** respectively. This process occurs before the protein moves out of the RER.



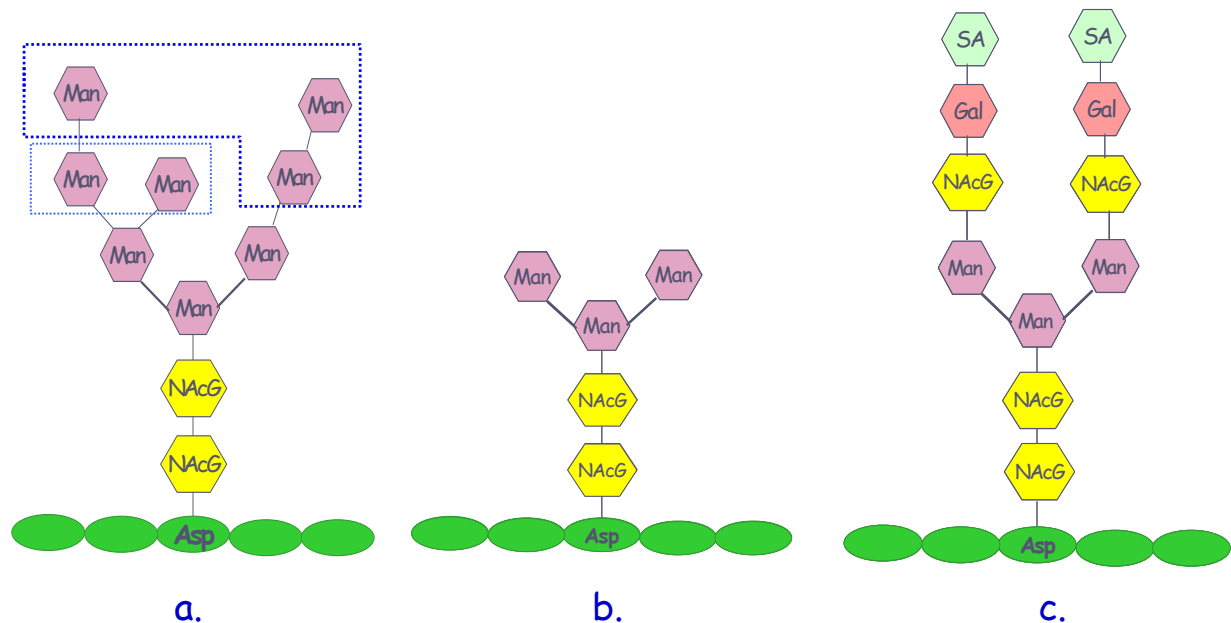
2. **Terminal glycosylation** occurs in the Golgi complex as the proteins move from the cis face, through the medial cisternae to the trans face. It involves modifications of the initial glycoproteins to serve as signals for directing the proteins to their final destinations.

Proteins destined for lysosomes receive two terminal PO_4 groups (as shown in the adjacent diagram), which constitute the signal for packaging in primary lysosomes.



For other destinations, modification usually involves further pruning of the mannosidase branches (shown boxed in diagram a) by mannosidase I and mannosidase II.

Further modification involves the addition of various carbohydrate units, including N-acetylglucosamine, galactose, sialic acid and sometimes fucose, generating complex oligosaccharides (c).



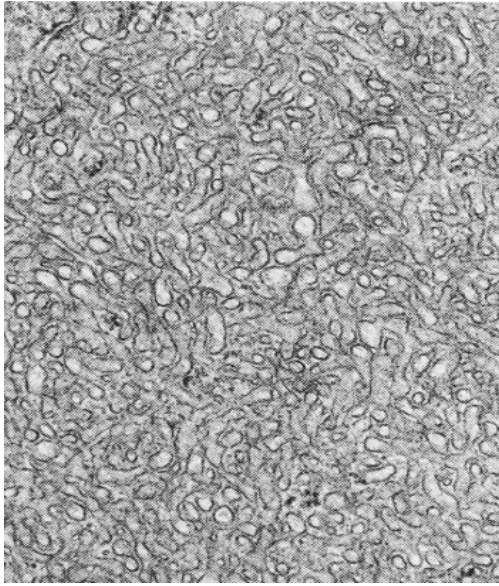
The Golgi complex may also receive vesicles from the smooth endoplasmic reticulum.

Lipids formed in the SER are transferred to the Golgi complex to synthesise lipoproteins.

Sorting and packaging of proteins occurs in the trans face of the Golgi Complex.

- The sorting depends on the tag that has been attached to the protein.
- The tag may be an oligosachharide, a side-group, lipid component or a specific amino acid sequence.
- The tags are necessary for the final packaging in specific vesicles, according to their destination e.g. lysosomes or secretory vesicles.
- They also help to exclude the intrinsic enzymes of the different Golgi compartments from being enclosed in vesicles.

Smooth Endoplasmic Reticulum (SER)



SER is structurally & functionally different from RER

- SER consists of branched tubules and lacks ribosomes.
- The enzymes contained in the lumen and membranes of the SER are different from those of RER reflecting their different functions

Functions of Smooth Endoplasmic Reticulum

1. Lipid synthesis:

- a. Phospholipids for membranes
- b. Adrenal cortical hormones
- c. Sex hormones

2. Carbohydrate metabolism:

- a. Glycogen granules lie in close proximity to SER
- b. Glycogenolysis - Glucose 6-phosphate dehydrogenase is present in the SER membrane

3. Detoxification of metabolites or extraneous chemicals. This may occur by:

- a. conjugation
- b. hydroxylation
- c. methylation
- d. phosphorylation

This function occurs primarily in liver cells:

- Detoxification of drugs and chemicals
- Conjugation of bilirubin

3. A reservoir of Ca^{2+} ions

- This occurs mainly in skeletal muscle cells:

Ca^{2+} pumps in the SER membrane pump ions into the SER lumen;

Ca^{2+} channels in the SER membrane regulate the flow of ions into the cytosol

SER occurs in abundance in steroid - secreting cells. They have three distinctive cytoplasmic features:

- a) Abundant SER
- b) Mitochondria with tubular cristae
- c) Lipid droplets

In these cells part of the biosynthesis of the steroid hormones occurs in the mitochondrial cristae, which have a similar appearance to the SER tubules.

Steroid-secreting cell include:

- a) Adrenal cortical cells - secrete cortisol
- b) Interstitial cells of the testes - secrete testosterone
- c) Corpus Luteum - secretes progesterone

Clinical Applications

Barbiturates are a group of drugs that were used for the treatment of insomnia, as short-acting anaesthetics and for the treatment of epilepsy. Their use is now restricted because they induce dependence and have several undesirable side effects. Prolonged use of barbiturates induced proliferation of the SER in the liver and consequent impairment of liver function. Barbiturates were used extensively in experimental animals for the study of SER.

Barbiturates happens to have an unusually marked effect in inducing SER proliferation, but most medications including antibiotics and drugs, are broken down in the liver. Most drugs are administered in divided doses two or three times a day because of their rate of inactivation in the liver. Conversely, most medications have to be administered with caution and in reduced dosage in patients who have liver damage or impairment because the drug remains active for a long time.