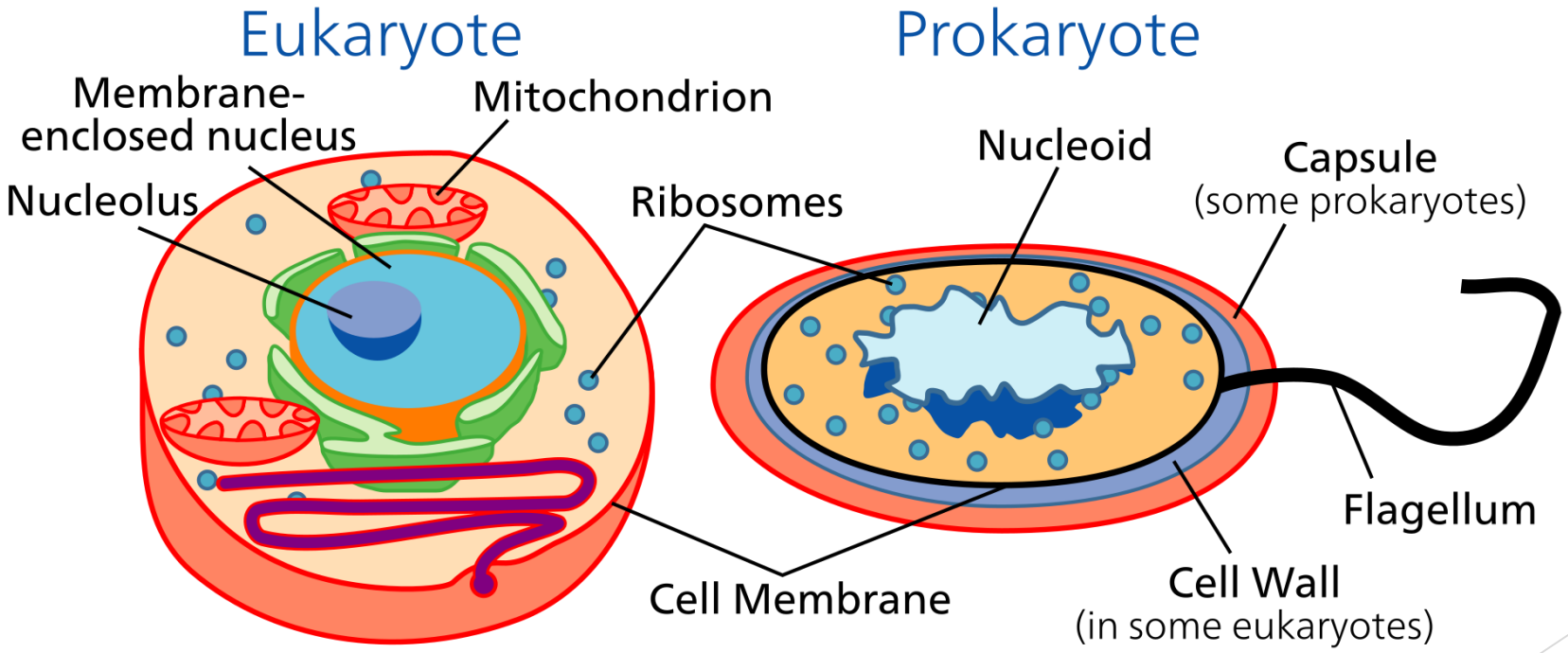
5TH SEM MAJOR

ASSEMBLY OF MACROMOLECULAR COMPLEXES…PLASMA MEMBRANE, RIBOSOME, CHROMATIN

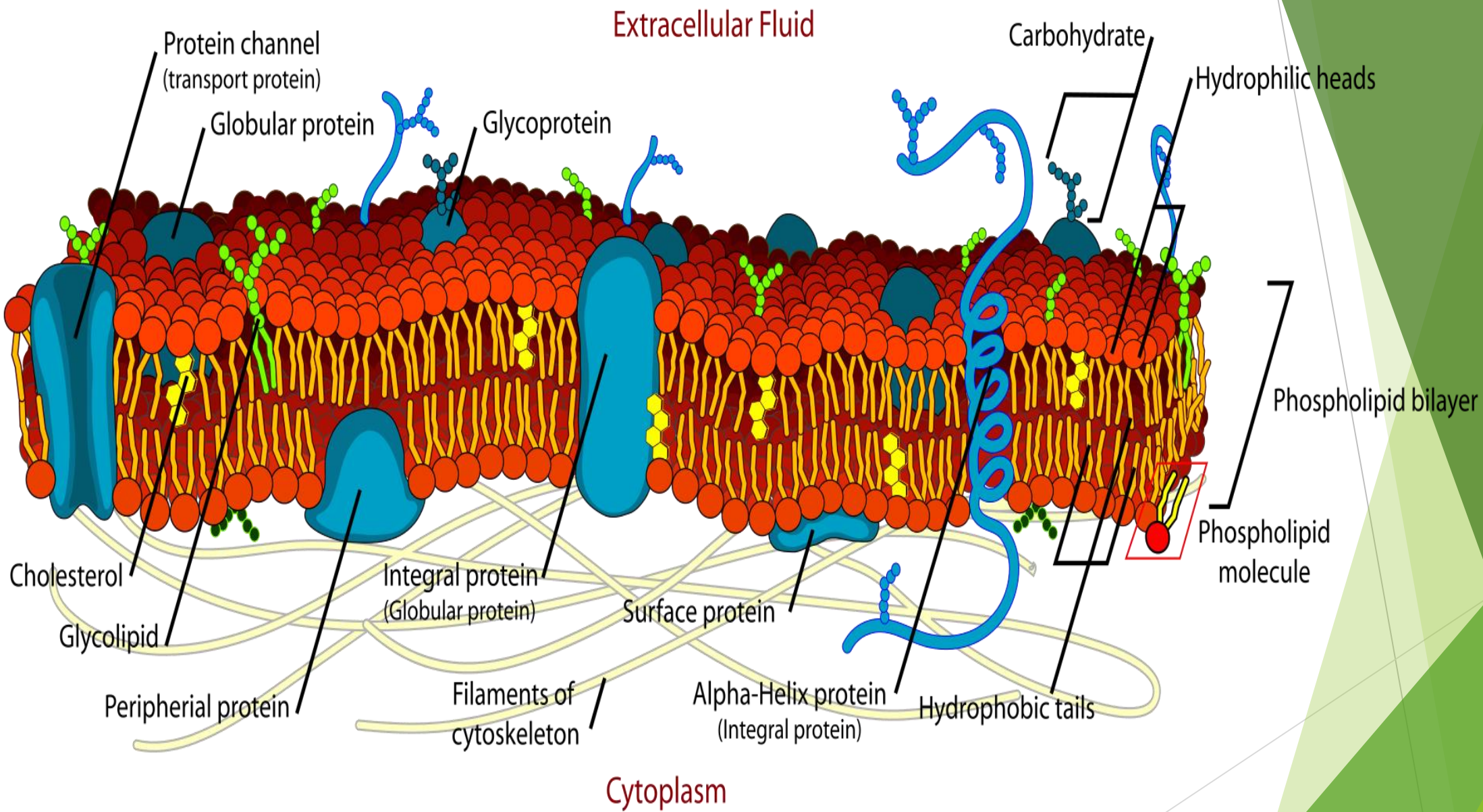
DR. LUNA PHUKAN

Cell membrane or Plasma membrane

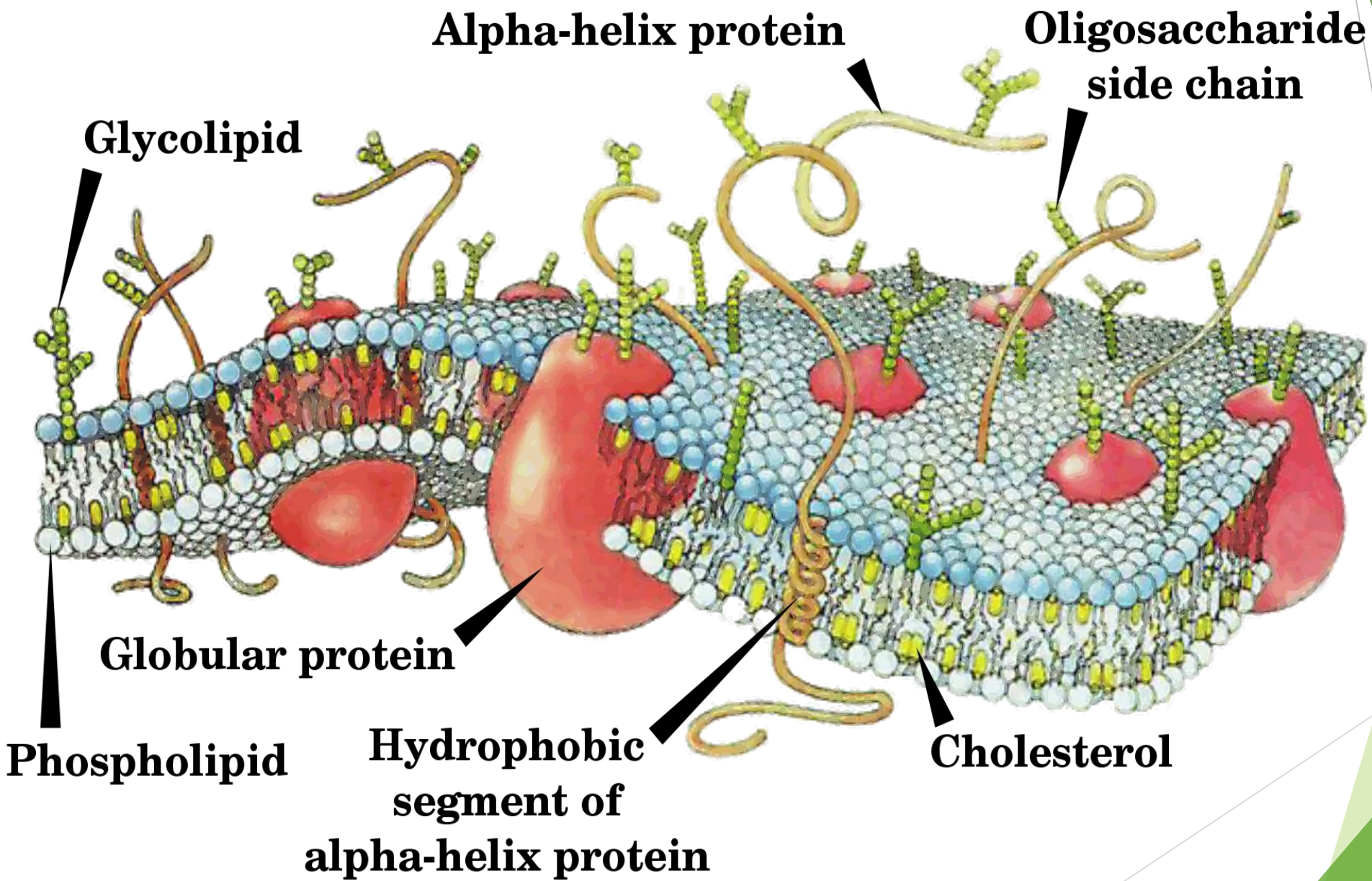
The cell membrane (also known as the plasma membrane, or cytoplasmic membrane, and historically referred to as the plasmalemma) is the semipermeable membrane of a cell that surrounds and encloses its contents of cytoplasm and nucleoplasm. The cell membrane separates the cell from the surrounding interstitial fluid the main component of the extracellular fluid



The cell membrane consists of a lipid bilayer, including cholesterols (a lipid component) that sit between phospholipids to maintain their fluidity at various temperatures. The membrane also contains membrane proteins, including integral proteins that go across the membrane serving as membrane transporters, and peripheral proteins that loosely attach to the outer (peripheral) side of the cell membrane, acting as enzymes shaping the cell. The cell membrane controls the movement of substances in and out of cells and organelles. In this way, it is selectively permeable to ions and organic molecules. In addition, cell membranes are involved in a variety of cellular processes such as cell adhesion, ion conductivity and cell signalling and serve as the attachment surface for several extracellular structures, including the cell wall, the carbohydrate layer called the glycocalyx, and the intracellular network of protein fibers called the cytoskeleton. In the field of synthetic biology, cell membranes can be artificially reassembled



A detailed diagram of the cell membrane


Alpha-helix protein
Oligosaccharide side chain
Glycolipid
Globular protein
Phospholipid
Hydrophobic segment of alpha-helix protein
Cholesterol

Composition

- Cell membranes contain a variety of biological molecules, notably lipids and proteins. Composition is not set, but constantly changing for fluidity and changes in the environment, even fluctuating during different stages of cell development. Specifically, the amount of cholesterol in human primary neuron cell membrane changes, and this change in composition affects fluidity throughout development stages

- Material is incorporated into the membrane, or deleted from it, by a variety of mechanisms:

Fusion of intracellular vesicles with the membrane (exocytosis) not only excretes the contents of the vesicle but also incorporates the vesicle membrane's components into the cell membrane. The membrane may form blebs around extracellular material that pinch off to become vesicles (endocytosis).

If a membrane is continuous with a tubular structure made of membrane material, then material from the tube can be drawn into the membrane continuously.

Although the concentration of membrane components in the aqueous phase is low (stable membrane components have low solubility in water), there is an exchange of molecules between the lipid and aqueous phases.

Lipids

Examples of the major membrane phospholipids and glycolipids: phosphatidylcholine (PtdCho), phosphatidylethanolamine (PtdEtn), phosphatidylinositol (PtdIns), phosphatidylserine (PtdSer).

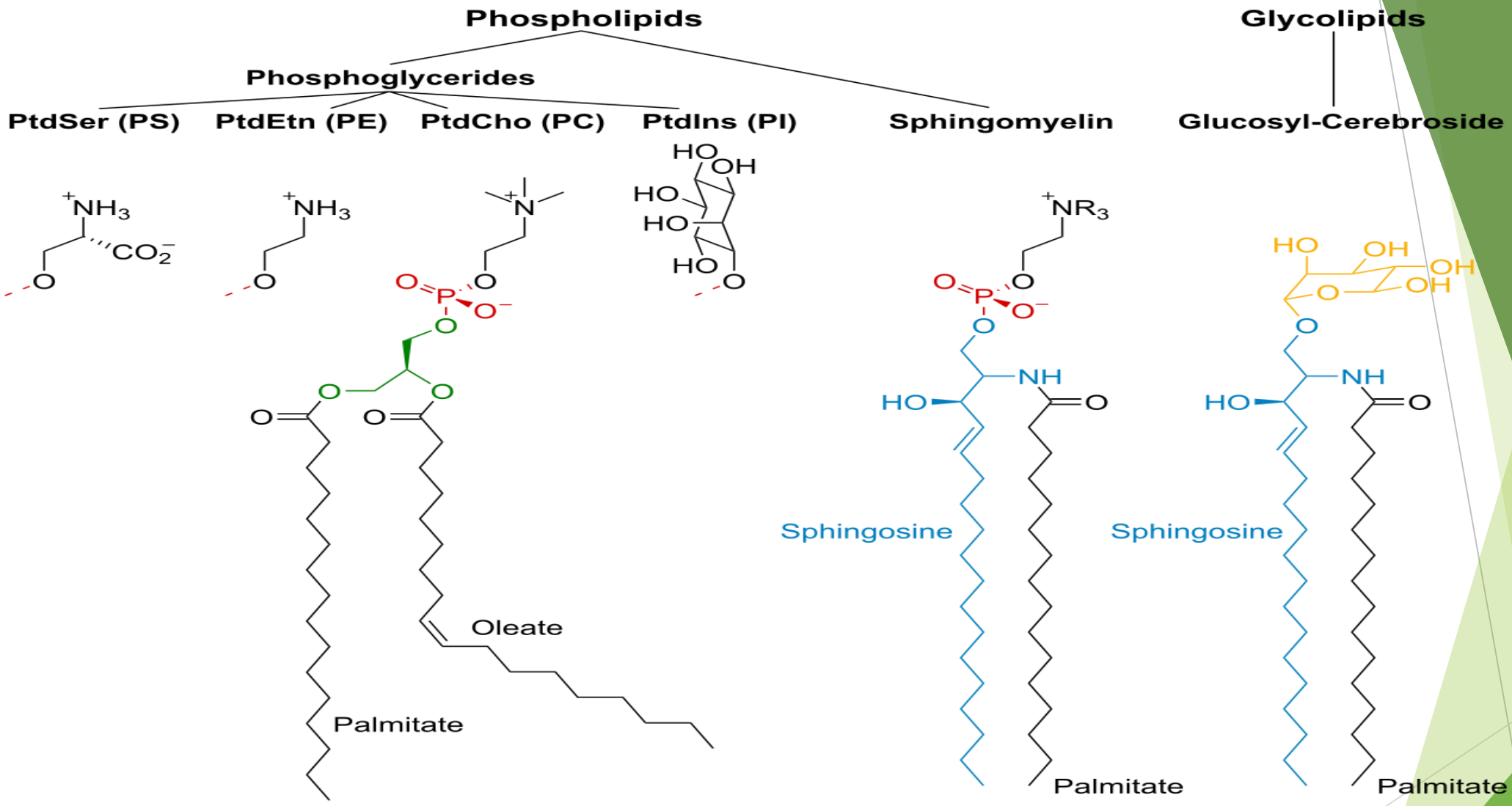
The cell membrane consists of three classes of amphipathic lipids: phospholipids, glycolipids, and sterols. The amount of each depends upon the type of cell, but in the majority of cases phospholipids are the most abundant, often contributing for over 50% of all lipids in plasma membranes. Glycolipids only account for a minute amount of about 2% and sterols make up the rest. In RBC studies, 30% of the plasma membrane is lipid. However, for the majority of eukaryotic cells, the composition of plasma membranes is about half lipids and half proteins by weight.

Phospholipids forming lipid vesicles

Lipid vesicles or liposomes are approximately spherical pockets that are enclosed by a lipid bilayer.These structures are used in laboratories to study the effects of chemicals in cells by delivering these chemicals directly to the cell, as well as getting more insight into cell membrane permeability. Lipid vesicles and liposomes are formed by first suspending a lipid in an aqueous solution then agitating the mixture through sonication, resulting in a vesicle. By measuring the rate of efflux from that of the inside of the vesicle to the ambient solution, allows researcher to better understand membrane permeability. Vesicles can be formed with molecules and ions inside the vesicle by forming the vesicle with the desired molecule or ion present in the solution. Proteins can also be embedded into the membrane through solubilizing the desired proteins in the presence of detergents and attaching them to the phospholipids in which the liposome is formed. These provide researchers with a tool to examine various membrane protein functions.

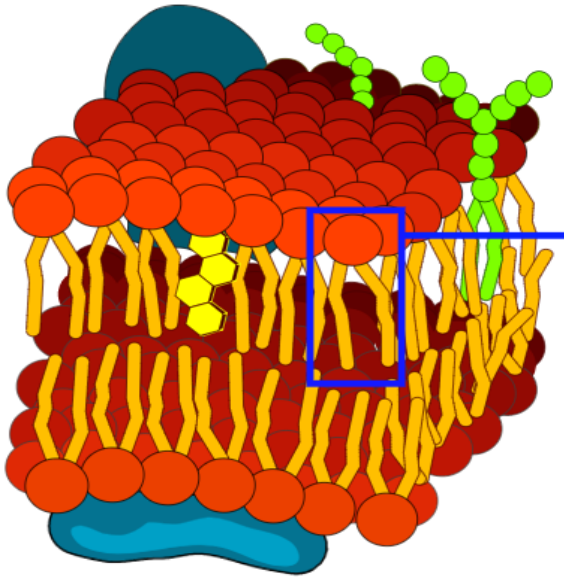
Carbohydrates

Plasma membranes also contain carbohydrates, predominantly glycoproteins, but with some glycolipids (cerebrosides and gangliosides). Carbohydrates are important in the role of cell-cell recognition in eukaryotes; they are located on the surface of the cell where they recognize host cells and share information, viruses that bind to cells using these receptors cause an infection [24] For the most part, no glycosylation occurs on membranes within the cell; rather generally glycosylation occurs on the extracellular surface of the plasma membrane. The glycocalyx is an important feature in all cells, especially epithelia with microvilli. Recent data suggest the glycocalyx participates in cell adhesion, lymphocyte homing,[24] and many others. The penultimate sugar is galactose and the terminal sugar is sialic acid, as the sugar backbone is modified in the Golgi apparatus. Sialic acid carries a negative charge, providing an external barrier to charged particles.

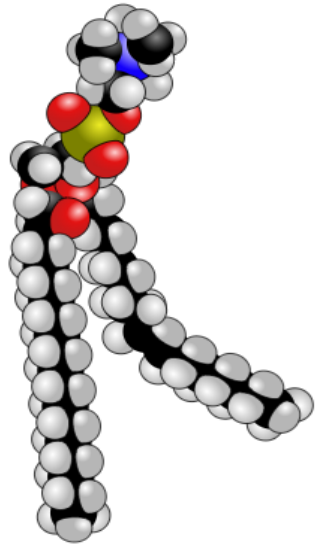


Examples of the major membrane phospholipids and glycolipids: phosphatidylcholine (PtdCho), phosphatidylethanolamine (PtdEtn), phosphatidylinositol (PtdIns), phosphatidylserine (PtdSer).

Phospholipid bilayer



Phospholipid
(Phosphatidylcholine)



Hydrophilic head

Hydrophobic tail

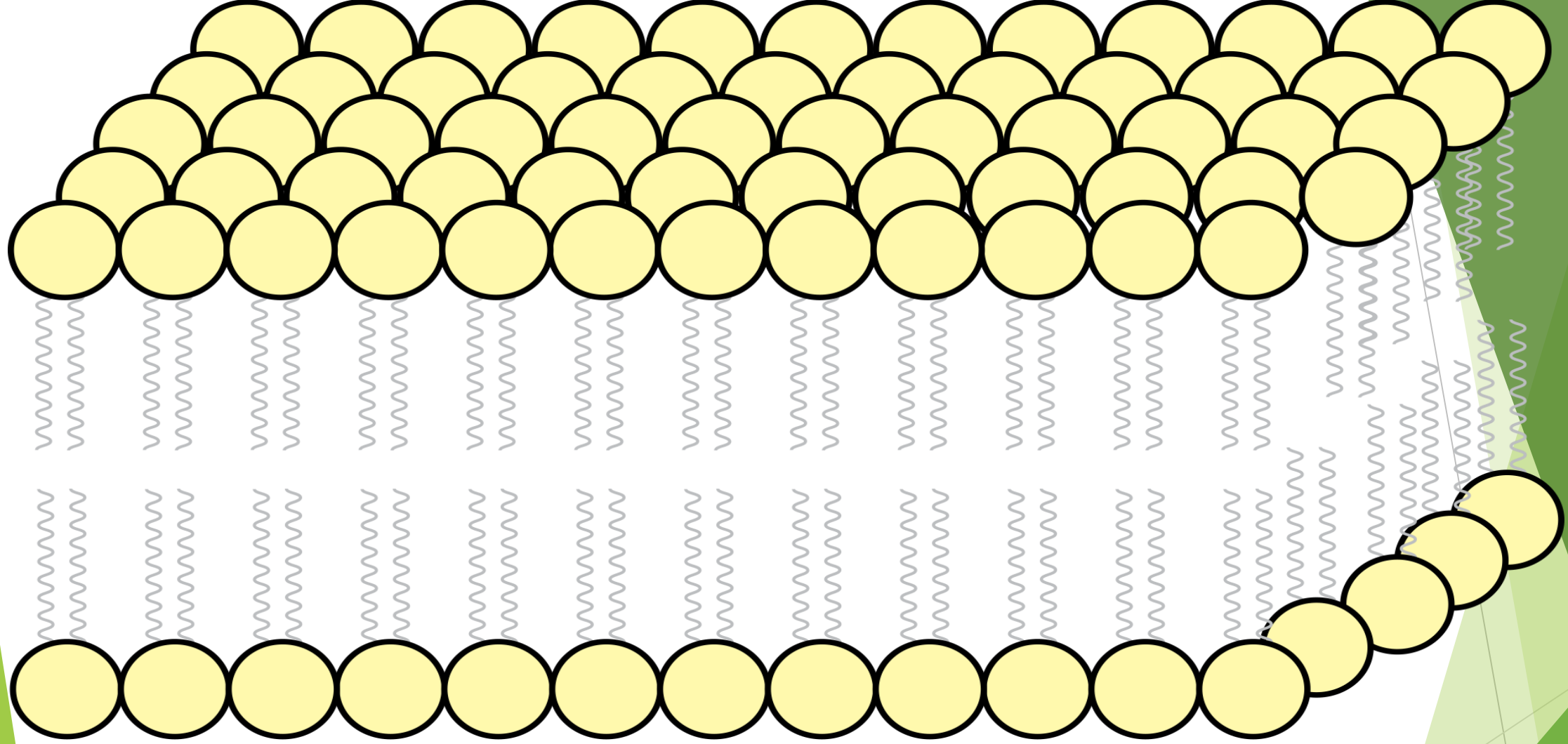


Diagram of the arrangement of amphipathic lipid molecules to form a lipid bilayer. The yellow polar head groups separate the grey hydrophobic tails from the aqueous cytosolic and extracellular environments.

Proteins

The cell membrane has large content of proteins, typically around 50% of membrane volume[25] These proteins are important for the cell because they are responsible for various biological activities. Approximately a third of the genes in yeast code specifically for them, and this number is even higher in multicellular organisms.[23] Membrane proteins consist of three main types: integral proteins, peripheral proteins, and lipid-anchored proteins.[4]

As shown in the adjacent table, integral proteins are amphipathic transmembrane proteins. Examples of integral proteins include ion channels, proton pumps, and g-protein coupled receptors. Ion channels allow inorganic ions such as sodium, potassium, calcium, or chlorine to diffuse down their electrochemical gradient across the lipid bilayer through hydrophilic pores across the membrane. The electrical

Type	Description	Examples
Integral proteins or transmembrane proteins	Span the membrane and have a hydrophilic cytosolic domain, which interacts with internal molecules, a hydrophobic membrane-spanning domain that anchors it within the cell membrane, and a hydrophilic extracellular domain that interacts with external molecules. The hydrophobic domain consists of one, multiple, or a combination of α-helices and β sheet protein motifs.	Ion channels, proton pumps, G protein-coupled receptor
Lipid anchored proteins	Covalently bound to single or multiple lipid molecules; hydrophobically insert into the cell membrane and anchor the protein. The protein itself is not in contact with the membrane.	G proteins
Peripheral proteins	Attached to integral membrane proteins, or associated with peripheral regions of the lipid bilayer. These proteins tend to have only temporary interactions with biological membranes, and once reacted, the molecule dissociates to carry on its work in the cytoplasm.	Some enzymes, some hormones

behavior of cells (i.e. nerve cells) are controlled by ion channels. Proton pumps are protein pumps that are embedded in the lipid bilayer that allow protons to travel through the membrane by transferring from one amino acid side chain to another. Processes such as electron transport and generating ATP use proton pumps. A G-protein coupled receptor is a single polypeptide chain that crosses the lipid bilayer seven times responding to signal molecules (i.e. hormones and neurotransmitters). G-protein coupled receptors are used in processes such as cell to cell signaling, the regulation of the production of cAMP, and the regulation of ion channels.

The cell membrane, being exposed to the outside environment, is an important site of cell-cell communication. As such, a large variety of protein receptors and identification proteins, such as antigens, are present on the surface of the membrane. Functions of membrane proteins can also include cell-cell contact, surface recognition, cytoskeleton contact, signaling, enzymatic activity, or transporting substances across the membrane.

Most membrane proteins must be inserted in some way into the membrane. For this to occur, an N-terminus "signal sequence" of amino acids directs proteins to the endoplasmic reticulum, which inserts the proteins into a lipid bilayer. Once inserted, the proteins are then transported to their final destination in vesicles, where the vesicle fuses with the target membrane.

RIBOSOME

- In eukaryotes, ribosome assembly is a complex process involving more than 200 assembly factors and spreads from the nucleolus to the cytoplasm. The process starts with the synthesis by the RNA Pol-I of the 35S pre-rRNA, which is co-transcriptionally bound and folded by ribosomal proteins and assembly factors.

Ribosomes are the macromolecular machines that are responsible for mRNA translation into proteins. The eukaryotic ribosome, also called the 80S ribosome, is made up of two subunits – the large 60S subunit (which contains the 25S [in plants] or 28S [in mammals], 5.8S, and 5S rRNA and 46 ribosomal proteins) and a small 40S subunit (which contains the 18S rRNA and 33 ribosomal proteins)[5]. The ribosomal proteins are encoded by ribosomal genes.

rRNA found in prokaryotic and eukaryotic ribosomes

Type	Size	Large subunit (LSU rRNA)	Small subunit (SSU rRNA)
Prokaryotic	70s	50S (5S : 120 nt, 23S : 2906 nt)	30S (16S : 1542 nt)
eukaryotic	80S	60S (5S : 121 nt,[6] 5.8S : 156 nt,[7] 28S : 5070 nt[8])	40S (18S : 1869 nt[9])

Prokaryotes

There are 52 genes that encode the ribosomal proteins, and they can be found in 20 operons within prokaryotic DNA. Regulation of ribosome synthesis hinges on the regulation of the rRNA itself.

First, a reduction in aminoacyl-tRNA will cause the prokaryotic cell to respond by lowering transcription and translation. This occurs through a series of steps, beginning with stringent factors binding to ribosomes and catalyzing the reaction:

GTP + ATP --> pppGpp + AMP

The γ-phosphate is then removed and ppGpp will bind to and inhibit RNA polymerase. This binding causes a reduction in rRNA transcription. A reduced amount of rRNA means that ribosomal proteins (r-proteins) will be translated but will not have an rRNA to bind to. Instead, they will negatively feedback and bind to their own mRNA, repressing r-protein synthesis. Note that r-proteins preferentially bind to its complementary rRNA if it is present, rather than mRNA.

The ribosome operons also include the genes for RNA polymerase and elongation factors (used in RNA translation). Regulation of all of these genes at once illustrate the coupling between transcription and translation in prokaryotes.

Eukaryotes

- The DNA is transcribed, at a high speed, in the nucleolus, which contains all 45S rRNA genes. The only exception is the 5S rRNA which is transcribed outside the nucleolus. After transcription, the rRNAs associate with the ribosomal proteins, forming the two types of ribosomal subunits (large and small). These will later assemble in the cytosol to make a functioning ribosome. See nuclear export for more about the movement of the ribosomal subunits out of the nucleus.

Processing

Eukaryotic cells co-transcribe three of the mature rRNA species through a series of steps. The maturation process of the rRNAs and the process of recruiting the r-proteins happen in precursor ribosomal particles, sometimes called pre-ribosomes, and takes place in the nucleolus, nucleoplasm, and cytoplasm. The yeast, S. cerevisiae is the eukaryotic model organism for the study of ribosome biogenesis. Ribosome biogenesis starts in the nucleolus. There, the 18S, 5.8S, and 25S subunits of the rRNA are cotranscribed from ribosomal genes as a polycistronic transcript by RNA polymerase I,[3] and is called 35S pre-RNA.

Transcription of polymerase I starts with a Pol I initiation complex that binds to the rDNA promoter. The formation of this complex requires the help of an upstream activating factor or UAF that associates with TATA-box binding protein and the core factor (CF). Together the two transcription factors allow the RNA pol I complex to bind with the polymerase I initiation factor, Rrn3. As the pol I transcript is produced, approximately 75 small nucleolar ribonucleoparticles (snoRNPs) facilitate the co-transcriptional covalent modifications of >100 rRNA residues. These snoRNPs control 2'-O-ribose methylation of nucleotides and also assist in the creation of pseudouridines. At the 5' end of rRNA transcripts, small subunit ribosomal proteins (Rps) and non-ribosomal factors assemble with the pre-RNA transcripts to create ball-like knobs. These knobs are the first pre-ribosomal particles in the small (40S) ribosomal subunit pathway. The rRNA transcript is cleaved at the A2 site, and this separates the early 40S pre-ribosome from the remaining pre-rRNA that will combine with large subunit ribosomal proteins (Rpl) and other non-ribosomal factors to create the pre-60S ribosomal particles.

40S subunit

The transcriptional assembly of the 40S subunit precursor, sometimes referred to as the small subunit processome (SSU) or 90S particle happens in a hierarchical fashion – essentially a stepwise incorporation of the UTP-A, UTP-B, and UTP-C subcomplexes. These subcomplexes are made up of over 30 non ribosomal protein factors, the U3 snoRNP particle, a few Rps proteins, and the 35S pre-rRNA. Their exact role, though has not been discovered. The composition of the pre-40S particle changes drastically once cleavage at the U3 snoRNPA dependent sites (sites A0, A1, and A2) are made. This cleavage event creates the 20S pre-rRNA and causes ribosomal factors to dissociate from the pre-40S particle. U3 is displaced from the nascent 40S by the helicase Dhr1. At this point in the ribosome biogenesis process,

the 40S pre-ribosome already shows the “head” and “body” structures of the mature 40S subunit. The 40S pre-ribosome is transported out of the nucleolus and into the cytoplasm. The cytoplasmic 40S pre-ribosome now contains ribosomal proteins, the 20s rRNA and a few non-ribosomal factors. The final formation of the 40S subunit “beak” structure occurs after a phosphorylation and dephosphorylation event involving the Enp1-Ltv1-Rps3 complex and the kinase, Hrr25. Cleavage of the 20S pre-rRNA at the D-site creates the mature 18s rRNA. This cleavage event is dependent on several non-ribosomal factors such as Nob1, Rio1, Rio2, Tsr1 and Fap7

60S subunit

The maturation of the pre-60S subunit into a mature 60S subunit requires many biogenesis factors that associate and disassociate. In addition, some assembly factors associate with the 60S subunit while others only interact with it transiently. As an overall trend, the maturation of the pre-60S subunit is marked a gradual decrease in complexity. The subunit matures as it moves from the nucleolus to the cytoplasm and gradually the number of trans-acting factors are reduced.The maturation of the 60S subunit requires the help of about 80 factors. Eight of these factors are directly involved with the processing of the 27S A3 pre-rRNA, which actually completes the formation of the mature 5’end of the 5.8S rRNA. The A3 factors bind to distant sites on the pre-RNA as well as to each other. Subsequently, they bring areas of rRNA close together and promote the processing of pre-rRNA and the recruitment of ribosomal proteins. Three AAA-type ATPases work to strip the factors from the maturing 60S pre-ribosome.

One of the ATPases is a dynein-like Rea1 protein made up of 6 different ATPase domains that form a ring structure. The ring structure is attached to a flexible tail that happens to have a MIDAS (Metal ion-dependentant adhesion site) tip. The Rea1 interacts with the 60S pre-ribosome via its ring while two substrates, Ytm1 and Rsa1, interact with Rea1 through its MIDAS tip. The role of these substrates has not yet been defined. Both though, along with their interactions, are removed in the maturation process of the 60S pre-ribosome. The other two ATPases, Rix7 and Drg1 also function to remove assembly factors from the maturing 60S subunit. Helicases and GTPases are also involved in the removal of assembly factors and the rearrangement of RNA to form the completed 60S subunit. Once in the cytoplasm (see nuclear export), the 60S subunit further undergoes processing in order to be functional. The rest of the large subunit ribosomal particles associate with the 60S unit and the remaining non-ribosomal assembly factors disassociate. The release of the biogenesis factors is mediated mostly by GTPases such as Lsg1 and ATPases such as Drg1. The precise sequence of these events remains unclear. The pathway of 60S cytoplasmic maturation remains incomplete as far as current knowledge is concerned.[

Chromatin assembly

- The simplest definition of chromatin assembly is the process by which DNA is packaged into nucleosomes. ... The basic chromatin assembly process is mediated by histone chaperones and ATP-utilizing factors that catalyse the deposition of the histones onto DNA to yield periodic arrays of nucleosomes

The poster shows the general steps in chromatin assembly and the diversity of chromatin organization. At each step, variation in the basic constituents of chromatin leads to the establishment of distinct final structures. The left-hand column of text provides examples of such variation; the right-hand column lists stimulatory factors that can promote progression through the assembly steps. Assembly begins with the deposition of the histone H3-H4 tetramer onto DNA, followed by the addition of two histone H2A-H2B dimers to form the core particle. The newly synthesized histones are specifically modified; typically histone H4 is acetylated at Lys5 and Lys12. During the maturation step ATP is required to establish a regular spacing, and histones are de-acetylated. The incorporation of linker histones is accompanied by folding of the nucleofilament, which is represented here as a solenoid structure containing six nucleosomes per gyre. Further folding events produce a defined organization within nuclear domains.⇓

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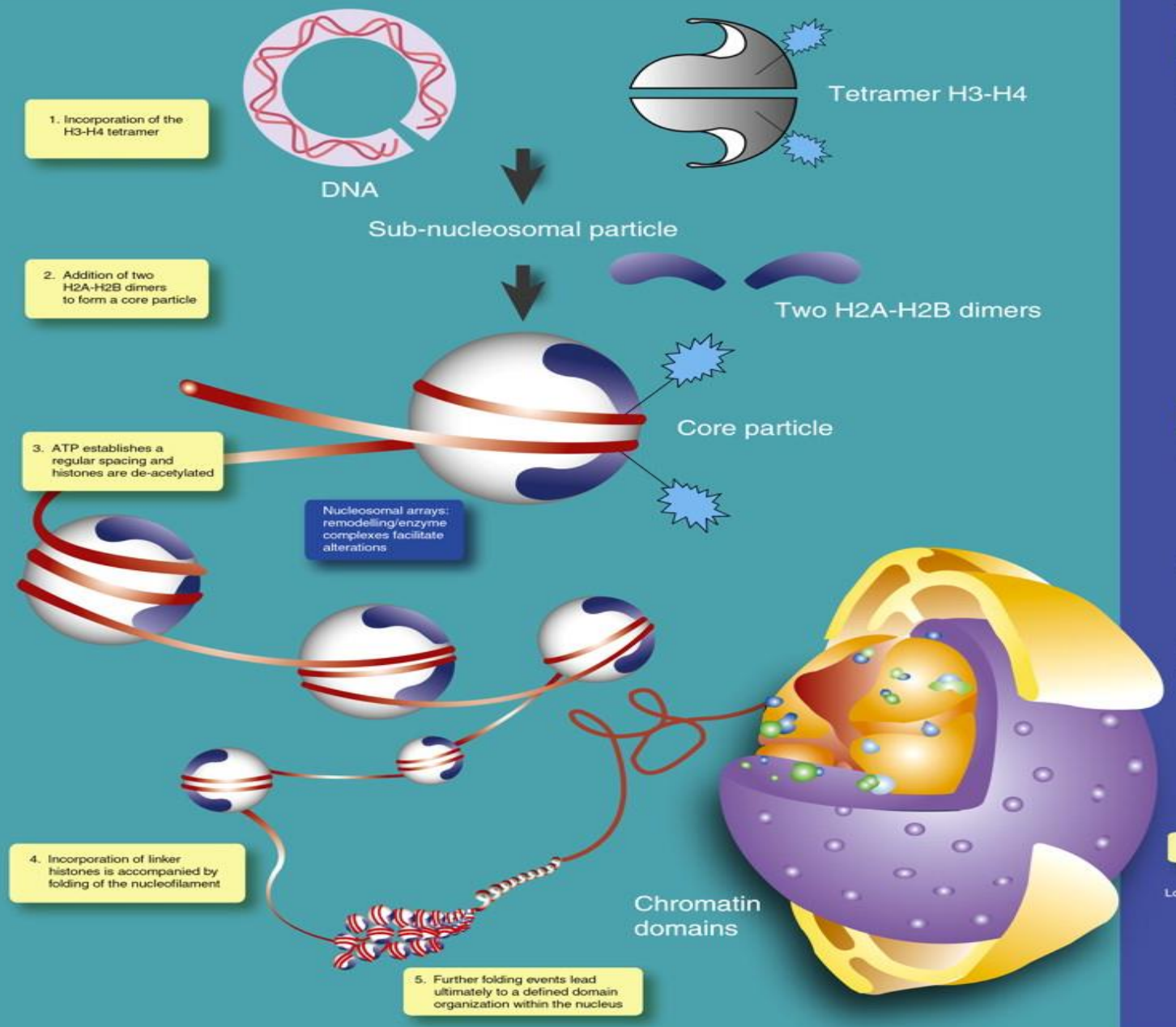
Chromatin Assembly and Organization

Patricia Ridgway and Geneviève Almouzni



Variation in basic constituents

DNA and histones

DNA modification (e.g. methylation)

"Histone code" of modifications during cell cycle progression define chromatin states

- interplay between acetylation, phosphorylation, methylation, ubiquitination and poly-ADP-ribosylation
- acetylation/deacetylation cycle of H3-H4 during S phase; phosphorylation of H3/CENP-A in G2/M

Histone variants (H2A-X, CENP-A, etc.)

- incorporation of variants can impact on final localization into specialized chromatin domains
- phosphorylated H2A-X localized in regions of double-strand breaks is induced by ionizing radiation
- H3 variant CENP-A is primarily associated with silent centromeric region

Maturation

Chromatin maturation

- remodelling and histone-modifying enzyme complexes facilitate histone modifications (acetylation/deacetylation) or introduce histone variants that alter chromatin composition

Histone modifications can operate later in the assembly process

- phosphorylation of H3 family
- H3 methylation on Lys9 allows HP1 binding to mediate chromatin silencing

Differential incorporation of linker histones, HMG proteins and specific DNA-binding factors helps to space and fold the nucleosomal array

- DNA bending to promote or destabilize assembly

Concentration of constituents

Local concentration of specific constituents in domains

Stimulatory assembly factors

Histone-interacting factors

Coordinated action of chaperones, specialized chaperones provide temporal and regional regulation

Xenopus nucleoplasmin and dNLP form complexes with H2A-H2B

N1/N2 interacts with H3 and H4

CAF-1 preferentially assembles histones onto newly synthesized DNA during replication and repair; p48 and p150 interact with H3-H4

RCAF: Asf1p/H3-H4 synergizes with CAF-1 to promote nucleosome assembly during DNA replication in vitro

NAP-1, NAP-2 (interacts with linker and core histones)
NAP-1 promotes nucleosome assembly in presence of all 4 core histones and cooperates with remodeller ACF to assemble nucleosomal arrays

Spt-6 interacts with H3 and H4

DF 31 thought to be a histone chaperone from *Drosophila*

mis6 protein in *S. pombe* localizes CENP-A to centromeres

Mobility factors and remodelling machines

ISWI family (ACF, CHRAC, ISW1&2, NURF, RSF, INO80)

SNF2 family (SWI/SNF, RSC, Brahma,?ATRX)

Mi-2/CHD family (Mi-2 complex, NuRD) linked to silencing via DNA methylation

Repair factor Cockayne syndrome B

Histone-modifying enzymes

Sir2 (NADase activity linked to deacetylation of H3 and H4)

Histone acetyl transferase (HAT)

Histone deacetylase (HD) brings about modification of histones and other proteins

SUV39H1 HMT methylates H3 and contributes to propagation and localization of heterochromatin leading to silencing

Targeting of proteins to specialized domains

PCNA 'marks' DNA for assembly

Tissue-specific chromatin assembly factors: Nap1/2 specific to neurons

Heterochromatin proteins: HP1, Polycomb, Sir3p/Sir4p, ATRX
Transcription factor: Ikaros
Assembly factor: CAF-1

During the first steps of assembly, incorporation of differentially modified (e.g. methylated) DNA and histones modified in numerous ways (e.g. acetylation, phosphorylation, methylation, ubiquitination and poly-ADP-ribosylation) can produce differences in chromatin structure and activity. These modifications signal the progression of defined chromatin states, such as the acetylation/deacetylation cycle of H3-H4 during S phase or the phosphorylation patterns of H3/CENP-A in G2/M phase. The incorporation of histone variants such as H2A-X or CENP-A can also impact on the final localization of chromatin into specialized chromatin domains. More specifically, phosphorylated histone H2A-X is localized in regions of double-strand breaks induced by ionizing radiation, whereas the histone H3 variant CENP-A is primarily associated with silent centromeric regions.

In the maturation step, the differential incorporation of linker histones, HMG proteins and other specific DNA-binding factors helps to space and fold the nucleosomal array. Histone modifications can also operate at this later stage. In this context, histone H3 phosphorylation has been demonstrated to play a role in chromosome condensation and segregation, and methylation on Lys9 of histone H3 mediates chromatin silencing. Therefore, these early steps in assembly can have a great impact on the later steps, leading to localization of chromatin into specialized nuclear domains.

Stimulatory factors are implicated in assembly at several levels, directly binding to histones, acting as mobility/remodelling machines, functioning as histone-modifying enzymes or targeting chromatin to specialized domains.

Histone-interacting factors or chaperones may impart specificity on chromatin assembly. Xenopus nucleoplasmin, for example, forms complexes with histones H2A and H2B, as does Drosophila nucleoplasmin-like protein (dNLP). Xenopus N1/N2, however, interacts with histones H3 and H4. Chromatin assembly factor 1 (CAF-1) preferentially assembles nucleosomes onto newly synthesized DNA during replication and repair. RCAF promotes nucleosome assembly during DNA replication in vitro by a synergy between Asf1p/H3/H4 and CAF-1. Of the histone-interacting proteins, NAP-1 can associate with all core histones (depending on the study system), and NAP-2 interacts with both linker and core histones. Other factors include Spt-6, which interacts with histones H3 and H4, DF 31 (a putative Drosophila histone chaperone) and the S. pombe mis6 protein, which localizes CENP-A to centromeres.

Mobility factors and remodelling machines regulate DNA accessibility and are good candidates for mediators of ATP-dependent chromatin maturation. These include Drosophila ATP-utilizing chromatin-assembly and -modifying factor (ACF), chromatin-accessibility complex (CHRAC), nucleosome-remodelling factor (NURF) and yeast remodelling and spacing factor (RSF). Other examples are INO80, which is involved in transcription and DNA processing, SNF2 family members, and the Mi-2 complex and nucleosome-remodelling and histone-deacetylase complex (NuRD), which are linked to silencing by DNA methylation.

Histone-modifying enzymes also affect chromatin structure. Sir2, for example, has NADase activity linked to deacetylation of H3 and H4, and histone acetyltransferases (HAT) and histone deacetylases (HD) modify both histones and other interacting proteins. Significantly, the HD involved in post-replicative deacetylation is not yet identified, and several enzymes are capable of acting on the nucleosome in concert. SUV39H1 HMT, for instance, methylates H3, which has been implicated in silencing through an interaction with heterochromatin protein 1

The final group of stimulatory factors is targeting proteins, which may help to bring specialized proteins to specific domains in the nucleus. They might act through a `marking' mechanism (e.g. PCNA) or by exhibiting tissue specificity (e.g. neuron-specific Nap1/2). Hypothetically, heterochromatin-associated proteins, transcription factors and assembly factors may ensure these functions and examples are given for each class. Naturally, the timing of modifications, regulatory factor expression and protein localization may provide an additional level of regulation. Detailed information on this topic can be found in a number of recent reviews (Annunziato and Hansen, 2000; Cheung et al., 2000; Mello and Almouzni, 2001; Moazed, 2001; Ridgway and Almouzni, 2000; Strahl and Allis, 2000; Wu and Grunstein, 2000).