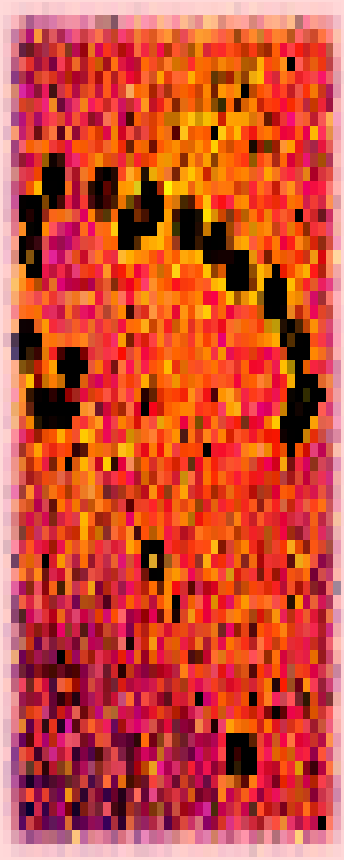
CHAPTER

14

Ribosomes



Ribosomes may be found free in the cytoplasm either singly or strung along messenger RNA molecules as they participate in protein synthesis. Ribosomes also stud the rough endoplasmic reticulum, giving it a rough appearance and allowing the synthesis of proteins within the ER.

The ribosomes are small, dense, rounded and granular particles of the ribonucleoprotein. They occur either freely in the matrix of mitochondria, chloroplast and cytoplasm (*i.e.*, cytoplasmic matrix) or remain attached with the membranes of the endoplasmic reticulum and nucleus. They occur in most prokaryotic and eukaryotic cells and are known to provide a scaffold for the ordered interaction of all the molecules involved in protein synthesis.

HISTORICAL

Ribosomes are remarkable organelles of cell. They were studied before they were discovered. Thus, ribosomes were studied in the early 1930s, discovered and isolated in the early 1940s, scrutinized in 1950s and baptized in 1958. In 1960s they were dissociated and reconstituted; in 1970s sequenced and studied topographically; and in 1980s they continue to be object of considerable research.

Before 1930s, it was the prevailing view that DNA was found only in animal cells and RNA only in plant cells. In 1930s various direct studies, employing basic staining teachniques that discriminated between DNA and RNA, and spectrophotometric measurements of absorption in different cell regions, confirmed that RNA is present in the cytoplasm of both plant and animal cells and suggested that DNA is found exclusively

in the nucleus (**Brachet** and **Caspersson**). Using quantitative techniques in 1940s, a very significant observation was made regarding the ribosome function. It was reported that cells were rich in RNA when they were active in protein synthesis. For example, secretory cells such as pancreas cells and silk gland cells, were noted to be RNA-rich, whereas cells of other types (non-secretory cells such as heart muscle cells which make little new proteins) are relatively RNA-poor. In 1940s **Albert Claude** homogenized chick and mammalian embryos and obtained a fraction containing what he called **microsomes**— particles of ribonucleoprotein and lipid visible with the dark field microscope. He, thus, showed that the cytoplasmic RNA was included in tiny particles of ribonucleoprotein later to be called "ribosomes'. In 1952, **G.E. Palade** described the ribosome. Their presence in both free and membrane attached form was confirmed by **Palade** and **Siekevitz** by the electron microscopy. In 1950s another technique, namely ultracentrifugal analysis was employed to study the ribosomes. This showed that ribosomes sedimented at discrete peaks in the 40S—70S range. When purified by centrifugation and electrophoresis, they were found to contain half RNA and half protein. As $MgCl_2$ was known to precipitate RNA, **Siekevitz** suggested that RNA might somehow be involved in protein synthesis. In 1952, **Siekevitz** and **Zamecnik** showed clearly that radioactive amino acids first were incorporated into proteins on ribosomes and then were released to the soluble portions of the cell.

In 1958, the papers presented at a meeting of the Biophysical Society at the Massachusetts Institute of Technology were published in a book form. **R.B. Roberts** edited this collection of papers and coind the name **ribosome** in his introductory comments. The term ribosome is due to rich RNA content of this organelle. **Tissieres** and **Watson** (1958) isolated 70S *E.coli* ribosomes and showed that they consist of two subunits, 50S and 30S. In 1960s ribosomes were subjected to exhaustive electrophoretic and chromatographic procedures, this time not to purify them but to examine their parts. It soon became clear that ribosomes contain three or four kinds of RNA and scores of proteins.

Recently, various workers such as **Lake**, **Nomura**, **Wittman**, **Traut** , **Stoffler**, **Kurland**, etc., have studied the relationship between rRNAs and ribosomal proteins to work out the topology of ribosomes (Topology includes study of detailed shape and positions of the individual proteins and rRNA molecules relative to each other; see **King**, 1986).

OCCURRENCE AND DISTRIBUTION

The ribosomes occur in cells, both prokaryotic and eukaryotic cells. In prokaryotic cells the ribosomes often occur freely in the cytoplasm. In eukaryotic cells the ribosomes either occur freely in the cytoplasm or remain attached to the outer surface of the membrane of endoplasmic reticulum. The yeast cells, reticulocytes or lymphocytes, meristamatic plant tissues, embryonic nerve cells and cancerous cells contain large number of ribosomes which often occur freely in the cytoplasmic matrix. The cells in which active protein synthesis takes place, the ribosomes remain attached with the membranes of the endoplasmic reticulum. Such cells are the pancreatic cells, plasma cells, hepatic parenchymal cells, Nissls bodies, osteoblasts, serous cells, or the submaxillary gland, chief cells of the glandular stomach, thyroid cells and mammary gland cells. The cells which synthesize specific proteins for the intracellular utilization and storage often contain large number of free ribosomes. Such cells are the erythroblasts, developing muscle cells, skin and hair.

METHOD OF ISOLATION

The ribosomes are usually isolated from the cell by the differential centrifugation method in which an analytical centrifuge is employed. The sedimentation coefficient of the ribosomes is determined by the various optical and electronic techniques. The sedimentation coefficient is expressd in the **Svedberg unit**, *e.g.*, **S** unit. The **S** is related with the size and molecular weight of the ribosomal particles.

TYPES OF RIBOSOMES

Recently accroding to the size and the sedimentation coefficient (S) two types of ribosomes have been recognised (Fig. 14.1).

1. 70S Ribosomes. The 70S ribosomes are comparatively smaller in size and have sedimentation coefficient 70S and the molecular weight 2.7× 106 daltons. (**Dalton** is the unit of molecular weight **(MW)**; one dalton equals the weight of hydrogen atom. For example, a water molecule weighs 18 daltons, see **De Robertis** *et al*., 1970). According to the data of electron microscopy the dimension of the dry particles of 70S ribosomes are 170 × 170 × 200 A° (**Hall** and **Stayter**, 1959, **Huxley** and **Zubay**, 1960). They occur in the prokaryotic cells of the blue green algae and bacteria and also in mitochondria and chloroplasts of eukaryotic cells.

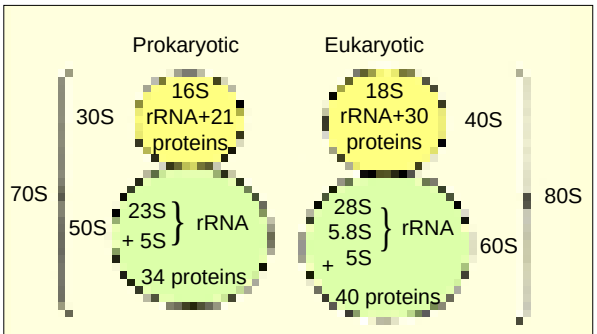


Fig. 14.1. Various components of prokaryotic (70S) and eukaryotic (80S) ribosomal subunits (after De Robertis and De Robertis, Jr. 1987).

2. 80S Ribosomes. The 80S ribosomes have the sedimentation coefficient of 80S and the molecular weight 40 × 10^6 daltons. The 80S ribosomes occur in eukaryotic cells of the plants and animals.

The ribosomes of mitochondria and chloroplasts are always smaller than 80S cytoplasmic ribosomes and are comparable to prokaryotic ribosomes in both size and sensitivity to antibiotics, although their sedimentation values vary in different phyla, *e.g.*, 77S in mitochondria of fungi, 60S in mitochondria of mammals and 60S in mitochondria of animals in general. The ribosomes of chloroplasts are 70S type.

NUMBER OF RIBOSOMES

An *E. coli* cell contains 10,000 ribosomes, forming 25 per cent of the total mass of the bacterial cell. In contrast, mammalian cultured cells contain 10 million ribosomes per cell, each of which is about twice as large as a prokaryotic ribosome.

STRUCTURE OF RIBOSOMES

The ribosomes are oblate spheroid structures of 150 to 250A° in diameter. Each ribosome is porous, hydrated and composed of two subunits. One ribosomal subunit is large in size and has a dome-like shape, while the other ribosomal subunit is smaller in size and occurring above the larger subunit and forming a cap-like structure.

The 70S ribosome consists of two subunits, *viz*., 50S and 30S. The 50S ribosomal subunit is larger in size and has the size of 160 A° to 180 A°. The 30S ribosomal subunit is smaller in size and occurs above the 50S subunit like a cap (Fig. 14.1A).

The 80S ribosome also consists of two subunits, *viz*., 60S and 40S. The 60S ribosomal subunit is dome-shaped and larger in size. In the ribosomes which remain attached with the membranes of endoplasmic reticulum and nucleus, etc., the 60S subunit remains attached with the membranes. The 40S ribosomal subunit is smaller in size and occurs above the 60s subunit forming a cap-like structure. Both the subunits remain separated by a narrow cleft (Fig. 14.1B).

The two ribosomal subunits remain united with each other due to high concentration of the Mg^{++}(.001M) ions. When the concentration of Mg^{++}ions reduces in the matrix, both ribosomal subunits get separated. Actually in bacterial cells the two subunits are found to occur freely in the cytoplasm and they unite only during the process of protein synthesis. At high concentration of Mg++ ions in the matrix, the two ribosomes (called **monosomes**) become associated with each other and known as the **dimer**. Further, during protein synthesis many ribosomes are aggregated due to common messenger RNA and form the **polyribosomes** or **polysomes**.

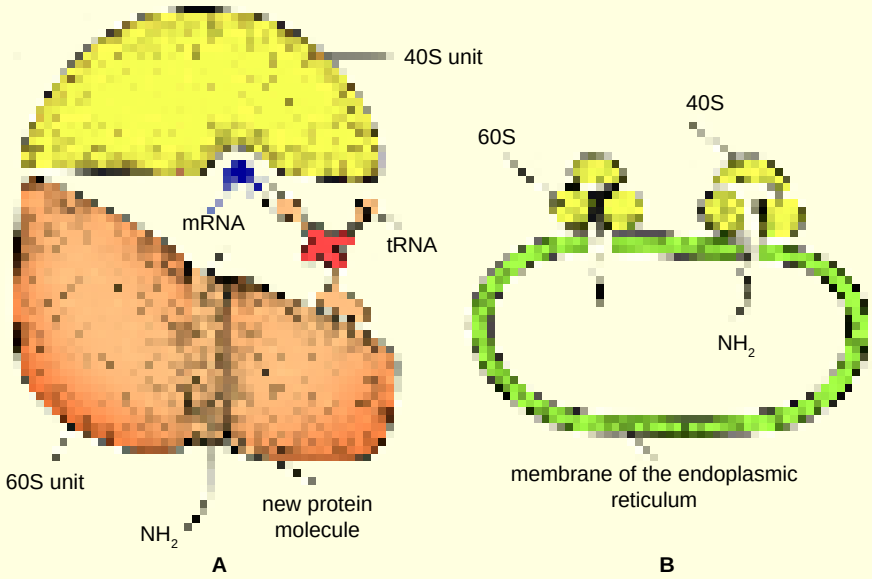


Fig. 14.2. Functional 80S ribosomes : A—Diagram of a ribosome showing the two subunits and the probable position of mRNA and tRNA. The nascent polypeptide chain passes through a kind of tunnel within the large subunit; B—Diagram showing the relationship between the ribosome and the membrane of endoplasmic reticulum and the entrance of the polypeptide chain into the centre of endoplasmic reticulum during the process of protein synthesis.

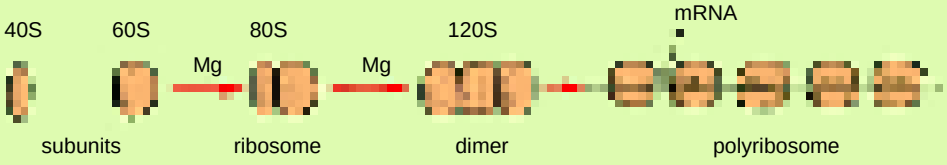
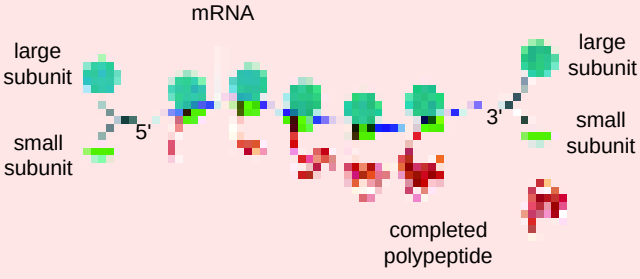


Fig. 14.3. Diagram of the subunit structure of the ribosome and the influence of Mg^{++}ions.



Polyribosome in action.

CHEMICAL COMPOSITON

The ribosomes are chemically composed of RNA and proteins as their major constituents ; both occurring approximately in equal proportions in smaller as well as larger subunit. However, the 70S ribosomes contain more RNA (60 to 40%) than the proteins (36 to 37%), *e.g.*, the ribosomes of *E. coli* contain 63% rRNA and 37% protein. While the 80S ribosomes contain less RNA (40 to 44%) than the proteins (60 to 56%), *e.g.*, yeast ribosomes have 40 to 44% RNA and 60 to 56% proteins ; ribosomes of pea seedling contain 40% RNA and 60% proteins. There is no lipid content in ribosomes.

1. Ribosomal RNAs

The 70S ribosomes contain three types of rRNA, *viz*., **23S rRNA**, **16S rRNA**, **5S rRNA**. The 23S and 5S rRNA occur in the larger 50S ribosomal subunit, while the 16S rRNA occurs in the smaller 30S ribosomal subunit. Assuming an average molecular weight for one nucleotide to be 330 daltons, one can calculate the total number of each type of rRNA. Thus, the 23S rRNA consists of 3300 nucleotides, 16S rRNA contains 1650 nucleotides and 5S rRNA includes 120 nucleotides in it (**Brownlee**, 1968 ; **Fellner**, 1972).

The 80S ribosomes contain four types of rRNA, *viz*., **28S rRNA** (or **25-26 rRNA** in plants, fungi and protozoa), **18S rRNA**, **5S rRNA** and **5.8S rRNA**. The 28S, 5S and 5.8S rRNAs occur in the larger 60S ribosomal subnit, while the 18S rRNA occurs in the smaller 40S ribosomal subunit. About 60 per cent of the rRNA is helical (*i.e*., double stranded) and contains paired bases. These double stranded regions are due to hairpin loops between complimentary regions of the linear molecule.

The 28S rRNA has the molecular weight 1.6×10^6 daltons and its molecule is double stranded and having nitrogen bases in pairs. The 18S rRNA has the molecular weight 0.6×10^6 daltons and consists of 2100 nucleotides. The 18S and 28S ribosomal RNA contain a characteristic number of methyl groups, mostly as 2'-O-methyl ribose. The molecule of 5S rRNA has a clover leaf shape and a length equal to 120 nucleotides (**Forget** and **Weissmann**, 1968). The 5.8S rRNA is intimately associated with the 28S rRNA molecule and has, therefore, been referred to as **28S-associated ribosomal RNA** (**28S-A rRNA**) (**Avers**, 1976).

The 55S ribosomes of mammalian mitochondria lack 5S rRNA but contain **21S** and **12S rRNAs**. The 21S rRNA occurs in larger or 35S ribosomal subunits, while 12S rRNA occur in smaller or 25S ribosomal subunit. The sedimentation coefficient of ribosomes, ribosomal subunits, rRNAs and number of ribosomal proteins of certain representative organisms have been tabulated in Table 14.1.

It is thought that each ribosomal subunit contains a highly folded ribonucleic acid filament to which the various proteins adhere (**Hart**, 1965). But as the ribosomes easily bind the basic dyes so it is concluded that RNA is exposed at the surface of the ribosomal subunits, and the protein is assumed to be in the interior in relation to non-helical part of the RNA.

2. Ribosomal Proteins

According to **Nomura** (1968, 1973) and **Garett** and **Wittmann** (1973) each 70S ribosome of *E. coli* is composed of about 55 **ribosomal proteins**. Out of these 55 proteins, about 21 different molecules have been isolated from the 30S ribosomal subunit, and some 32 to 34 proteins from the 50S ribosomal subunit. The primary structure of several of these proteins has been elucidated. Most of the recent knowledge about the structure of ribosomal proteins has been achieved by dissociation of ribosomal subunits into their component rRNA and protein molecules. When both 50S and 30S ribosomal subunits are dissociated by centrifuging both of them in a gradient of 5 M cesium chloride, then there are two inactive core particles (40S and 23S, respectively) which contain the RNA and some proteins called **core proteins** (**CP**) at the same time several other proteins—the so-called **split proteins** (**SP**) are released from each particle (Fig. 14.3). There are SP50 and SP30 proteins which may reconstitute the functional ribosomal subunit when added to their corresponding core. Some of the split proteins are apparently specific for each ribosomal subunit. The split proteins have been further fractionated and divided into acidic (A) and basic (B) proteins. **Nomura** *et al*., (1968) fractionated at least six different groups of proteins in the ribosome (Fig. 14.4).

In all, 21 types of proteins have been isolated in smaller subunit (30S) of ribosome of *E. coli*. These are designated as S1 to S21. Similarly, in larger subunit (50S) 34 different proteins designated as L1 to L34, have been isolated. Thus, the 70S ribosome was thought to consist of 55 different proteins. However it was later shown that protein S20 is identical to L26, thus, the correct number of S proteins is 20. Likewise, L8 was shown to be an aggregate of proteins L7, L12 and L10; thus, the correct number of L proteins is 33. Thus, the prokaryotic 70S ribosome consists of 53 different proteins

(20S + 33L = 53 proteins). Similar organization of ribosomal proteins and RNA is found in 80S ribosomes (Fig. 14.5).

Table 14.1. **Some characteristics of ribosomes of various organisms (Avers, 1976).**

Source	Intact ribosomes	Ribosome subunits	rRNA in subunit	Number of proteins in subunit
Prokaryotes	70S	30S 50S	16S 23S, 5S	21 32-34
Eukaryotes	80S	40S 60S		~30 ~50
Animals		40S 60S	18S 28S,5S, 5.8S	
Plants		40S 60S	18S 25-26S, 5S, 5.8S	
Fungi		40S 60S	18S 25-26S, 5S 5.8S	
Protozoa (some other protists)		40S 60S	18S 25-26S, 5S 5.8S	

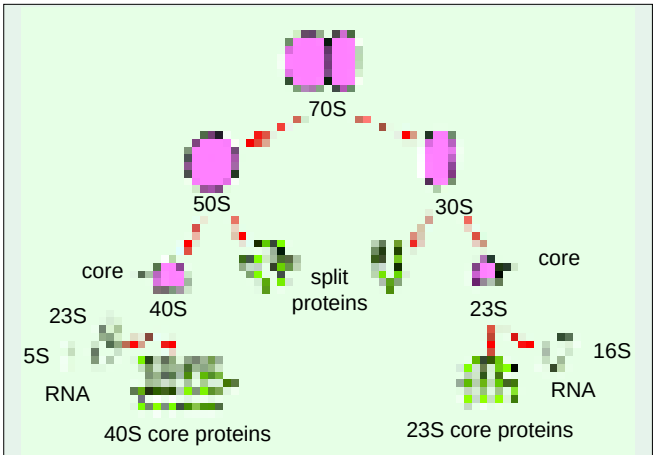


Fig. 14.4. Diagram showing the stepwise dismantling of the two subunits of 70S ribosome. Note that the proteins may be separated into split and core proteins. The 50S subunit contains 23S and 5S RNAs, and the 30S subunit has 16S RNA (after De Robertis and De Robertis, Jr., 1987).

Different rRNA molecules evidently play a central role in the catalytic activities of ribosomes in the process of protein synthesis. Various ribosomal proteins have been found to mainly enhance the catalytic function of the rRNA in the ribosomes (see **Alberts** *et al.*,1989).

3. Metallic Ions

The most important low molecular weight components of ribosomes are the divalent metallic ions such as Mg^{++}, Ca^{++} and Mn^{++}

ULTRASTRUCTURE

Molecular organization and function of ribosomes have been studied more intensively in prokaryotes than in eukaryotes. Fine or ultra-structure of 70S ribosome is very complex. In it, the RNA and proteins are intertwined and arranged in a complex manner in the two subunits. Since the positive protein charges are not sufficient to balance the many negative charges in the phosphates of the RNA, so ribosomes are strongly negative and bind cations and basic stains. Consequently, **negative staining** of ribosomes has led to better understanding

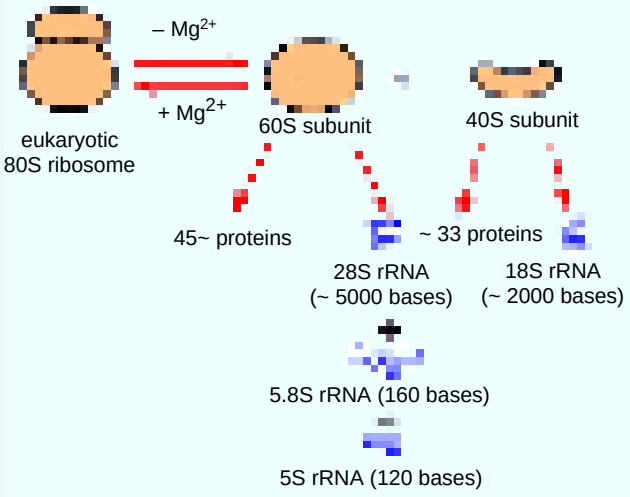


Fig. 14.5. Different RNA and protein components of eukaryotic ribosomes.

of the fine structure of these organelles. Recently following two models have been suggested to explain the three-dimensional structure of prokaryotic or 70S ribosomes :

1. Stoffler and Wittmann's Model (Quasi-symmetrical Model, 1977)

According to this model the 30S ribosomal subunit has an elongated, slightly bent prolate shape (Fig. 14.6). It is a bipartite structure. A transverse hollow or cleft divides the 30S subunit into two parts, a smaller **head** and larger **body**, giving it the appearance of a telephone receiver or embryo. In electron microscopy 50S ribosomal subunit showed various shapes depending on structure seen in different views such as frontal-maple leaf, lateral-kidney shaped or rear view-rounded. In a frontal view, the 50S subunit appears bilaterally symmetrical and shows three protuberances arising from a rounded base (maple leaf structure). The **central protuberance** being the most prominent. The 50S subunit is often compared with an armchair, with the rounded base forming a **vaulted seat**, the central protuberance forming the **back** and the lateral protuberances the **arms** of chair.

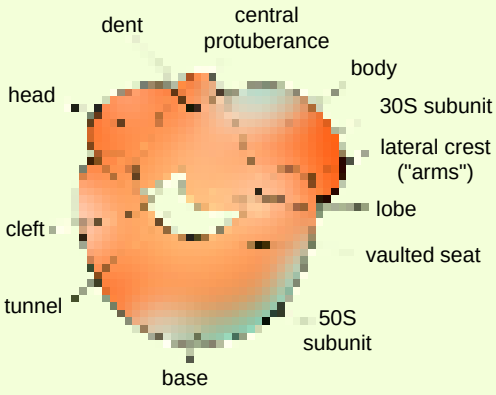


Fig. 14.6. Stoffler and Wittman's model of 70S ribosome.

When 30S and the 50S subunits become associated to form the 70S ribosome, the frontal face of the 30S subunit with its hollow faces the vaulted seat of the 50S subunit. The long axis of 30S subunit is oriented transversely to the central protuberance of the 50S subunit. A **tunnel** is formed between the hollow of the small subunit and vaulted seat of the large subunit.

2. Lake's Model (Asymmetrical Model, 1981)

This completely asymmetrical model of ribosome has been suggested by **James A. Lake** (1981). The smaller subunit has a **head**, a **base** and a **platform**. The platform separates the head from the base by the help of a **cleft**. This cleft is an important functional region ; it is suggested to be the site of codon-anticode interaction and as a part of binding site for initiation factors of protein synthesis.

The large subunit consists of a **ridge**, a **central protuberance** and a **stalk**. The ridge and the central protuberance are separated with the help of a valley (Fig. 14.7)

Three Dimensional Model of 80S Ribosome

In spite of the difference in overall sizes (as manifested in the greater molecular weights, sedimentation constants, sizes and numbers of rRNAs and proteins), the cytoplasmic ribosomes of

eukaryotes (80S) are remarkably similar in morphology to those of prokaryotes. As in 30S subunits of prokaryote ribosomes, the 40S ribosomal subunit of eukaryotes is divided into **head** and **base** segments by a transverse groove (Fig. 14.8). The 60S ribosomal subunit is generally rounder in shape than the small subunit, although its one side is flattened ; this is the side that becomes confluent with the small subunit during the formation of the monomer or monosome (*i.e.*, functional 80S ribosome).

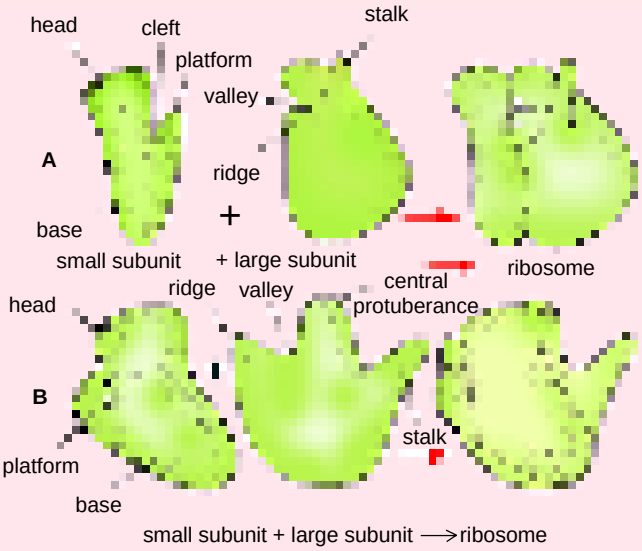


Fig. 14.7. Lake's model of the 70S ribosome. Note the three dimensional structure of the ribosome in two different orientations.

Dissociation and reconstitution (self-assembly) of the ribosomes. To understand the three-dimensional organization of ribosomal proteins in the ribosomes and also for the investigation of interactions between the molecules of rRNA and proteins, following classical experiment of dissociation and reconstitution of **Nomura** and **Traub** (1968) can be considered. This experiment involves to take purified 30S ribosomal subunits, dissociate them by chemical means into their component RNAs and proteins and then allow them to reassociate under appropriate ionic conditions. Dissociation of 30S subunit may be achieved by treatment with four molar urea and two molar LiCl, which separate the proteins. If the 16S rRNAs previously extracted with phenol is placed in the presence of 20 different protein molecules of 30S ribosomal subunit, the reconstitution or self-assembly of 30S ribosomal subunit takes place in two steps :

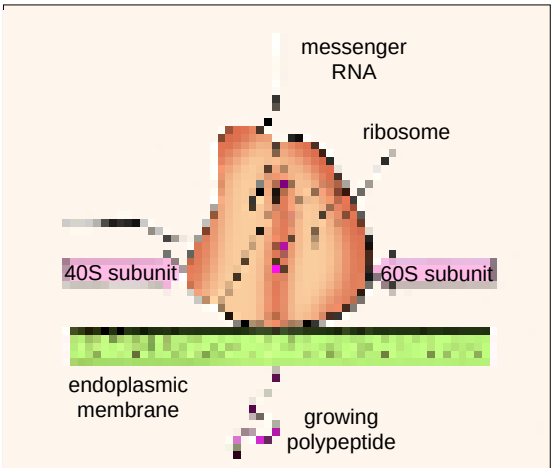


Fig. 14.8. A three dimensional model of eukaryotic cytoplasmic ribosome (after Sheeler and Bianchi, 1987).

$$\text{16S RNA} + \xrightarrow[\text{proteins}]{R_1} \text{RI particles} \xrightarrow{\text{heat}} \text{RI* particles} \xrightarrow{+\text{ S proteins}} \text{30S ribosomal subunit.}$$

In the first step, performed at a low temperature, the 16S RNA binds some of the 30S ribosomal proteins, forming an RI particles (*i.e.*, a reconstitution intermediate) that is inactive. In the second step, the RI particles are heated at 40ºC in the presence of the other proteins that have remained in the supernatant (*i.e.*, S proteins) thereby forming an excited intermediate, RI*, within 20 minutes fully active 30S ribosomal subunits are formed. The self-assembly of 30S subunits is highly specific. It can be achieved with 16S RNA of other bacteria, but not with 16S RNA from yeast or the 23S RNA from *E. coli.*

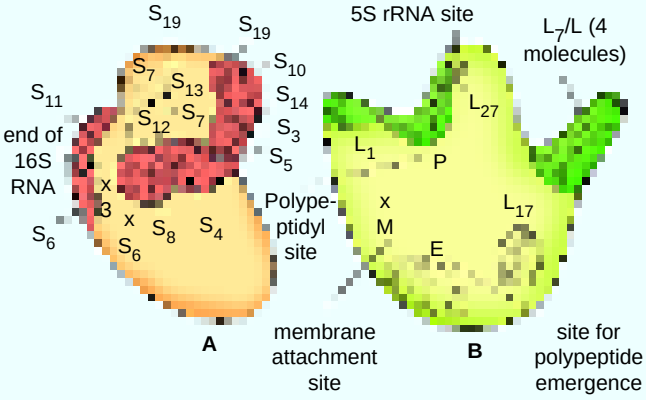


Fig. 14.9. Map of proteins in the ribosome showing their position in small subunit (A) and large subunit (B).

In similar manner, reconstitution of 50S ribosomal subunit is achieved. Finally, a complete functional ribosome is reconstituted spontaneously. In some of these experiments, when ribosomal protein is omitted (or modified) at a time, they show that certain ribosomal proteins require prior to the attachment of other proteins in order to become incorporated in a stepwise manner. For example, some ribosomal proteins, called **initial** or **primary binding proteins** (*e.g.*, L4 protein) bind at specific sites on the naked rRNA and without them the other proteins, called **secondary binding proteins** cannot bind. Initial binding proteins have also been found essential in the control of synthesis of ribosomal proteins.

Further, all ribosomal proteins of the 70S ribosomes also have been isolated and specific antibodies against them have been produced. Various immunological and chemical cross-linking procedures have made possible the construction of maps of the topographical distribution of ribosomal proteins within the ribosomal subunits (Fig. 14.9). Some important sites or centres for specific functions have also been indicated in some of these maps (Fig. 14.10).

COMPARISON OF 70S AND 80S RIBOSOMES

Eukaryotic 80S ribosomes differ from prokaryotic 70S ribosomes in the following respects : (1) they are considerbly larger; (2) they contain a large number of proteins (70–80 types of proteins instead of 53) ; (3) they have four types of RNA molecules instead of three types; (4) their proteins and nucleic acids are large-sized; (5) the RNA-protein ratio is near to 1 : 1 instead of 2 : 1 and (6) several antibiotics, such as **chloramphenicol**, inhibits bacterial but not eukaryotic ribosomes (this is the basis of the use of many antibiotics in medical treatment). Protein synthesis by eukryotic ribosomes is inhibited by **cycloheximide**.

However, eukaryotic ribosomes do not differ functionally from those in prokaryotes in a fundamental way; they perform the same functions, by the same set of chemical reactions. The genetic code is the same for all living organisms, and eukaryotic ribosomes are able to translate bacterial mRNAs efficiently, provided that a "cap" is added enzymatically (**Paterson** and **Rosenberg**, 1979).

Ribosomes from mitochondria and chloroplasts show resemblance to 70S ribosomes of bacteria. Their functions are also inhibited by chloramphenicol. Further, **hybrid ribosomes** containing one bacterial subunit and one subunit from the chloroplast ribosomes are found fully active in protein synthesis, but if hybrid ribosomes contain one subunit of bacteria and another subunit from any eukaryote, they are found to be inactive or non-functional in protein synthesis. However, ribosome constitution experiments have shown some homology between 70S and 80S ribosmes, *e.g.*, proteins L7 and L12 of *E. coli* can replace the homologous proteins in mammalian ribosomes.

BIOGENESIS OF RIBOSOMES

Ribosomes are not self-replicating particles. Synthesis of various component of ribosomes such as rRNAs and proteins, are under genetic control, *i.e.*, rRNAs and mRNAs (for various ribosomal proteins) are transcribed by genes (DNA). Since the mechanism of biogenesis of 70S and 80S ribosomes differ greatly, so can be studied separately as follows :

1. Biogenesis of 70S Ribosomes

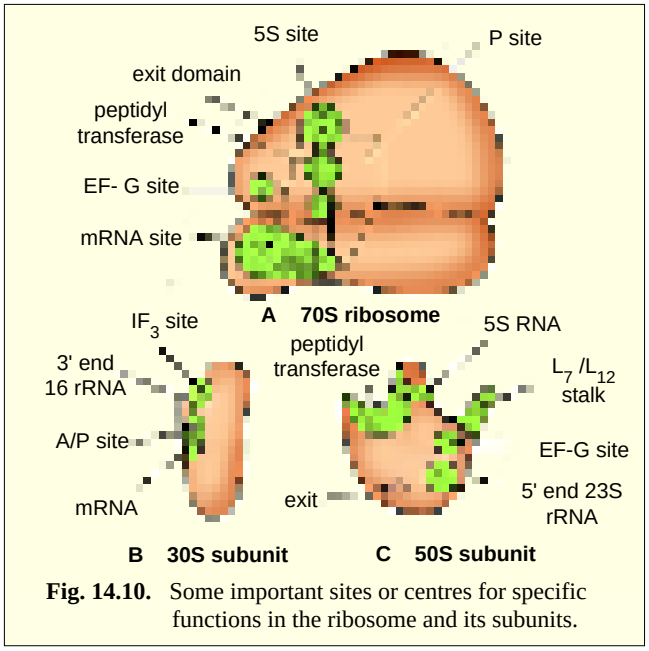


Fig. 14.10. Some important sites or centres for specific functions in the ribosome and its subunits.

Smith *et al.*, (1968) have suggested that in bacteria the RNA genes coding for the 5S, 23S, and 16S ribosomal RNAs are tightly clustered in a region of the chromosome and are present in only few copies. In other words, the ribosomal genes are in a single operon which is transcribed as a unit to synthesize a large molecule of RNA containing the 16S, 23S and 5S rRNA sequences. About 0.4 per cent of a *E.coli* chromosome is devoted to carrying rRNA sequences. In contrast, about 80 per cent of the RNA in an *E. coli* cell is rRNA. This discrepancy in percentages can be explained as follows—First, rRNA is greatly stabilized and protected by ribosomal proteins, and thus any rRNA molecule that is synthesized is expected to have a far longer lifetime than other RNA species (*i.e.*, tRNA, mRNA). Second, in contrast to other genes that may be transcribed only a few times or perhaps not at all during the life of a cell, the rRNA genes are in a state of perpetual transcription.

Thus, in bacteria a single gene transcript containing the sequences of 16S, 23S and 5S rRNAs, is synthesized by a rRNA operon and this larger molecule is thought to undergo both tailoring and chemical modifications before each rRNA molecule assumes its mature form. During tailoring of larger rRNA molecule, 16S rRNA sequence is first of all cleaved off and is separated from the 23S and 5S sequences. The fragment containing 16S information is still larger than the mature 16S rRNA by at least 100 bases and is not methylated : both the methylation and the tailoring of this molecule takes place after it has associated with a number of proteins to form the precursor ribosomal subunits. The 5S rRNA is found not to undergo the processes of tailoring and methylation before it becomes mature (see **Good-enough** and **Levine**, 1974). The whole process of biogenesis of 70S ribosomes takes place in cytoplasm.

Regulation of synthesis of 70S ribosomal proteins. For *E. coli* the task of coordination of synthessis of 54 ribosomal proteins and 3 rRNAs may be quite difficult. Since synthesis of excess of proteins would be wasteful and if some of them were missing, ribosomes would not be assembled properly. Recent findings have indicated that control of synthesis of exact amount of proteins at the proper time is achieved mainly through blocking the translation of mRNAs for ribosomal proteins, when there is an excess of free ribosomal proteins.

For example, in *E. coli* mRNAs tend to be **polycistronic**, *i.e*, they contain the information for several proteins in a single mRNA molecule. Such a transcriptional unit for multiple proteins is called an **operon** and *E. coli* has six operons which contain genes for the ribosomal proteins. The longest of these operons contains genes for 11 ribosomal proteins. It has been shown that L4 ribosomal protein can inhibit the translation of several of these ribosomal proteins from the polycistronic mRNA (Fig., 14.11) (**Yates** and **Nomura**, 1980 ; **Dean** *et al*., 1981). This is called **autogenous regulation of translation**, because, the protein blocks translation by binding to its own mRNA. This inhibition is overcome by addition of purified 23S rRNA, which binds to protein L4. *In vivo* experiments of **Lindahl** and **Zengel** (1982) have investigated that induction of L4 protein overproduction has greatly

reduced the synthesis of all ribosomal proteins by this longest operon. Similar properties have been reported for the proteins of other operons (**Nomura**, 1986). All such ribosomal proteins which are involved in autogenous regulation of translation have one common property—they are primary or initial binding proteins, *i.e.*, they can bind directly to rRNA.

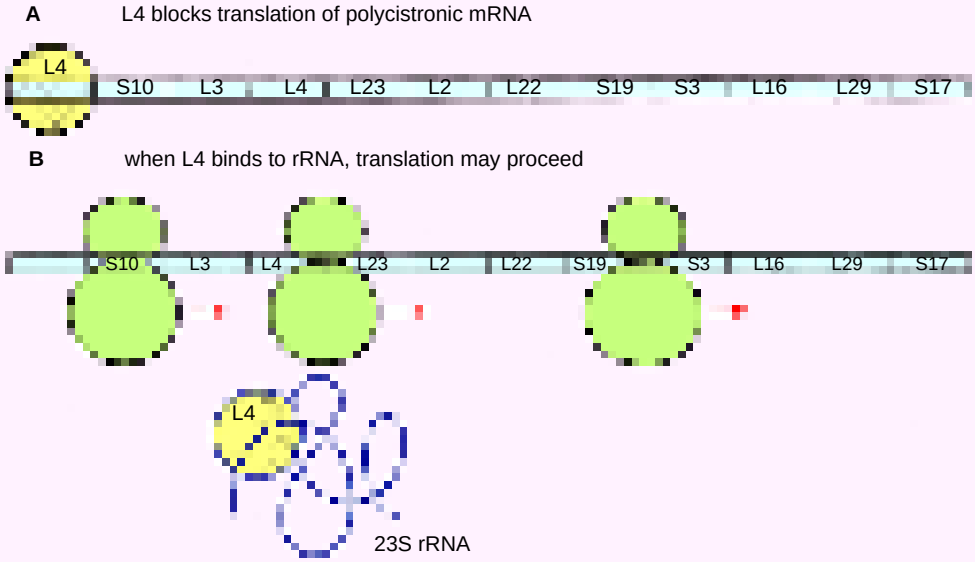


Fig. 14.11. Autogenous regulation of the translation of a polycistronic mRNA coding for 11 ribosomal proteins (after De Robertis and De Robertis, Jr., 1987).

2. Biogenesis of 80S Ribosomes

In eukaryotes, the biogenesis of ribosomes is much more complex and involves a long-lasting process in which several regions of cell are involved. The 5.8S, 18S and 28S rRNAs are transcribed as a much larger molecule in the **nucleolar organizer (NO)** which contains many copies 5.8S, 18S and 28S rRNA genes or **ribosomal DNA** (*i.e.*, there is gene redundancy or amplification). The DNA coding for the 5S rRNA is also highly repetitive, but the molecule is synthesized outside the nucleolus. It is in the nucleolus that newly synthesized rRNA accumulates and becomes associated, presumably by a self-assembly process, with 50 or more ribosomal proteins that have been synthesized in the cytoplasm by usual mechanism of protein synthesis and then migrate to the cytoplasm of cell, in the form of ribosomal subunits.

Biogenesis of 80S ribosomes involves the following three main events :

A. Ribosomal RNA synthesis by nucleolar organizer ;

B. Biosynthesis of ribosomal proteins ;

C. 5S RNA (or 5S rRNA) synthesis.

A. Ribosomal RNA synthesis inside nucleolus. Direct evidence that the nucleolus is responsible for the synthesis of rRNA was obtained in 1964, when it was discovered that an anucleolate mutant (O-nu) of the South African frog *Xenopus laevis*, was unable to synthesize rRNA **Brown** and **Gurdon**, 1964). Since, O-nu embryos were able to continue synthesizing 5S rRNA (**Miller**, 1973), it indicated that these genes were not located in the nucleolar organizer. In *Xenopus* chromosomes, the genes for 5S rRNA are found located at the telomeres.

(1) Ribosomal RNA genes. All organisms have multiple rRNA genes. In case of *Xenopus*, each nucleolar organizer contains 450 rRNA genes. These genes are **tandemly repeated** or **reiterated** along the DNA molecule (in a head to tail arrangement) and are separated from each other by stretches of **spacer DNA**, which is not transcribed. These rRNA genes are being actively transcribed and the

nascent RNA chains are spread perpendicularly to the DNA axis. Each gene is transcribed into a long RNA molecule (which varies in size from 40S to 45S according to species) which will eventually be processed to give rise to 18S, 28S and 5.8S rRNA. Because each rRNA gene has a fixed initiation site (promoter) and a fixed termination site, the transcripts adopt the characteristic "Christmas tree" or "fern leaf" configuration (Fig. 14.12). Nucleolar rRNA genes are transcribed by **RNA polymerase I** (about 100 enzymes per gene). RNA polymerase I molecules are found to remain bound to the nucleolar organisers during mitotic metaphase and anaphase (**Scheer** and **Rose**, 1984). During this period there is no RNA synthesis and so the enzyme molecules must remain in an inactive state.

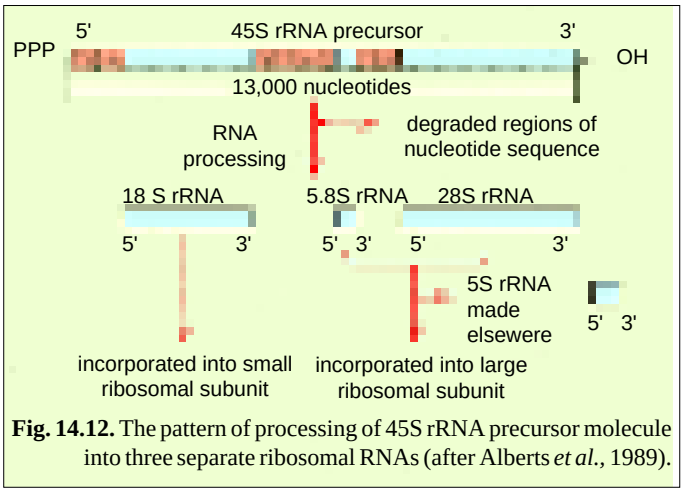


Fig. 14.12. The pattern of processing of 45S rRNA precursor molecule into three separate ribosomal RNAs (after Alberts *et al.*, 1989).

The spacer DNA can be subdivided into a **nontranscribed spacer** and a **transcribed spacer**, the latter is copied into RNA but does not give rise to mature rRNA. Evidently spacer DNA provides multiple functional binding sites that attract factors needed to activate the promoter (**Busby** and **Reeder**, 1983). In electron microscopy, spacers have acted as **sinks** or storage areas for gene-specific binding proteins.

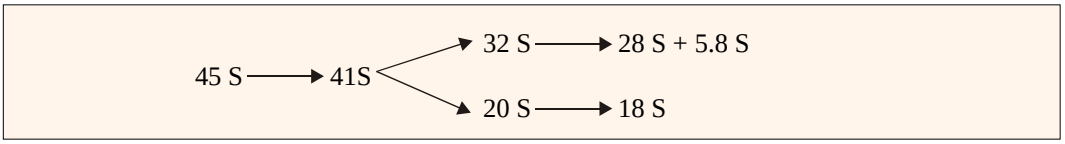
Gene amplification is the process by which a set of genes is selectively replicated. The rDNA in the amphibian oocyte undergoes this process to accumulate in the egg the huge number of ribosomes (10^{12}) that are used in the first stages of development. During pachytene there is an active replication of the nucleolar organizers and the rDNA is amplified 1000-fold. In *Xenopus* egg 25 pg of extra DNA with 2, 000,000 rRNA genes is accommodated by between 1000 to 1500 nucleoli. This amplified DNA is ultimately lost during development. The amplification of specific genes is not a common event. The rDNA is amplified in oocytes of amphibians, some beetles and spiders, as well as in the macromolecules of ciliate protozoons such as *Tetrahymena* and *Stylonichia*. Gene amplification also occurs in the DNA puffs of *Sciarid* dipterans and in chorion (egg shell) genes of *Drosophila*. In all these cases the amplified genetic material is not passed on the future cell generations.

(2) Processing of rRNAs inside nucleolus. As already described, rRNA genes are transcribed into a long precursor RNA (which is 40S in *Xenopus* and 45S in HeLa and other human cells) ; this precursor must be cleaved into 18S, 28S and 5.8 rRNA. In the cleavage process about 50 per cent of the precursor RNA is degraded. In HeLa cell, the processing of rRNA involves the following steps (**Weinberg** *et al*,. 1967 ; **Maden**, 1977) :

(i) The first ribosomal RNA in HeLa cells is a large 45S molecule of 14,000 nucleotides. Within this precursor molecule the rRNAs are separated by stretches of spacer RNA and the order of transcription is : 5' end — 18S—5.8S—28S—3' end. On a fully active gene about 100 RNA polymerase I enzymes (along with transcription factor I or TFI) are transcribing simultaneously on the rRNA gene.

(ii) In nucleolus, 45S RNA is rapidly **methylated**, even before transcription is completed. Methylations occur mostly on the ribose moiety (producing 2'-O- methyribose) and occur only in the 18S (46 methylations) and 28S (71 methylations) sequences that have to be conserved. Those segments of 45S which have to be degradde are not methylated.

(iii) 45S RNA has a lifetime of about 15 minutes and is then cleaved into smaller components as follows :



(iv) 20S RNA is rapidly processed in 18S rRNA and probably due to this reason the small ribosomal subunits appper in the cytoplasm earlier than the large ribosomal subunits. The large ribosomal subunits have a slower RNA processing.

(v) 32S RNA remains in the nucleolus for about 40 minutes and is then cleaved into 28S rRNA and 5.8S rRNA. Both of these rRNAs persist in the nucleolus for another 30 minutes before entering the cytoplasm as part of the large ribosomal subunit.

Thus, about half of 45S rRNA molecule is lost by the successive degradations. This degradation occurs in the regions that are non-methylated and have a higher content of GC. In this way processing of ribosomal RNA results into an increase in methyl groups and decrease in GC content.

Further, all these processing steps do not take place on naked RNA, but rather on RNA-protein complexes. Ribosomal proteins bind to rRNA at the nucleolus, and electron microscopic observations on "christmas tree" spreads stained with antibodies against specific ribosomal proteins have shown that the primary or initial ribosomal proteins bind before the synthesis of 45S rRNA is completed (**Chooi** and **Leiby**, 1981). In addition, a smaller nuclear ribonucleoprotein (U_3 sn RNP) becomes tightly bound to the 32S RNA precursor and is believed to be involved in its processing.

B. Biosynthesis of ribosomal proteins. There is a great possibility that *E. coli*-like translational regulation also exists in eukaryotes. For example, the early embryo of frog is found to contain the mRNAs for all the 70 ribosomal proteins, and except four of these mRNAs, all are not translated until the midblastula stage when synthesis of rRNAs is switched on in the nucleolus (**Pierandrei-Amaldi**, 1982). The remaining four mRNAs are translated at all times.

C. 5S RNA synthesis. The 5S rRNA is synthesized from 20,000 genes in the oocytes but only from 400 genes in the somatic cells, which differ slightly in sequence (six nucleotides out of 120). A gene for 5S rRNA contains an **internal control region** (**ICR**) in its middle region which is found essential for transcription. To this control region of gene remains attached a special protein, called **transcription factor IIIA** or **TF III A** which permits RNA polymerase III enzyme to recognize the promoter of a 5S rRNA gene (*i.e.*, TF III A initiates the synthesis of 5S rRNA).

FUNCTIONS

Ribosomes play a very significant role during biosynthesis of proteins and that will be discussed in a separate chapter.

REVISION QUESTIONS

1. What are the ribosomes ? What is meant by a 70S and 80S ribosome ? Describe the structure of both types of ribosomes.
2. How many types of RNA and proteins are found in the 70S and 80S ribosomes ?
3. Describe the process of biogenesis of ribosomes.
4. Describe the Nomura's experiment of ribosomal self-assembly.
5. Write short notes on the following :
   (i) Transcription factors ; (ii) Three-dimensional structure of ribosome.