CYTOLOGY

Premed 2018 - JU

◉ Sheet ○ Slides

Number

19

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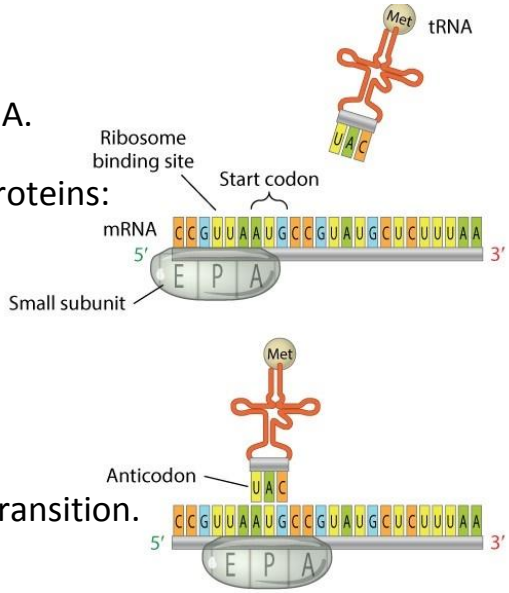
Translation



Translation: the process of making proteins out the mRNA.

We need three major components in order to produce proteins:

1-mRNA template.

2-tRNA.

3-rRNA molecule which is a part of ribosome.

Also, there is micro RNA which has an important role in transition.

****We will talk about these three components in the next pages.**

"More than hundred years ago, scientists thought that the hereditary material is enzyme (proteins), until they did several experiments and they then discovered that the DNA is the hereditary material. This was a great discovery ,but there was a mind problem : they said that proteins are found in all over the cell and also in the cytoplasm while the DNA is found in the nucleus .So, how would information be transferred ?

Also, many molecules were discovered in that time :

- tRNA was discovered as the molecule carries amino acids and works like mediators.

- mRNA that can exist in the nucleus and out of it.

Briefly :Translation is the process of synthesizing proteins from mRNA strand (the result of transcription) by the help of ribosomes and tRNA.

-What is codon?

It is a certain sequence in mRNA that will be translated into amino acid.

-There are many codons in the mRNA strand and each one of them represents certain amino acid ,but there may be more than one codon represents the same amino acid .

-How many codons do we need?

OK, the story is that we have 4 nucleotides (A,T,G,C) and we want to produce 20 amino acids ….the question is how ??? think about the way that we can make MILLIONS of words and sentences although we have only 28 letters ;it is the same principle.

So, if we have only 1 nucleotide in each codon ,we will get 4 amino acids.

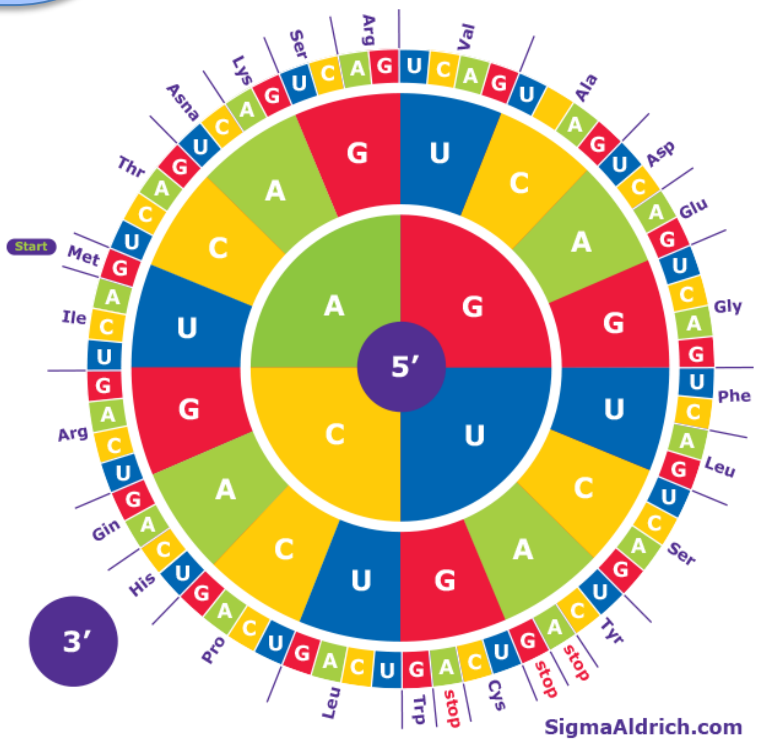
-when we have 2 nucleotides in each codon the result will be 16 amino acids.

Actually ,the answer is 3 (4*4*4=64), note that there are more than 20 amino acids, so more than one codon can be translated into the same amino acid.

These codons are within the mRNA NOT tRNA…..

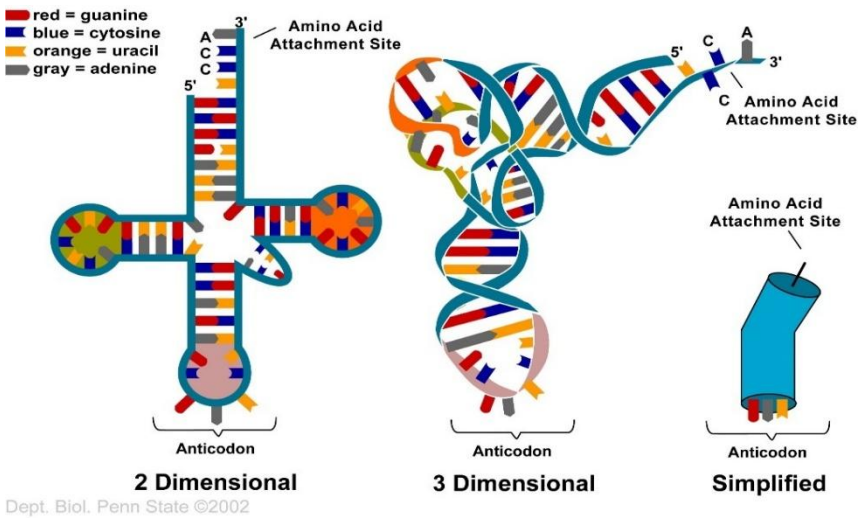
The inner ring represents the first nucleotide in the mRNA (the first part of the codon), the middle one is the second nucleotide and the

NOTE: the third nucleotide can be variable , so it can be changed and will result in the same amino acid. *We call this silent mutation . For example, CG(G/A/C/U)=Arg



-tRNA (transfer RNA): is a short single-stranded RNA molecule that is made of 80 nucleotides and carries amino acids.

Amino acids are attached to **the 3' of the tRNA,** where there is CCA region).



-The enzyme that attaches amino acid to the 3' of the tRNA is called as **Aminoacyl-tRNA synthetases,** and there are 20 Aminoacyl-tRNA synthetases, one for each amino acid .SO, What do these enzymes do ?

They look to the anticodon [which is the part of tRNA that will interact with the codon on the mRNA], also they look to the other regions (sequences) in tRNA. SO, they Know that this tRNA is specific to that amino acid .

What is the special about the tRNA ?

It is the structure of it Becouse the following :

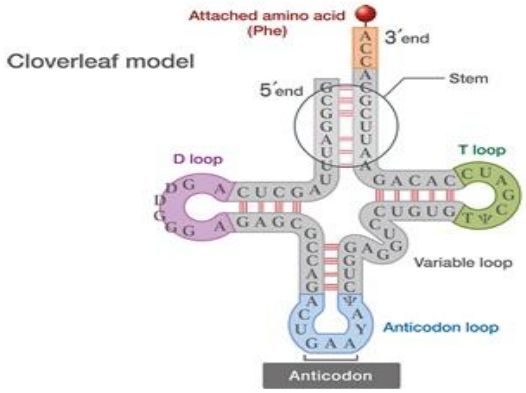
1-It is single chain RNA .

2-It has internal hydrogen bonds.

3-It has loops .

Actually , it has three stem loops .

Active site of each aminoacyl-tRNA synthetase fits only a **specific combination** of aa and tRNA.



Also , it may contain awkward nucleotides, particularly inosine ,so it is not as the usual RNA.

When tRNA is carrying an amino acid , we call it **charged or activated tRNA .**

Note: the amino acid is covalently attached to the ribose of the terminal adenosine at CCA (which is on the 3' of the tRNA).

In reality, it looks like a cloverleaf (because of the presence of complementary stretches of nucleotides) with internal base pairing.

So, these tRNAs have this practicular structure ,generally , RNA molecule takes up a certain structure and this structure can be predicted (For example : A polypeptide is made of 100 amino acids will form one structure which is **the structure that required the least amount of energy to keep it stable -the most stable structure-)**

For example , the aptamers (which are small RNA molecule that can synthise millions of different types of RNA molecules and each one of them will take its own 3D structure and we can predict the structure. These aptamers can be used as targets for proteins and then they bind to these proteins and either activite or inhibit them.

Consequently , RNA molecules can be used as drugs to target proteins to inhibit them instead of using chemical drugs; this RNA molecule can target a certain protein, fit the active site of it and then ditribute in it causing inhibition of this protein.

-Molecules of tRNA are not identical:

* Each carries a specific amino acid on one end (charged tRNA)

** Each has an anticodon on the other end; the anticodon base-pairs with a complementary codon on mRNA .

We can simplify and show tRNA in different structures like L-shaped.

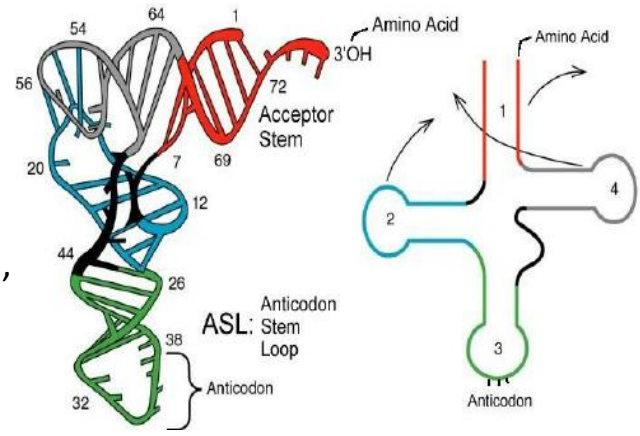
Because of hydrogen bonds, tRNA actually twists and folds into a three-dimensional molecule

tRNA is roughly L-shaped

Attachment of amino acids to the tRNA

Required ATP (just like building any molecule ,

we need ATP to combine them together).



Remember!!! We have 20 aminoacyl-tRNA synthetase(one for each amino acid) and each synthetase is able to bind all the different tRNAs that code for its particular amino acids.Also synthetase covalently join the aa to its tRNA resulting: aminoacyl tRNA/ charged tRNA.

Be attention , we have base-

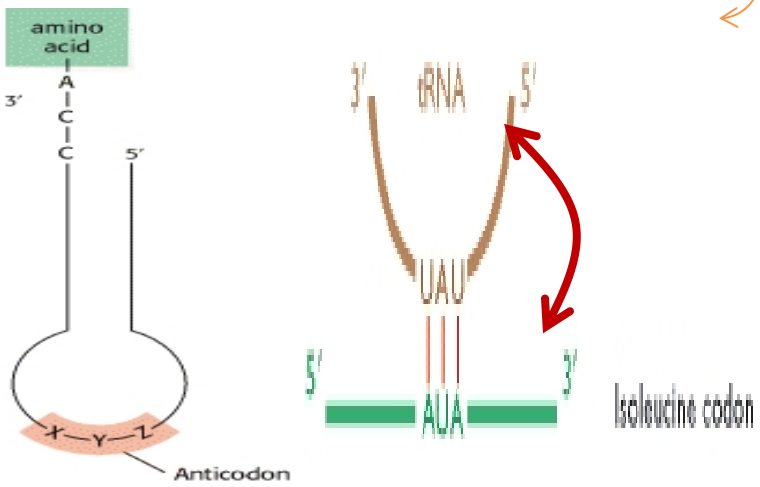
Pairing between the codon (on

The mRNA) and anticodon (on

The tRNA) **and they are antiparallel**

To each other ….here;

Codon 5'AUA 3'



While the anticodon 3' UAU 5'….SO, Be Attention In The EXAM !!!

You have to flip the anticodon to get the right codon and select the right amino acid.

- Translation must be accurate , otherwise disfunctional proteins will be produced

Fidelity (إخلاص) of translation means how the cell make sure that the right amino acid is added to the pepited ?

Fidelity stems from tow steps :

1- First: a correct match between a tRNA and an amino acid, done by the enzyme **aminoacyl-tRNA synthetase**.

(the correct match between the tRNA and amino acid is determined by the synthetase

Which selects the right tRNA for the right amino acid)

2- Second: a correct match between the tRNA anticodon and an mRNA codon

(The attachment between the tRNA and the mRNA)

Example , the antibiotics that we use when we get coryza (common cold) , what do they do exactly ?

[they target the bacterial translation process, **one of those** it attaches the wrong amino acid to the elengoted peptide making the bactira unable to produce functional proteins and bacteria will eventually die.]

The third nucleotide of the codon can be flexible

and this is known as **wobble base-pairing.**

So, the wobble base-pairing is the base-pairing

Between the third nucleotide of the codon of

The mRNA with the first nucleotide of the anticodon

Why the first nucleotide of the anticodon?

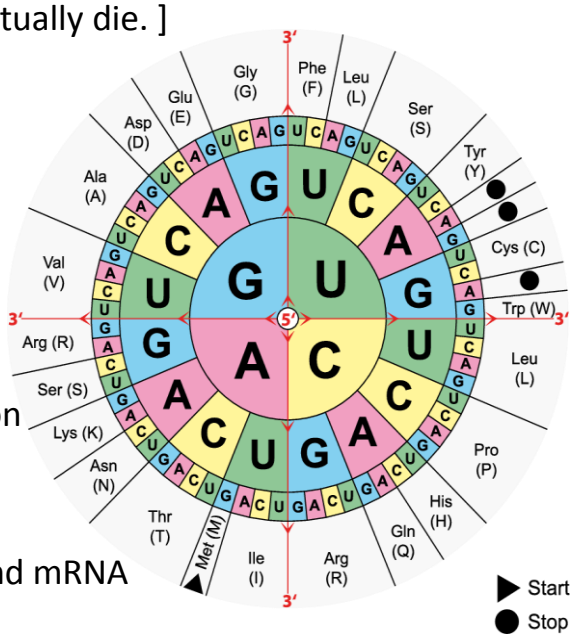
Becous it is on the 5’ of the anticodon (the tRNA and mRNA

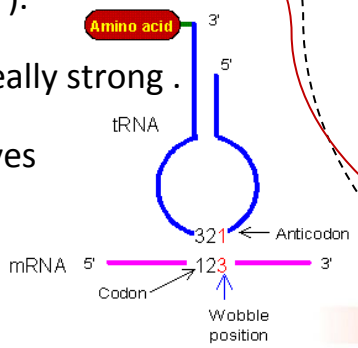
Are antiparallel to each other).

And this base-pairing is not really strong .

so, it can be verable and it gives

flexibility.





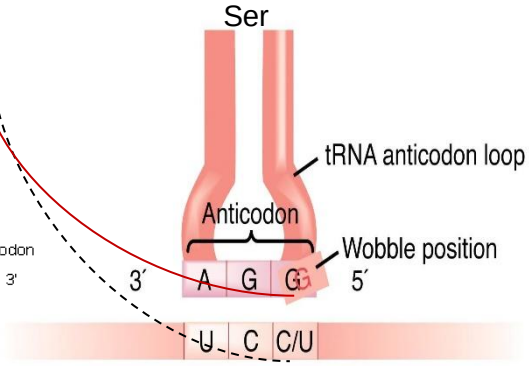


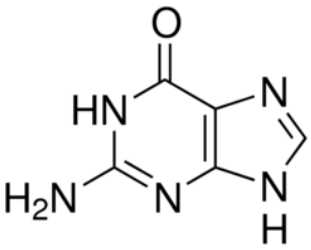
TABLE 1.6 RULES FOR BASE PAIRING CAN BE RELAXED (WOBBLE) AT POSITION 3 OF A CODON	
Base at 5′ end of tRNA anticodon	**Base recognized at 3′ end of mRNA codon**
A	U only
C	G only
G (or I)[a]	C or U
U	A or G

[a]Inosine (I) is a deaminated form of guanosine.

Table 1.6 Human Molecular Genetics, 4ed. (© Garland Science)

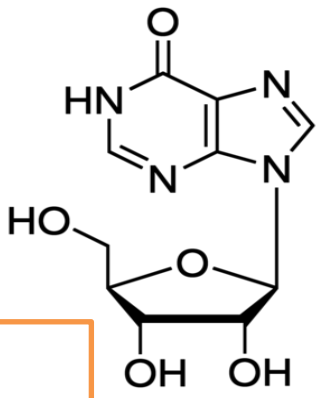
5′ Co

Guanine



This will be gone out in the Inosine.

Inosine



This table is not for memorizing

The table above shows how can an anticodon (in tRNA) bind to different codons (in mRNA) due to the flexibility of the third nucleotide of the codon .

The inosine ,which can exist on the anticodon , is a deaminated form of guanosine or guanin……And this is a sort of flexibility.

As the Dr mentioned, this genetic table will be given in the exam. We talk here about the **codons** on the mRNA (Not anticodon),the codon AUU will be encoded into Ile and if you change the third nucleotide to A or C it will give Ile ,too; this will give certain protection against mutation; if there is a mutation and the third nucleotide is changed the resultant amino acid will be the same and this is what we call :SILENT MUTATION.

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

Note: in Leu amino acid , If we change the first nucleotide like U to C ,it still be the same amino acid (this is for leu).However,if we change the first nucleotide from C to A ,it will give an another amino acid which is Ile ,**the idea is 💡 that when we change leucine(Leu) to isoleucine(Ile) ,hardly anything will happen to the protein as Ile and Leu are from the same class ,both of them are nonpolar amino acids and they are branched ,so the structure of the protein will not be changed so much. Again, this gives sort of protection against mutations. We can consider the Leu and Ile as isomers.**

-There are 4 codons that have to be memorized:

AUG [which is the first codon that will be translated and is encoded for Met]

- Stop codons which are: UAA , UAG and UGA here you finish/terminate translation .

Don't mix between the stop codon (translation) and termination sequences (transcription) .

Features of the genetic codon:

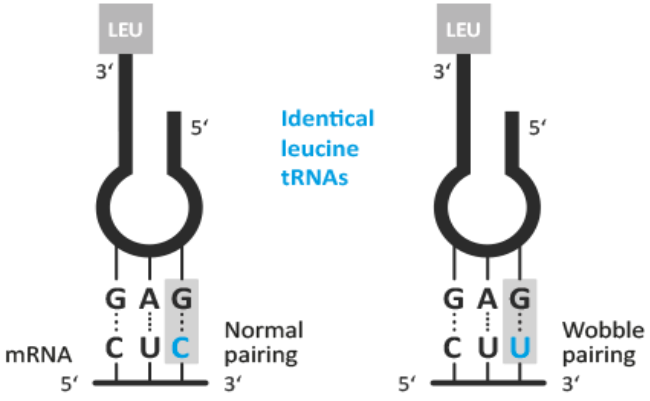
1-The previous genetic table is true for most organisms,but there are some exceptions in certain organisms and mitochondria**. So, the genetic codon is not universal**

Example: AUA in mitochondria represents (methionine), but in cytosol (isoleucine).(not to be memorized)

So, mitochondrial tRNA can read the mRNA as cytosolic tRNA.

2- Wobble base pairing and codon is degenerate (filthy),Why the codon is degenerate ? Becouse of the wobble base pairing ;third nucleotide can be different as the interaction is not strong (the idea that is act as a buffer [means a zone/region where we have no changes/mediatory activities. So, the degeneracy of the code acts as a buffer against deleterious mutations.

The bases that are common to several codons are usually the first and second bases, with more room for variation in the third base, which is called the "wobble" base.



	U	C	A	G	
U	UUU Phe UUC Phe UUA Leu UUG Leu	UCU Ser UCC Ser UCA Ser UCG Ser	UAU Tyr UAC Tyr UAA Stop UAG Stop	UGU Cys UGC Cys UGA Stop UGG Trp	U C A G
C	CUU Leu CUC Leu CUA Leu CUG Leu	CCU Pro CCC Pro CCA Pro CCG Pro	CAU His CAC His CAA Gln CAG Gln	CGU Arg CGC Arg CGA Arg CGG Arg	U C A G

Relaxed base pairing results from the formation of G-U base pairs.

Notes:

- All 64 possible codons of the genetic code and the amino acid specified by each, as read in the 5'→3' direction from the mRNA sequence.

-The interpretations of the 64 codons in the 'universal' genetic code are shown in black immediately to the right of the codons.

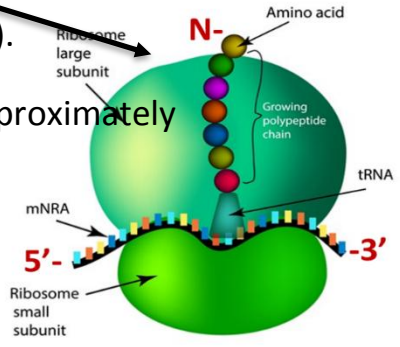
-Sixty-one codons specify an amino acid.

-Three STOP codons (UAA, UAG, and UGA) do not encode any amino acid.

The genetic code for mitochondrial mRNA (mtDNA) conforms to the universal code except for a few variants. (the details are not required)

Ribosomes

This is the factory of proteins synthesis in both prokaryotic and eukaryotic cells. It is made of large ribosomal subunit and small ribosomal subunit, in between we have the mRNA and here we have synthesis of polypeptide. Ribosomes are probably the most abundant protein in the cells (we need a lot of ribosomes inside the cell to handle all the proteins synthesis activities going on the cell).

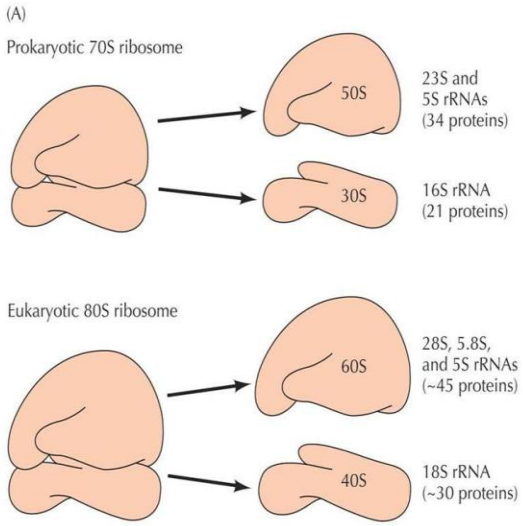


E. coli contain about 20,000 ribosomes, which account for approximately 25% of the dry weight of the cell. Also, rapidly growing mammalian cells contain about 10 million ribosomes.

The important thing that is the larger ribosomal subunit is the one that catalyzes the reaction itself (The peptides bonds formation between the amino acids …(peptidyl transferase reaction) specifically they are rRNA in the large ribosomal subunit that catalyzes this reaction. And this discovery was huge specifically to those interested in evolution (when they discovered that RNA molecules can act as both enzymes and genetic material, they said that the life started from RNA).

In eukaryotes and prokaryotes, ribosomes have the same structure ,and this shows you something about the importance of ribosomes (they have to be conserved ,so they are important and any change happens to these ribosomes, it will kill these organisms .

ribosome = large subunit + small subunit



The unit S represents to sedimentation (the rate of sedimentation),when we use the Centrifugation and the molecules move, large molecules will travel slower than the smaller molecules.So,where the large molecules stop through the Centrifugation process ,they are given the rate of movement called sedimentation.

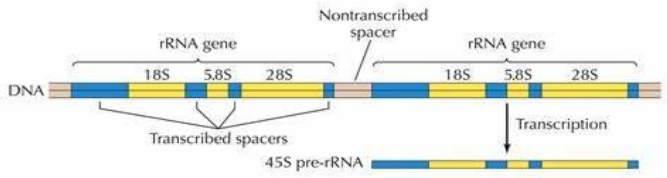
***The doctor mentioned that we don't have to know the details.

Notice that the 23S in combine with 55S doesn't equal 50S (In large subunit in prokaryotes),so we consider the Wight and the shape of the molecule to determine the sedimentation.

Again it is made of large and small subunits and it is made of rRNA molecules as well as proteins .

'Note: we have different rRNA molecules of different structures.'

The interesting thing that the 18S,5.8S and 28S are encoded by the same gene and they are all exist as tandem repeat of 5 different chromosomes ,so the same cluster is tandemly repeated on the same chromosome and the same cluster exists on different chromosomes ,why? Because cells need a lot of rRNA molecules to synthesis all of the proteins that are needed by the cells.



cells need to have multiple genes for rRNA molecules so they can produce a lot of ribosomes.The same story with histones (histones also exist as genes on multiple chromosomes so they can be produced all the time during DNA duplication).

So, these three (18S,5.8S and 28S) rRNAs are produced as a single rRNA and then cut into three different rRNA molecules.

this information is involved in the slides: (The major rRNA species are synthesized by cleavage of a shared primary transcription). (A) In human cells, the 18S, 5.8S, and 28S rRNAs are encoded by a single transcription unit that is **13 kb long.** It occurs within tandem repeat units of about **40 kb** that also includes a roughly **27 kb non-transcribed (intergenic) spacer**.

(B) Transcription by RNA polymerase I produces a **13 kb primary transcript** (45S rRNA) that then undergoes a **complex series of post-transcriptional cleavages**

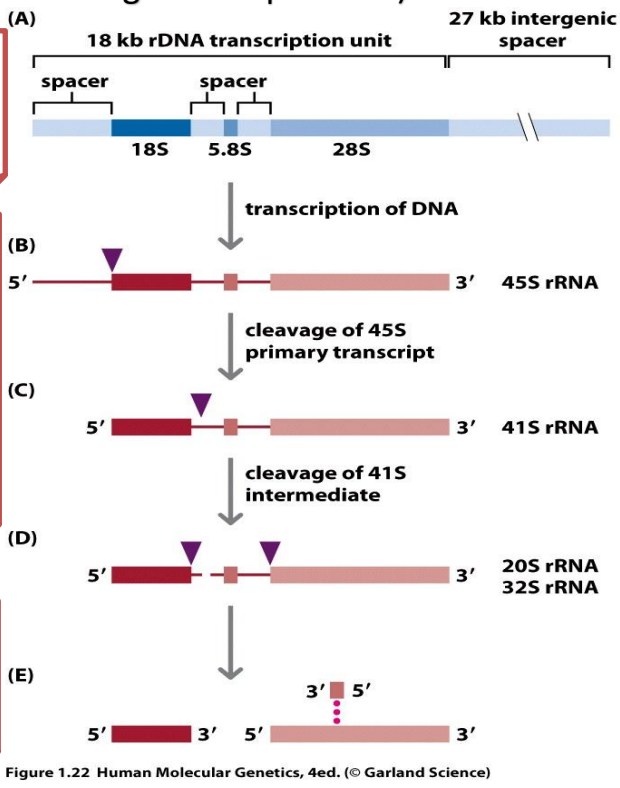
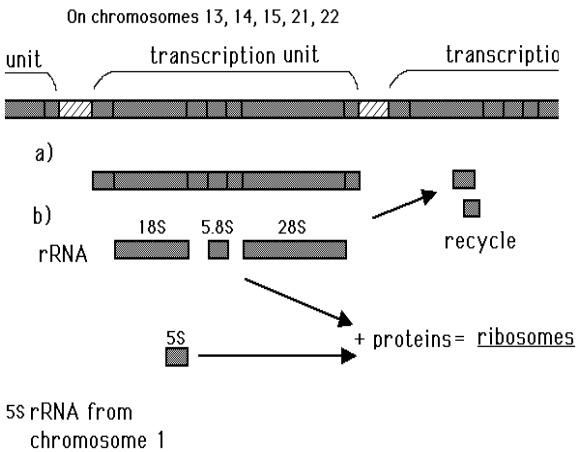


Figure 1.22 Human Molecular Genetics, 4ed. (© Garland Science)

(C–E) Ultimately, individual 18S, 28S, and 5.8S rRNA molecules are released.
The 18S rRNA will form part of the small ribosomal subunit.
The 5.8S rRNA binds to a complementary segment of the 28S rRNA; the resulting complex will form part of the large ribosomal subunit
The latter also contains 5S rRNA, which is encoded separately by dedicated genes transcribed by RNA polymerase III.

Note: the same clusters exist on 5 different chromosomes and are produced by the same promotor producing these 3 rRNA molecules.

5s rRNA is produced from a totally diffrernt gene than these three .In human cells, a cluster of around 250 genes on several chromosomes synthesize 5S rRNA using Pol III.



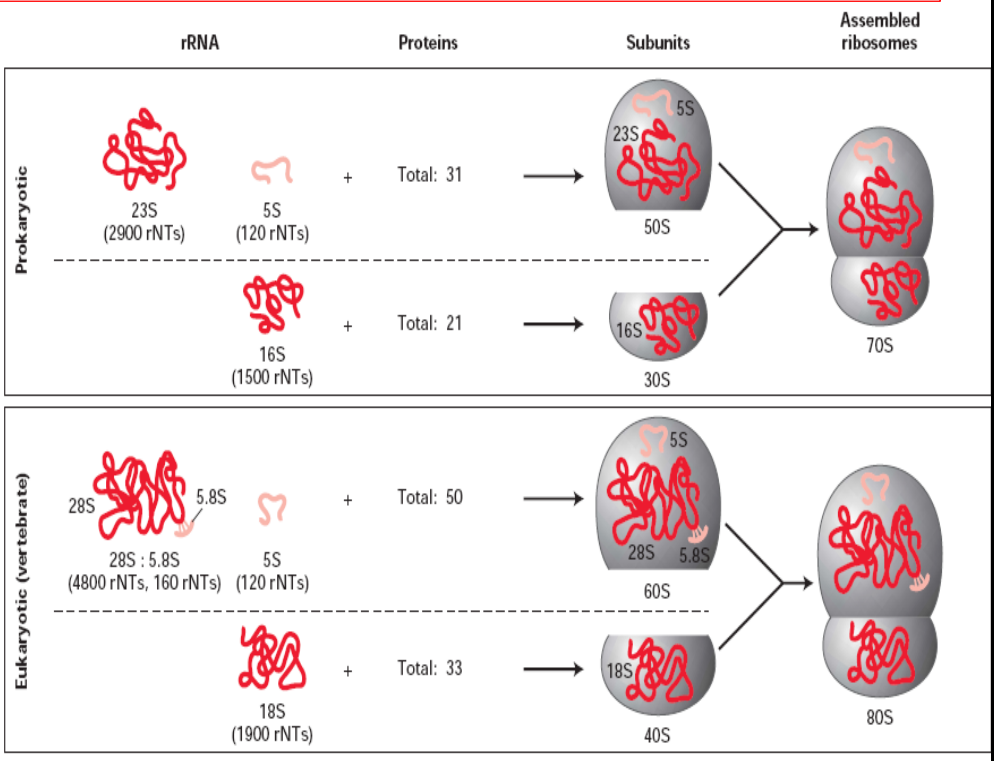
Remember!! In the first lecture in transcription; the doctor mentioned that in the eukaryotes; there are three types of RNA polymarases:1-RNA polymarase I(is responsible for synthesis of rRNA molecules [18S,5.8S AND 28S]) .

2-RNA polymarase II(is responsible for synthesis of mRNA and micro RNA.)

3-RNA polymarase III(is responsible for synthesis of tRNA as well as 5S rRNA molecule.)

Both in eukaryotes and prokaryotes we have combination between rRNA molecules and proteins .

Which rRNA molecule is responsible for proteins synthesis ? It is the 28S rRNA . when ever you have an RNA molecule acting as an enzyme we call it ribozyme . So, enzymes are mainly proteins (99%i,if not more, of enzymes are proteins)but you can have RNA molecules as enzymes (but the Dr said here proteins I think that he meant enzymes)like 28S rRNA that catalyses the peptides bonds formation .the RNA splicing is also catalyzed by RNA molecules NOT proteins .



-Note that:

-A combination between 28S,5.8Sand 5S rRNAs with proteins forms the large ribosomal subunit.

-A combination between 18S rRNA with proteins forms the small ribosomal subunit.

*rRNA is the most **abundant** type of cellular RNA

*rRNA genes are transcribed, the RNA is processed and assembled with proteins **imported** from the cytoplasm, the resulting ribosomal subunits are then **exported** via nuclear pores to the cytoplasm

*Large and small subunits **join** to form a functional ribomes only when they attach to the mRNA

*Bacterial and eukaryotic ribosomes are somewhat similar but have significant differences: some antibiotic drugs (tetracycline and streptomycin) specifically target bacterial ribosomes and inhibit protein synthesis without harming eukaryotic ribosomes.

*The large ribosomal subunit includes cavities and inside these cavities we have binding sites of tRNA molecule and we have 3 cavities ; they are known as A,P & E.

*A is where we have the tRNA (that is carrying the amino acid)binds to it .

*P is where we have the formation of the peptide bonds .

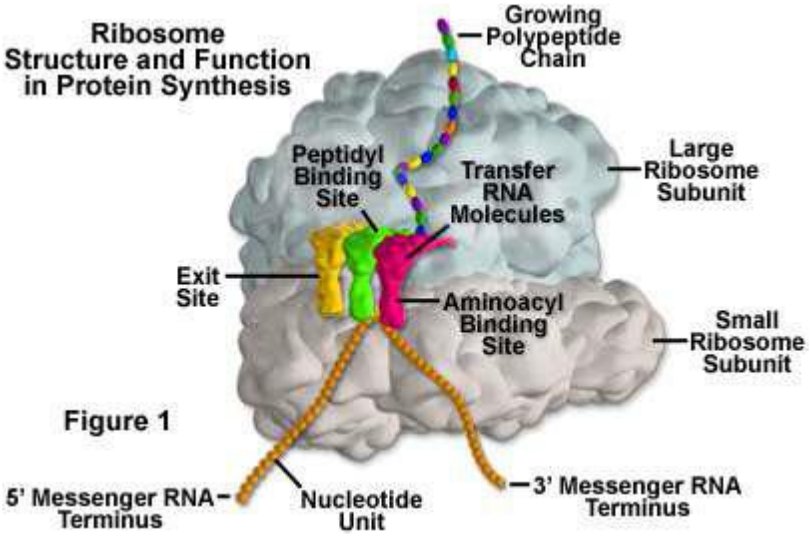
*E is from where the empty (uncharged)tRNA leaves the ribosome.

As mentioned in the slides :

-The **P site** holds the tRNA that carries the growing polypeptide chain

-The **A site** holds the tRNA that carries the next amino acid to be added to the chain

-The **E site** is the exit site, where discharged tRNAs leaves the ribosome



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