RNA

Ribonucleic Acid

- **Ribonucleic acid (RNA)- long, unbranched macromolecule consisting of nucleotides joined by 3/ - 5/ phosphodiester bonds.**
- **The number of ribonucleotides in RNA ranges from as few as 75 to many thousands.**

Differences with DNA

1. **The sugar moiety in RNA to which the phosphate and nitrogen bases are attached, is ribose rather than 2/ deoxyribose of DNA. Ribose contains a 2/ hydroxyl group not present in deoxyribose.**
2. **RNA contains the pyrimidine uracil(U) in place of thymine. Uracil like thymine can form a base pair with adenine by 2 hydrogen bonds. However, it lacks methyl groups in thymine.**

3. The native RNA is single stranded rather a double stranded helical structure characteristic of DNA. The single stranded of RNA may fold back on itself like a hairpin and thus acquire the double stranded pattern. In the region of hairpin loops, A pairs with U and G pairs with C.

G can also form a base pair with uracil but it is less strong than the G-C base pair. The base pairing in RNA hairpin is frequently imperfect.
Some of apposing bases may not be complementary and one or more bases along a single strand may be looped out to facilitate the pairing of the others.
The proportion of helical regions in various types of RNA varies over a wide range, although a value of 50% is typical.

4. Since the RNA molecule is single stranded and complementary to only one of the two strands of a gene. It need not have complementary base ratios.
Its adenine content does not necessarily equal its uracil content, nor does its guanine content necessarily equal its cytosine content.

5. RNA can be hydrolyzed by weak alkali (pH 9 at 100^{O}C) to $2^{/,}$ 3/-cyclic diesters of mononucleotides via an intermediate compound called $2^{/}$, $3^{/}$, $5^{/}$ triester. This intermediate however, cannot be formed in alkali treated DNA b/c of absence of $2^{/}$-hydroxyle group in its molecule. Thus RNA is alkali labile whereas DNA is alkali stable.

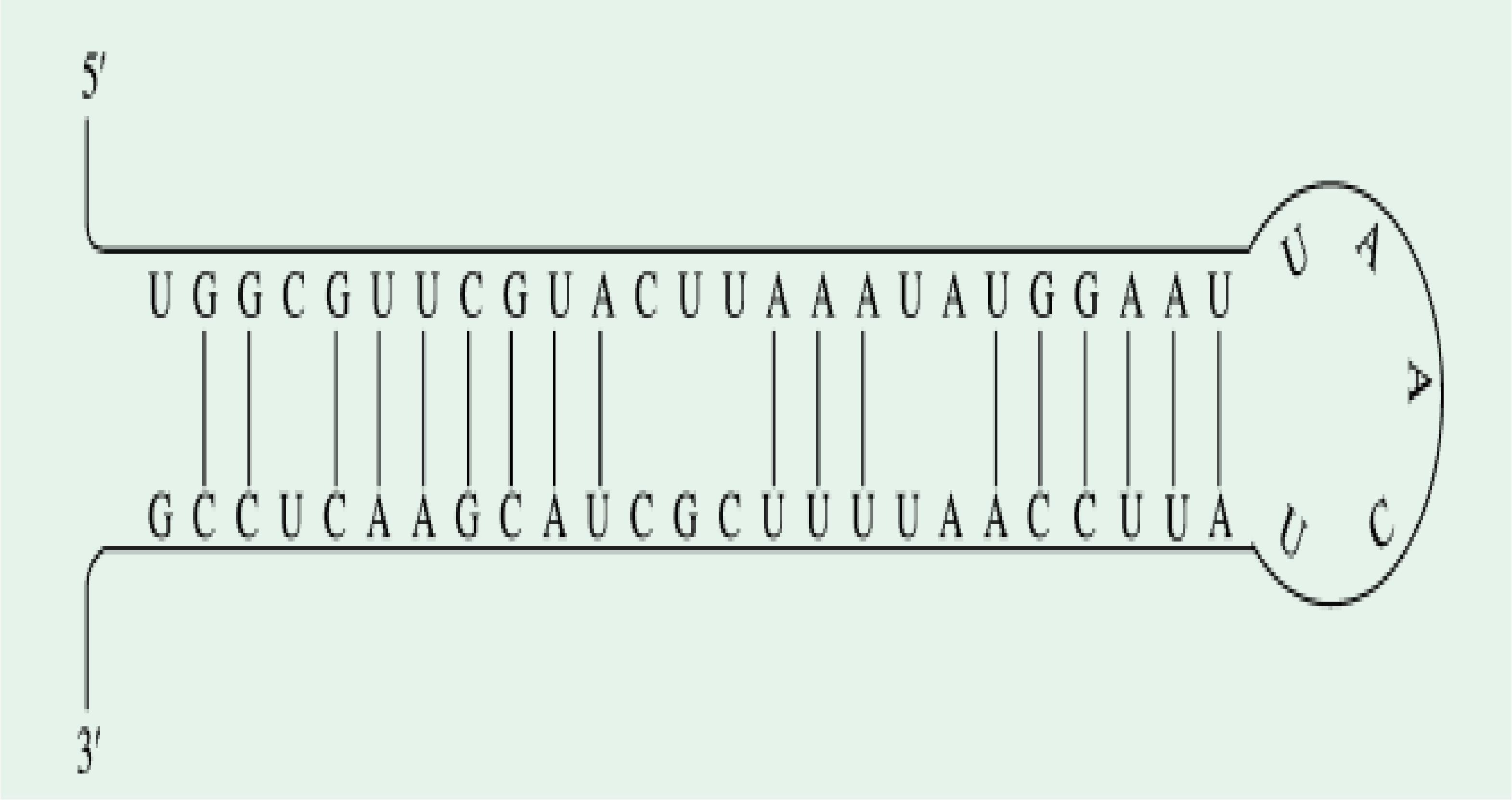


Fig. 15–35. Secondary structure of an RNA molecule

Comparison between DNA and RNA

DNA	*RNA*
1. Found mainly in the chromatin of the cell nucleus	1. Most of RNA (90%) is present in the cell cytoplasm and a little (10%) in the nucleolus.
2. Never present in free state in cytoplasm	2. May be present in free state.
3. Normally double-stranded and rarely single-stranded.	3. Normally single-stranded and rarely double-stranded.
4. DNA has both 'sense' and 'antisense' strands.	4. The sequence of an RNA molecule is the same as that of the 'antisense' strand.
5. Sugar moiety in DNA is 2′-deoxyribose (hence the nomenclature) which contains an H atom at C-2.	5. Sugar moiety in RNA is ribose (hence the nomenclature) which contains a 2′-hydroxyl group.

Comparison between DNA and RNA

6. Sugars in DNA are in the $C_{2'}$ - endo form.	6. Sugars in RNA are in the $C_{3'}$ - endo form.
7. The common nitrogenous bases are adenine, guanine, cytosine and thymine (but not uracil).	7. The common nitrogenous bases are adenine, guanine, cytosine and uracil (but not thymine)
8. Base pairing is inevitable during which adenine pairs with thymine and guanine with cytosine.	8. In case pairing takes place, adenine pairs with uracil and guanine with cytosine.
9. The base ratios (A/T and G/C) are necessarily around one.	9. It need not have complementary base ratios.
10. Base pairing involves the entire length of DNA molecule.	10. Base pairing takes place in only the helical regions of RNA molecule, which amount to roughly half (50%) of the entire RNA molecule.

Comparison between DNA and RNA

11. DNA contains only few unusual bases.	11. RNA contains comparatively more unusual bases.
12. DNA is of 3 types : filamentous (double helical or duplex), circular or single-stranded.	12. RNA is of 5 tyes : viral RNA, rRNA, tRNA, mRNA and double-stranded RNA.
13. It consists of a large number of nucleotides (up to 3-4 million) and has, therefore, high molecular weight.	13. It consists of fewer nucleotides (up to 12,000) and has, therefore, low molecular weight.
14. DNA is alkali-stable.	14. RNA is alkali-labile.
15. DNA stains blue with azureph thalate.	15. RNA stains red with azureph thalate.
16. DNA acts as a template for its synthesis.	16. RNA does not act as a template for its synthesis.
17. DNA on replication forms DNA and on transcription forms RNA.	17. Usually RNA does not replicate or transcribe.
18. During replicatin, exonuclease is needed.	18. During biosynthesis, exonuclease is not needed.
19. DNA is partially reversible only under certain conditions of slow cooling (= annealing).	19. RNA exhibits complete and practically instantaneous reversibility of the process of melting.
20. DNA undergoes mutation.	20. RNA does not undergo mutation.
21. DNA is the usual genetic material.	21. RNA is the genetic material of some viruses only.
22. DNA is stained green with a dye, pyronin.	22. RNA is stained red with pyronin.

Structure of RNA

- Primary structure of RNA is very similar to DNA structure.
- RNA is also synthesized by polymerization of four types of ribonucleotides.
- Phosphodiester bond
- Majority of RNA in cell is single stranded.
- Some dsRNA is also present.
- The structure of dsRNA is similar to A-DNA.
- Three major classes of RNA are present.
- rRNA
- tRNA
- mRNA

Structure of RNA

- **Ribosomal RNA**
- **It is most stable form of RNA and is found in ribosomes.**
- **It has the highest molecular weight and is sedimented when a cell homogenate containing 10^{-2} M of Mg^{2+} is centrifuged at high speed (100, 000gravity for 120 minutes).**
- **rRNA is most abundant of all cell types of RNAs and make up about 80% of the total RNA of a cell.**
- **Ribosomal RNA represents about 40-60% of the total weight of ribosomes.**
- **All types of rRNA except the 5S rRNA are processed from a single 45S precursor RNA molecule in the nucleolus.**
- **The 5S rRNA apparently has its own precursor which is independently transcribed.**
- **large in size**

Structure of RNA

- **It is remarkable to note that rRNA from all sources has G-C contents more than 50%. The rRNA molecule appears as single unbranched strand.**
- **At low ionic strengthen, the molecule shows a compact rod with random coiling.**
- **At high strengthen, the molecule reveals the presence of compact helical regions with complementary base pairing and looped outer region. The helical structure results from a folding back of a single stranded polymer at areas where hydrogen boding is possible because of short lengths of commentary structures.**

Structure of RNA

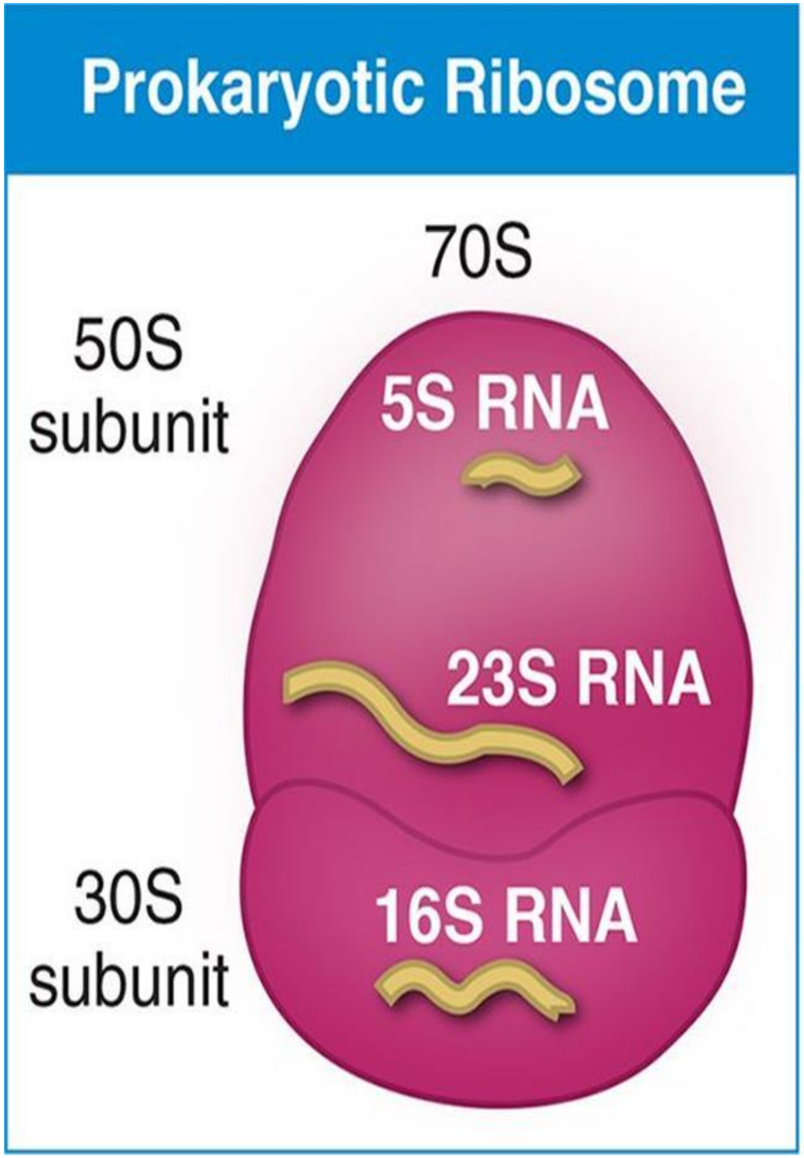
- **In the bacterium, *Escherichia coli***
- **predominant class of cellular RNA**
- **Prokaryotes (70S)**
- **small subunit(30S)- one RNA(16S, 1541 bases)and 21 proteins(S-1 to S-21).**
- **Large subunit(50S)- has two RNAs**
- **23S RNA(2904 bases) and 5S RNA(120 bases) and 31 proteins (L-1 to L-31).**

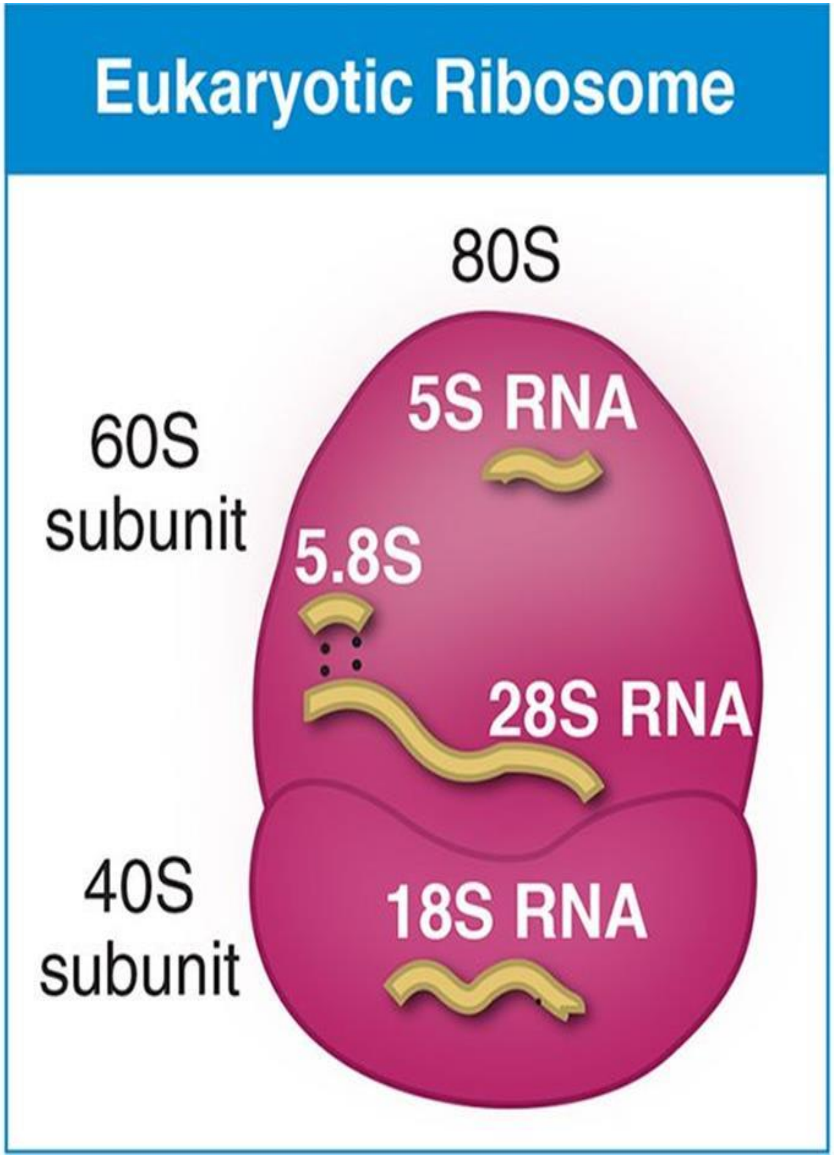
Structure of RNA

- **Eukaryotes (80S)**
- **small subunit(40S)- one RNA(18S, 1874 bases)and 33 proteins(S-1 to S-33).**
- **Large subunit(60S)- has three RNAs**
- **28S RNA, (4718 bases) and 5.8S RNA(160 bases), 5S(120 bases) and 49 proteins (L-1 to L-49).**

Structure of RNA

- **Ribosomal RNA**
- **predominant class of cellular RNA**
- **large in size**
- **28S and 18S(Eukaryotes)**
- **23S and 16S (Prokaryotes)**
- **along with minor component of 5S(both prokaryotes and Eukaryotes)**
- **Another small RNA of 5.8S(Eukaryotes ribosomes).**
- **tRNA**
- **Relatively small(4S, 75-100nucleotide)**


Prokaryotic Ribosome
70S
50S subunit
5S RNA
23S RNA
30S subunit
16S RNA


Eukaryotic Ribosome
80S
60S subunit
5S RNA
5.8S
28S RNA
40S subunit
18S RNA

Consequently, a formula for conversion is required to ensure that the appropriate setting is used in an experiment. The relationship between RPM and RCF is as follows: g = (1.118 × 10-5) R S2

Conversion Table

Speed (RPM)	Rotor Radius (from center of rotor to sample) in centimeters											
	4	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
1000	45	56	67	78	89	101	112	123	134	145	157	168
1500	101	126	151	176	201	226	252	277	302	327	352	377
2000	179	224	268	313	358	402	447	492	537	581	626	671
2500	280	349	419	489	559	629	699	769	839	908	978	1048
3000	402	503	604	704	805	906	1006	1107	1207	1308	1409	1509
3500	548	685	822	959	1096	1233	1370	1507	1643	1780	1917	2054
4000	716	894	1073	1252	1431	1610	1789	1968	2147	2325	2504	2683
4500	906	1132	1358	1585	1811	2038	2264	2490	2717	2943	3170	3396
5000	1118	1398	1677	1957	2236	2516	2795	3075	3354	3634	3913	4193
5500	1353	1691	2029	2367	2706	3044	3382	3720	4058	4397	4735	5073
6000	1610	2012	2415	2817	3220	3622	4025	4427	4830	5232	5635	6037
6500	1889	2362	2834	3306	3779	4251	4724	5196	5668	6141	6613	7085
7000	2191	2739	3287	3835	4383	4930	5478	6026	6574	7122	7669	8217
7500	2516	3144	3773	4402	5031	5660	6289	6918	7547	8175	8804	9433
8000	2862	3578	4293	5009	5724	6440	7155	7871	8586	9302	10017	10733
8500	3231	4039	4847	5654	6462	7270	8078	8885	9693	10501	11309	12116
9000	3622	4528	5433	6339	7245	8150	9056	9961	10867	11773	12678	13584
9500	4036	5045	6054	7063	8072	9081	10090	11099	12108	13117	14126	15135
10000	4472	5590	6708	7826	8944	10062	11180	12298	13416	14534	15652	16770
10500	4930	6163	7396	8628	9861	11093	12326	13559	14791	16024	17256	18489
11000	5411	6764	8117	9469	10822	12175	13528	14881	16233	17586	18939	20292
11500	5914	7393	8871	10350	11828	13307	14786	16264	17743	19221	20700	22178
12000	6440	8050	9660	11269	12879	14489	16099	17709	19319	20929	22539	24149
13000	7558	9447	11337	13226	15115	17005	18894	20784	22673	24562	26452	28341
13500	8150	10188	12225	14263	16300	18338	20376	22413	24451	26488	28526	30563
14000	8765	10956	13148	15339	17530	19722	21913	24104	26295	28487	30678	32869

Structure of RNA

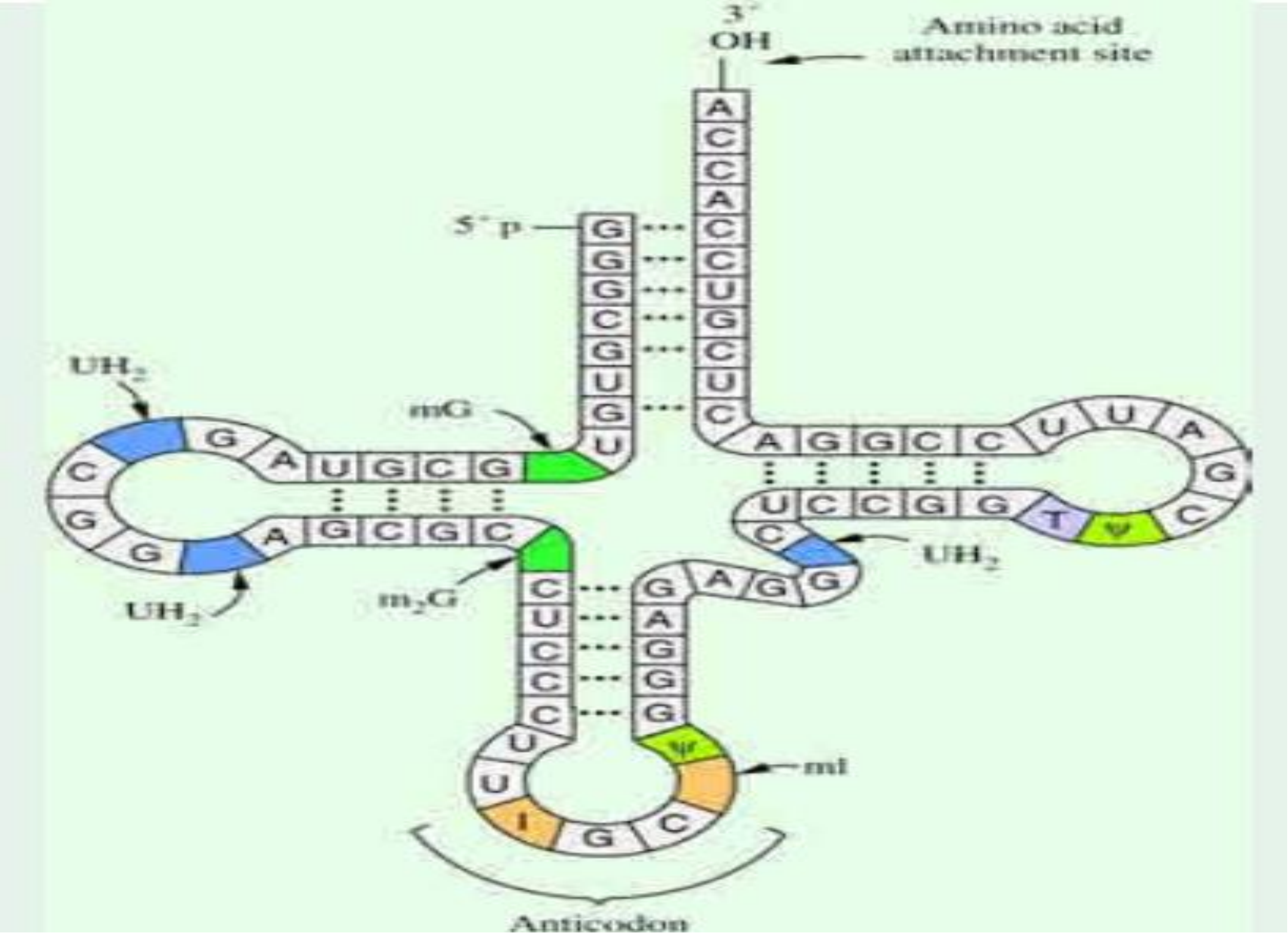
tRNA

- **Amounts to about 15% of the total RNA of the cell.**
- **tRNA remains dissolved in solution after centrifuging a broken cell suspension at 100, 000 X gravity for several hours.**
- **The tRNA act as specific carriers of activated amino acids to specific sites on the protein synthesizing templates.**
- **There are 20 types of tRNAs . Since the code is degenerate.**
- **There also may be more than one tRNA for a specific amino acid.**

Structure of RNA

- **tRNA**
- **In bacterial cells, there are more than 70 tRNA .**
- **in eukaryotic cells, this number is even greater.b/c there are tRNA specific of mitochondria and chloroplasts.**
- **Isoacceptor tRNAs: There are generally several tRNAs specific of the same amino acid (sometimes upto 4 or 5).**
- **Each tRNA is specific of an amino acid. E.g tRNA ^{Ala}, tRNA^{ser}.**
- **alanyle-tRNA ^{Ala} or alanyl-tRNA.**
- **tRNA are quite stable in prokaryotes, they are somewhat less stable in eukaryotic organisms.**

Primary structure of a tRNA molecule



Structure of RNA

- **Common structural features of tRNA**

1.All tRNA molecules have a common design and consist of 3 folds giving it a shape of the cloverleaf with four arms. The long longer tRNAs have short fifth or extra arm. The actual 3-D structural of a tRNA looks more like a twisted L than a cloverleaf.

2.All tRNA molecules are unbranched chains containing 73 to 93 ribonucleotides residues, (MW: 24,000 to 31000).

3. They contain 5 to 7 unusual basis. Many of these unusual bases are methylated or dimethylated derivatives of A, U, G and C. Includes pseudouridine, various methylated adenines and guanines, methylated pryimidines such as

➢ Common structural features of tRNA

4.The 5/ end of tRNA is phosphorylated. The The 5/ terminal residue is usually guanylate (pG).

5. The base sequence at the 3/ of all tRNA is CCA. All amino acids binds to this terminal adenosine via the 3/ OH group of its ribose.

6. About 50% of the nucleotides in tRNA are base paired to form double helices.

7. There are, however, 5 groups of bases which are not base-paired.

a. The 3/ CCA terminal region
b. The ribothymine-pseudouracil-cytosine loop.
c. the extra arm or little loop contains variable number of residues.

➢ Common structural features of tRNA

d. The dihydrouracil loop which contains several dihydrouracil residues

e.The anticodon loop, which consists of 7 bases with the sequence,

$5'$ pyrimidine-pyrimidine-X-Y-Z-modified purine-variable base-$3'$.

8. The four loops are recognition sites. Each tRNA must have at least two such recognition sites.

9. A unique similarity among all tRNA molecules is that overall distance from CCA at one end to the anticodon at the other end is constant. The difference in nucleotide numbers in various tRNA molecules is compensated for by size of extra arm .

mRNA

- The abundance of RNA in the cytoplasm and its role in protein synthesis suggested that genetic information of nuclear DNA is transmitted to an RNA which functions at the sites of protein synthesis.
- In 1961, two Nobel laureates, Francosis Jacob and Jacques Monod postulated that control of protein formation, at least certain microorganisms is determined by rate of synthesis of templates.

Messenger RNA should have the following properties.

1. Should be polynucleotide.
2. The base composition of the messenger should reflect the base composition of the DNA that specific it.

- **mRNA**

3. The mRNA should be very heterogeneous in size b/c genes or groups of genes vary in length. They also correctly assumed that 3 nucleotides code for one amino acid and that the molecular weight of an mRNA should be at least half million.

4. The messenger should be for a short period, associated with ribosomes.

5. The messenger should be synthesized and degraded very rapidly.

- **Messenger RNA is most heterogeneous in size and stability among all types of RNAs.**
- **It has large molecular weight approaching 2x106 and amounts to about 5% of total RNA of a cell.**

mRNA

- **It is synthesized on the surface of DNA template. Thus it has base sequence complementary to DNA and carries genetic information or message for assembly of amino acids from DNA to ribosomes, the site of protein synthesis.**
- **In prokaryotic cells, mRNA is metabolically unstable with high turnover rate where as it is rather stable in eukaryotes. It is synthesized by DNA-dependent RNA polymerase.**
- **on account of its heterogeneity, mRNA varies greatly in chain length.**
- **In E.coli, the average size of mRNA is 900 to 1500 nucleotides units.**

- **mRNA**
- **Monocistronic type**
- **polycistronic type**
- **The mRNA are unstable in bacterial systems with a half life from a few seconds to about 2 minutes.**
- **In mammalian system, however, mRNA molecules are more stable with a half life ranging from a few hours to one day.**
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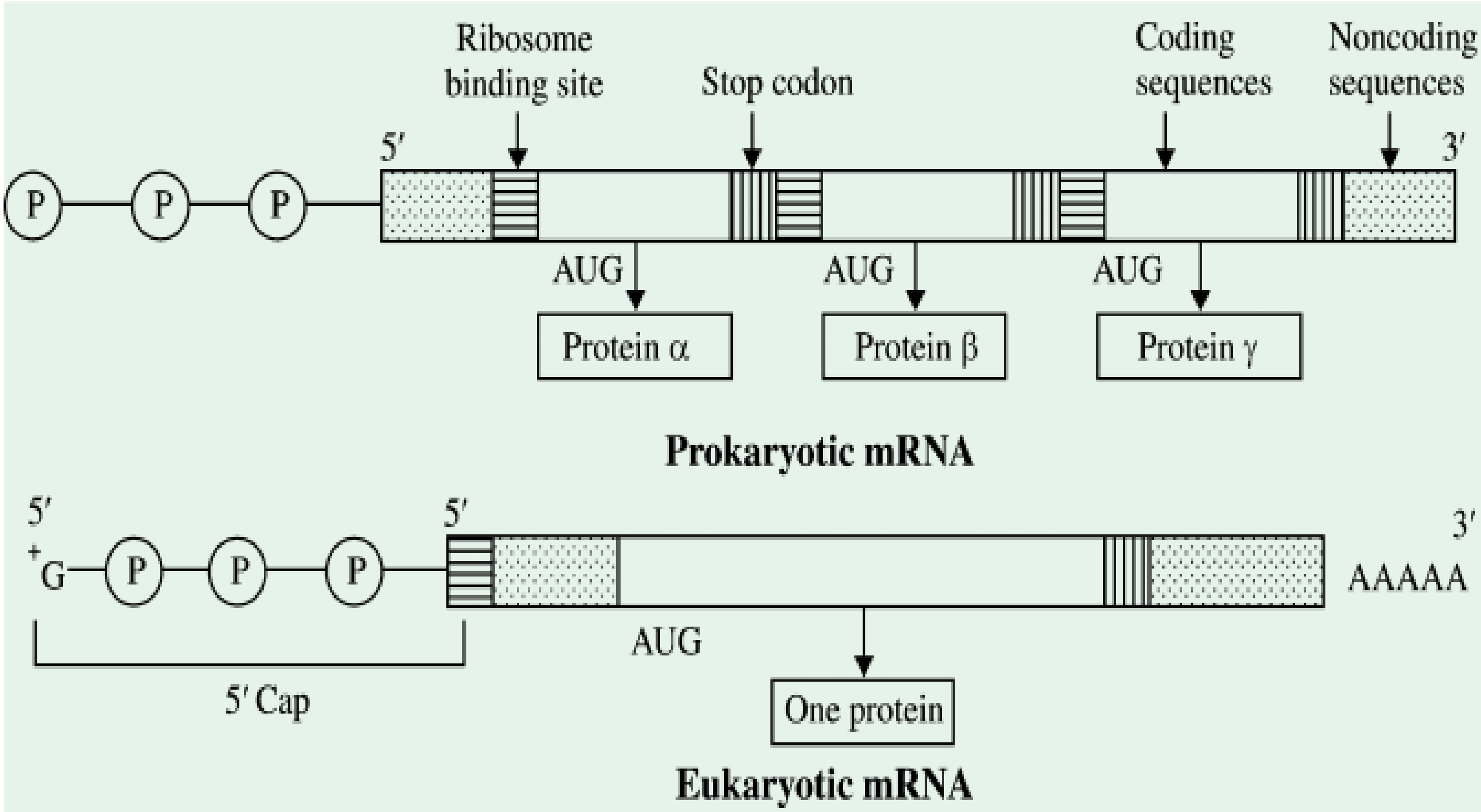

Fig. 15–44. A comparison of the structures of prokaryotic and eukaryotic messenger RNA molecules

- **mRNA**
- **Single stranded**
- **Both prokaryotic and eukaryotic mRNA are synthesized with a triphosphate group at the 5/ end, there is a basic difference between the two.**
- **In eukaryotes, mRNA molecule immediately acquires a $5^{/}$ cap, which is a part of structure recognized by small ribosomal subunit. Protein synthesis , therefore, begins at the start codon near the $5^{/}$ end of mRNA.**
- **in Prokaryotes, the $5^{/}$ end has no special significance, and there can be many ribosome binding sites(shine Dalgarno sequences) in interior of an mRNA chain, each resulting in the synthesis of a different protein.**

- **mRNA**
- **Eukaryotic mRNA molecules have some peculiar characteristics. The 5/ end of mRNA is capped by a 7-methylguanosine triphosphate which is linked to an adjacent 2/-O-methyribonucleotide at its 5/- hydroxyl through the 3 phosphates.**
- **The cap is probably involved in recognition of mRNA by translating machinery. The translation of mRNA into proteins begins at the capped 5/ end.**
- **The other end of most mRNA molecules, the 3/ hydroxyl of poly A tail at the 3/ End of mRNA is not under stood.**
- **It probably serves to maintain the intracellular stability of specific mRNA.**

Structure of RNA

- **Heterogeneous Nuclear RNA(hnRNA)**
- **In mammalian cells, including those of human, a precursor RNA is first synthesized in nucleoplasm by DNA-dependent RNA polymerase**
- **This precursor is then degraded by a nuclear nuclease to mRNA that is then translocated to cytoplasm where it becomes associated to ribosomal system.**
- **The hnRNA molecules may have molecular weights exceeding 10^7 Daltons where as the mRNA molecules are generally smaller than $2x10^6$ Daltons.**

Structure of RNA

- **Heterogeneous Nuclear RNA(hnRNA)**
- **Most mammalian mRNA molecules are 400-4000 nucleotides in length whereas a hnRNA molecule possesses 5000 to 50000 nucleotides.**
- **some uncertainty still exists concerning the precursor-product relationship between hnRNA and mRNA, the former being 10-100 times longer than the latter. Thus the hnRNA molecules appear to be processed to generate the mRNA which then enter the cytoplasm to serve as templates for protein synthesis.**
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Second Position

First Position	U		C		A		G		Third Position
U	UUU	Phe / F	UCU	Ser / S	UAU	Tyr / Y	UGU	Cys / C	U
	UUC		UCC		UAC		UGC		C
	UUA	Leu / L	UCA		*UAA*	*STOP*	*UGA*	*STOP*	A
	UUG		UCG		*UAG*	*STOP*	UGG	Trp / W	G
C	CUU	Leu / L	CCU	Pro / P	CAU	His / H	CGU	Arg / R	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	Gln / Q	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	Ile / I	ACU	Thr / T	AAU	Asn / N	AGU	Ser / S	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	Lys / K	AGA	Arg / R	A
	AUG	Met / M	ACG		AAG		AGG		G
G	GUU	Val / V	GCU	Ala / A	GAU	Asp / D	GGU	Gly / G	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	Glu / E	GGA		A
	GUG		GCG		GAG		GGG		G

RNA codon table

1st position	2nd position: U	2nd position: C	2nd position: A	2nd position: G	3rd position
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	stop	stop	A
	Leu	Ser	stop	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

Amino Acids

Ala: Alanine
Arg: Arginine
Asn: Asparagine
Asp:Aspartic acid
Cys:Cysteine

Gln: Glutamine
Glu: Glutamic acid
Gly: Glycine
His: Histidine
Ile: Isoleucine

Leu: Leucine
Lys: Lysine
Met: Methionine
Phe: Phenylalanine
Pro: Proline

Ser: Serine
Thr: Threonine
Trp: Tryptophane
Tyr: Tyrosisne
Val: Valine