BME 2901-BIOCHEMISTRY

Nucleic Acids - II

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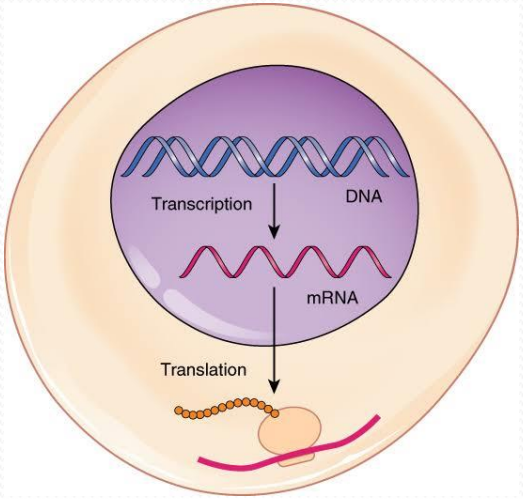
RNA

RNA, the second major form of nucleic acid in cells, has many functions. In gene expression, RNA acts as an intermediary by using the information encoded in DNA to specify the amino acid sequence of a functional protein.



Given that the DNA of eukaryotes is largely confined to the nucleus whereas protein synthesis occurs on ribosomes in the cytoplasm, some molecule other than DNA must carry the genetic message from the nucleus to the cytoplasm.

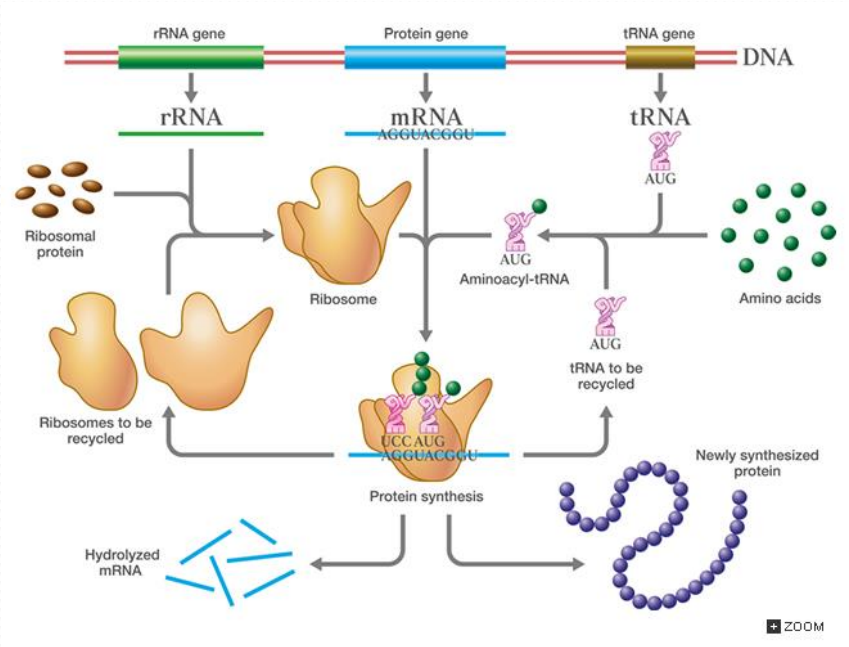
As early as the 1950s, RNA was considered the logical candidate: RNA is found in both the nucleus and the cytoplasm, and an increase in protein synthesis is accompanied by an increase in the amount of cytoplasmic RNA and an increase in its rate of turnover. These and other observations led several researchers to suggest that **RNA carries genetic information from DNA to the protein biosynthetic machinery of the ribosome.**

- However, being a template for protein synthesis in the translation process is not the only function of RNA.
- There are four major classes of RNA in all living cells:

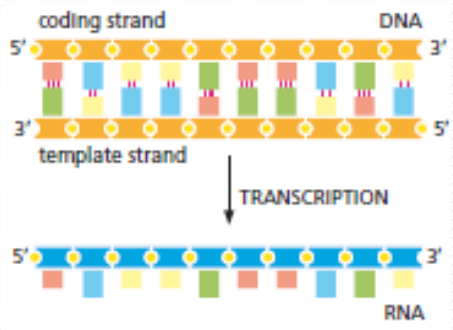
1. *Ribosomal RNA* (rRNA) molecules are an integral part of ribosomes (intracellular ribonucleoproteins that are the sites of protein synthesis). Ribosomal RNA is the most abundant class of ribonucleic acid accounting for about 80% of the total cellular RNA.
2. *Transfer RNA* (tRNA) molecules carry activated amino acids to the ribosomes for incorporation into growing peptide chains during protein synthesis. tRNA molecules are only 73 to 95 nucleotide residues long. They account for about 15% of the total cellular RNA.
3. *Messenger RNA* (mRNA) molecules encode the sequences of amino acids in proteins. They are the "messengers" that carry information from DNA to the translation complex where proteins are synthesized. In general, mRNA accounts for only 3% of the total cellular RNA. These molecules are the least stable of the cellular ribonucleic acids.
4. *Small RNA* molecules are present in all cells. Some small RNA molecules have catalytic activity or contribute to catalytic activity in association with proteins. Many of these RNA molecules are associated with processing events that modify RNA after it has been synthesized. Some are required for regulating gene expression.

Transcription

- mRNA, tRNA, rRNA or other small RNAs are synthesized by transcription process where RNA polymerases synthesize RNA using DNA as the template.

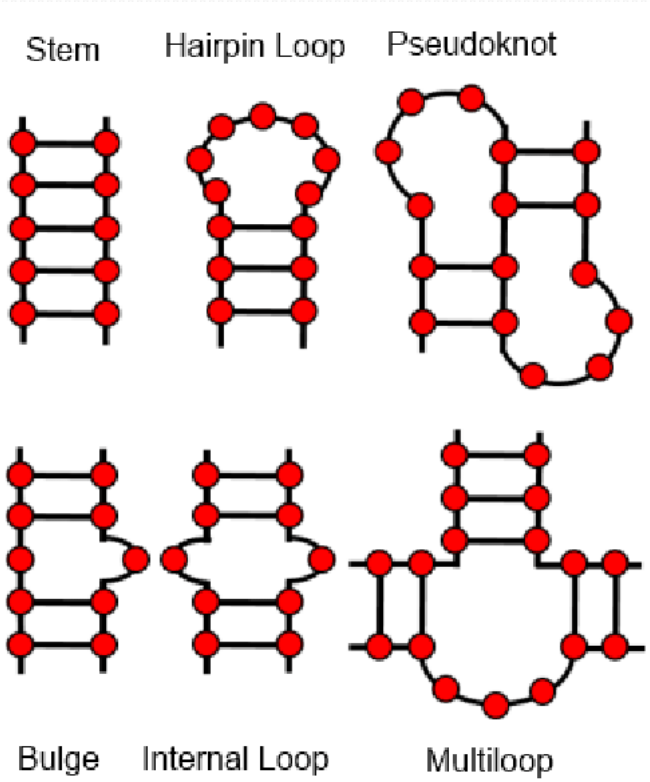


- Transcription begins with the opening and unwinding of a small portion of the DNA double helix to expose the bases on each DNA strand.
- One of the two strands of the DNA double helix then acts as a template for the synthesis of RNA.
- Ribonucleotides are added, one by one, to the growing RNA chain; as in DNA replication, the nucleotide sequence of the RNA chain is determined by complementary base-pairing with the DNA template.
- Transcription is catalyzed by RNA polymerases.



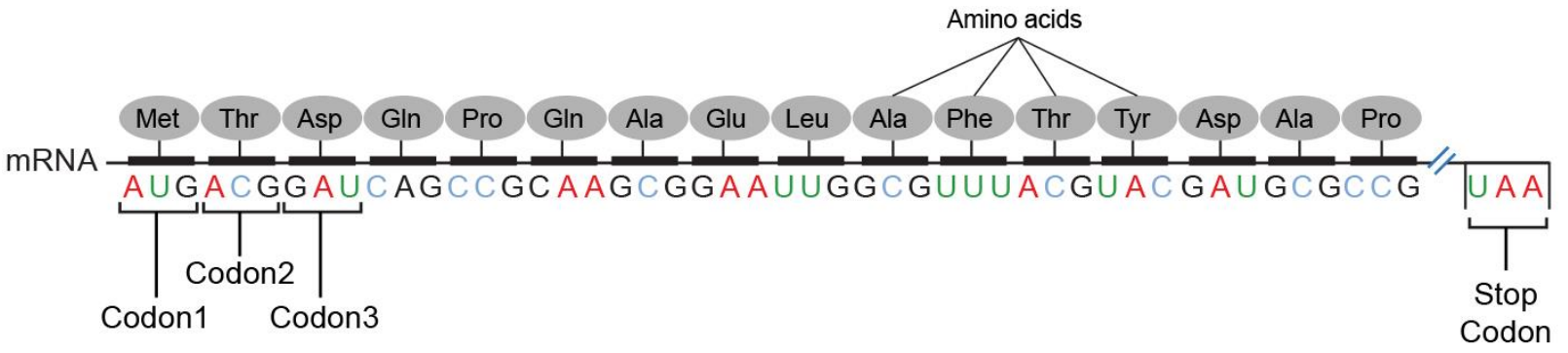
- The product of transcription of DNA is always single-stranded RNA. The single strand tends to assume a right-handed helical conformation dominated by base stacking interactions, which are stronger between two purines than between a purine and pyrimidine or between two pyrimidines.
- The purine-purine interaction is so strong that a pyrimidine separating two purines is often displaced from the stacking pattern so that the purines can interact.
- Any self-complementary sequences in the molecule produce more complex structures.

- Single-stranded RNA fold back on itself to form stable regions of base-paired, double-stranded RNA under physiological conditions.
- Base pairing matches the pattern for DNA: G pairs with C and A pairs with U.
- One difference is that base pairing between G and U residues—unusual in DNA—is fairly common in RNA.
- The paired strands in RNA or RNA-DNA duplexes are antiparallel, as in DNA.

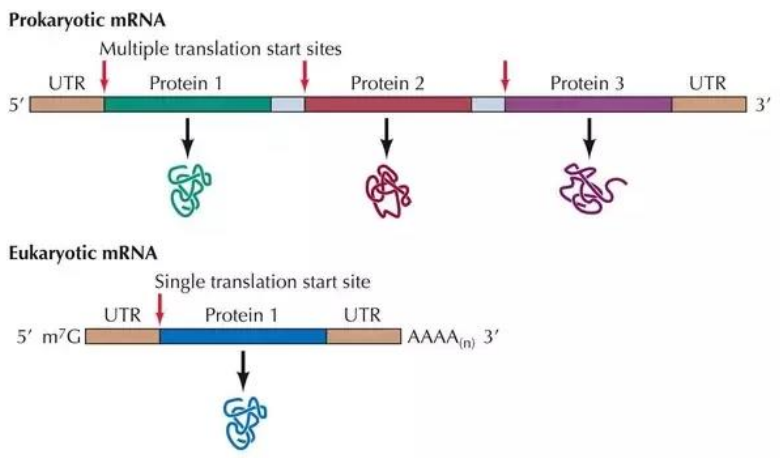


Messenger RNA (mRNA)

- mRNA carries information from DNA to the ribosomes where proteins are synthesized.
- They are the products of transcription of protein coding genes.
- They include codons which are sequences of three nucleotides that corresponds with a specific amino acid or stop signal during protein synthesis.



- In prokaryotes, a single mRNA molecule may code for one or several polypeptide chains. If it carries the code for only one polypeptide, the mRNA is **monocistronic;** if it codes for two or more different polypeptides, the mRNA is **polycistronic.**
- In eukaryotes, most mRNAs are monocistronic.



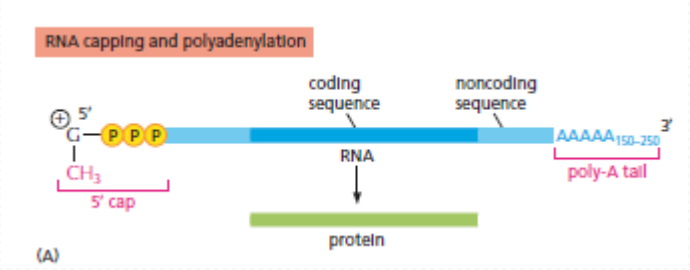
The minimum length of an mRNA is set by the length of the polypeptide chain for which it codes. For example, a polypeptide chain of 100 amino acid residues requires an RNA coding sequence of at least 300 nucleotides, because each amino acid is coded by a nucleotide triplet. However, mRNAs transcribed from DNA are always somewhat longer than the length needed simply to code for a polypeptide sequence (or sequences). The additional, noncoding RNA includes sequences that regulate protein synthesis.

mRNA processing

- The processing of mRNA precursors is one of the biochemical features that distinguishes prokaryotes from eukaryotes.
- In prokaryotes, the primary mRNA transcripts are translated directly, often initiating translation before transcription is complete. In eukaryotes, on the other hand, transcription occurs in the nucleus, and translation takes place in the cytoplasm.
- This compartmentalization of functions in eukaryotic cells allows nuclear processing of mRNA precursors without disrupting translation.

mRNA processing

- Mature eukaryotic mRNA molecules are often derived from much larger transcripts. A newly synthesized RNA molecule is called a **primary transcript.**
- Subsequent processing of these primary transcripts includes: ***capping***, ***splicing***, **and** ***polyadenylation***.



RNA capping

- RNA capping modifies the 5′ end of the RNA transcript, the end that is synthesized first. The RNA is capped by the addition of an atypical nucleotide—a guanine (G) nucleotide bearing a methyl group, which is attached to the 5′ end of the RNA in an unusual way.
- This capping occurs after RNA polymerase has produced about 25 nucleotides of RNA, long before it has completed transcribing the whole gene.

RNA Splicing

- Often, specific nucleotides (called **introns** – noncoding regions in the DNA) from the middle of an mRNA primary transcript are actually excised, or removed, and the resulting fragments (**exons –** coding regions in the DNA) are ligated together to produce a continuous sequence of mature mRNA that specifies a functional polypeptide.
- This process is called **splicing**.
- **Alternative splicing** produces different isoforms of a protein.

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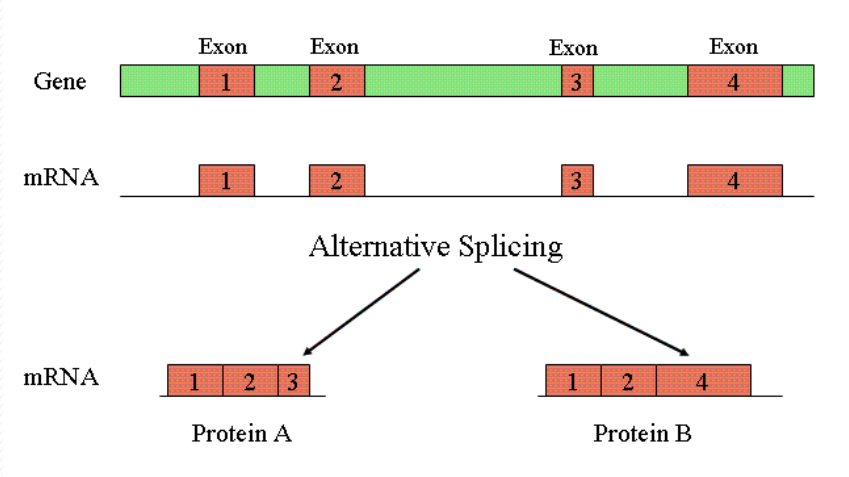
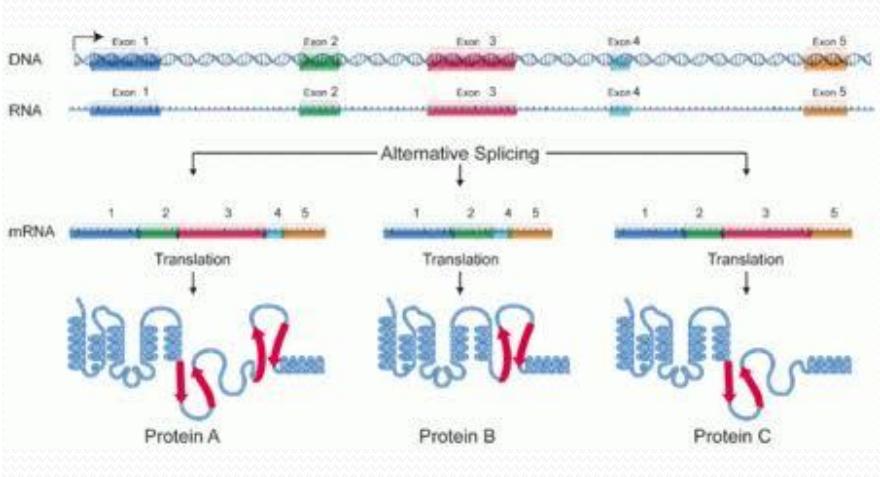


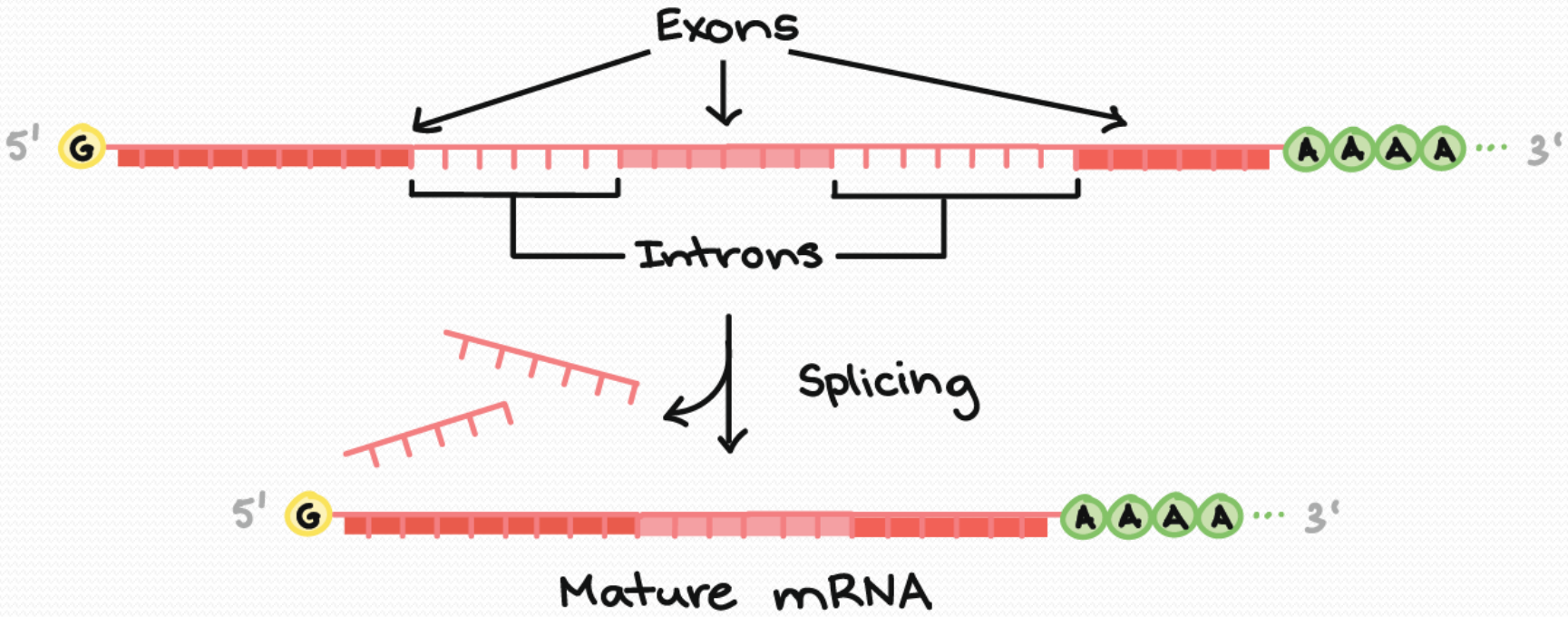


Polyadenylation

- Polyadenylation provides a newly transcribed mRNA with a special structure at its 3′ end.
- A series of repeated adenine (A) nucleotides are added to the 3’ end of the mRNA transcript.
- This **poly-A tail** is generally a few hundred nucleotides long.

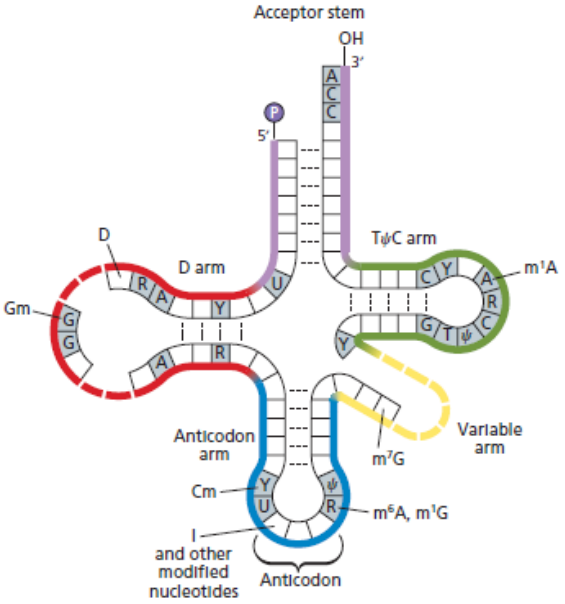
- Capping and polyadenylation—increase the stability of a eukaryotic mRNA molecule, facilitate its export from the nucleus to the cytoplasm, and generally mark the RNA molecule as an mRNA.
- They are also used by the protein-synthesis machinery to make sure that both ends of the mRNA are present and that the message is therefore complete before protein synthesis begins.

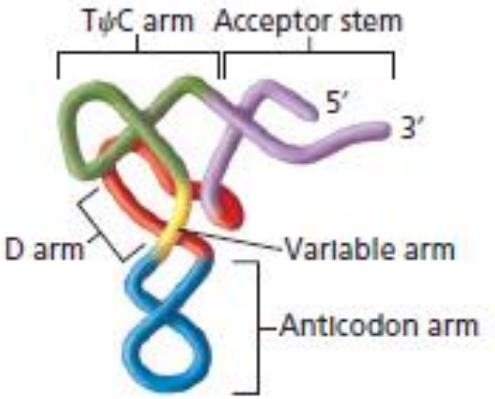
Mature mRNA



Transfer RNA (tRNA)

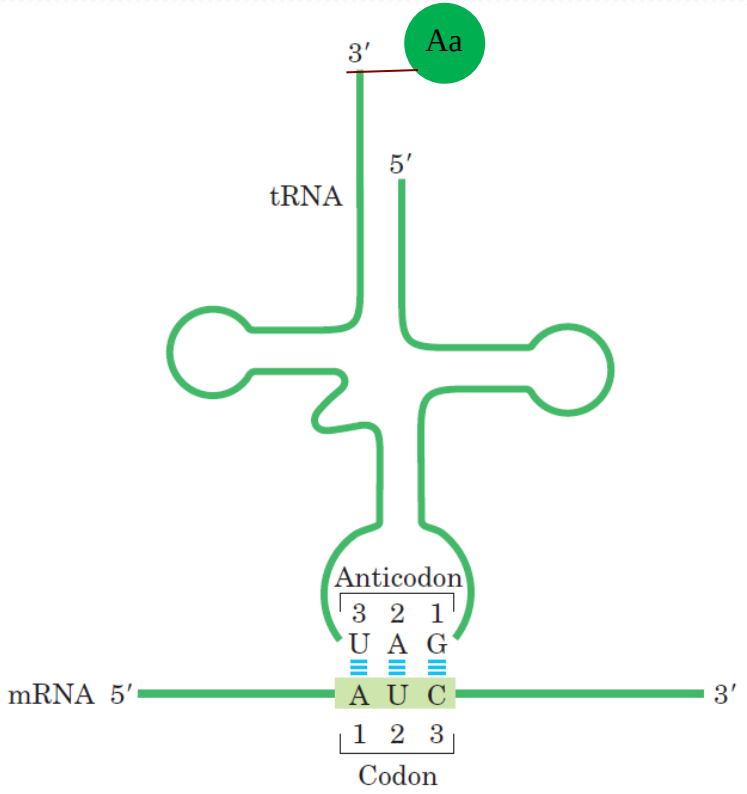
- Transfer RNA molecules are the interpreters of the genetic code.
- They are the crucial link between the sequence of nucleotides in mRNA and the sequence of amino acids in the corresponding polypeptide.
- In order for tRNA to fulfill this role, every cell must contain at least 20 different tRNA species (one for every amino acid) and each tRNA must recognize at least one codon.





tRNA

- Two regions of unpaired nucleotides situated at either end of the L-shaped tRNA molecule are crucial to the function of tRNAs in protein synthesis.
- One of these regions forms the **anticodon**, a set of three consecutive nucleotides that bind, through base-pairing, to the complementary codon in an mRNA molecule.
- The other is a short single-stranded region at the 3′ end of the molecule; this is the site where the amino acid that matches the codon is covalently attached to the tRNA.
- Aminoacid attached tRNAs are called aminoacyl-tRNA (Alanyl-tRNA, methionyl-tRNA…)

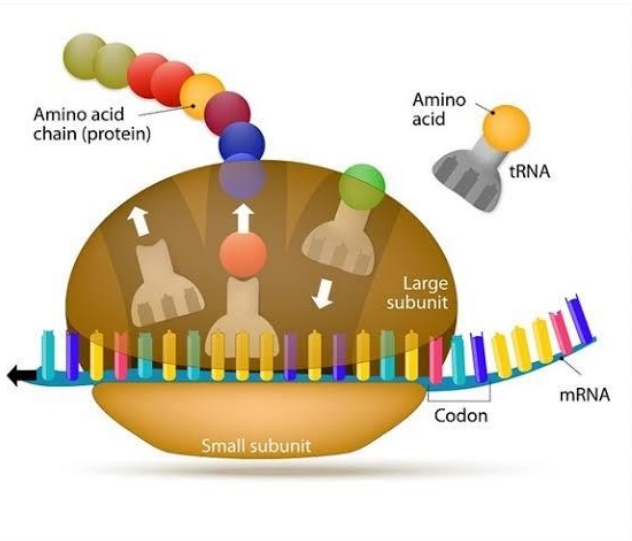


Translation

- Protein synthesis requires assembling four components that form an elaborate translation complex:
  - the ribosome, which catalyzes peptide bond formation;
  - its accessory protein factors, which help the ribosome in each step of the process;
  - the mRNA, which carries the information specifying the protein's sequence;
  - the tRNAs that carry the amino acids.

Ribosomal RNA (rRNA)

- Protein synthesis in cells takes place at ribosomes.
- Ribosomes are made of proteins and rRNAs.
- All ribosomes contain two subunits of unequal size one of which is called the small subunit and the other one is called large subunit.
- The two irregularly shaped ribosomal subunits fit together to form a cleft through which the mRNA passes as the ribosome moves along it during translation.



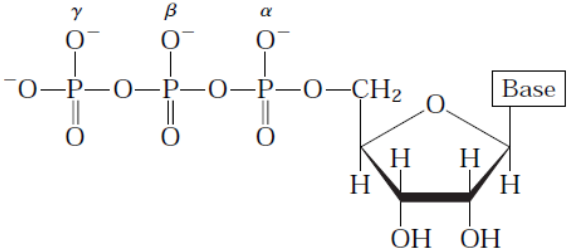
- mRNAs are translated into proteins in 5'→3' direction and protein synthesis begins at the amino-terminal end and proceeds by the stepwise addition of amino acids to the carboxyl-terminal end of the growing polypeptide.
- The AUG initiation codon specifies an *amino-terminal* methionine residue.
- Although methionine has only one codon, (5)AUG, all organisms have two tRNAs for methionine.
- One is used exclusively when (5)AUG is the initiation codon for protein synthesis. The other is used to code for a Met residue in an internal position in a polypeptide.
- Elongation continues until the ribosome adds the last amino acid coded by the mRNA.
- **Termination,** the fourth stage of polypeptide synthesis, is signaled by the presence of one of three termination codons in the mRNA (UAA, UAG, UGA), immediately following the final coded amino acid.

Other Functions of Nucleotides

- In addition to their roles as the subunits of nucleic acids, nucleotides have a variety of other functions in every cell: as energy carriers, components of enzyme cofactors, and chemical messengers.

Nucleotides Carry Chemical Energy in Cells

- The phosphate group covalently linked at the 5 hydroxyl of a ribonucleotide may have one or two additional phosphates attached. The resulting molecules are referred to as nucleoside mono-, di-, and triphosphates.
- Starting from the ribose, the three phosphates are generally labeled as α, β, and γ.
- Hydrolysis of nucleoside triphosphates provides the chemical energy to drive a wide variety of cellular reactions.
- Adenosine 5-triphosphate, ATP, is by far the most widely used for this purpose, but UTP, GTP, and CTP are also used in some reactions.
- Nucleoside triphosphates also serve as the activated precursors of DNA and RNA synthesis.



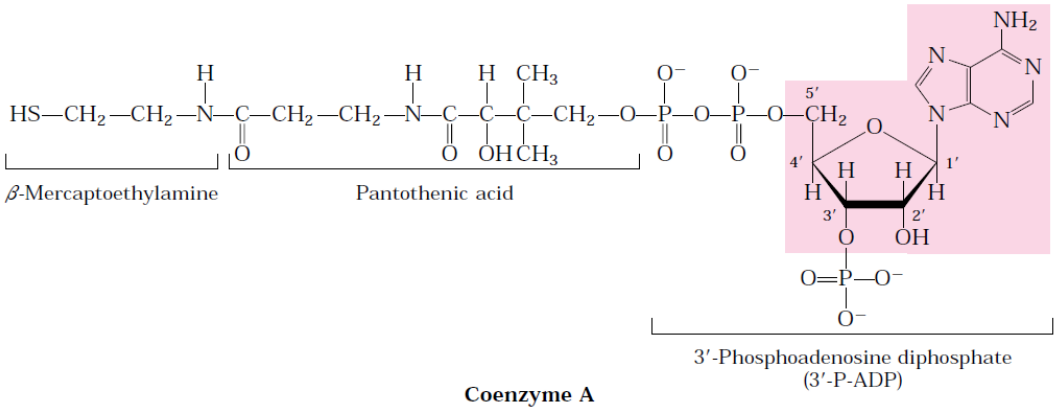
Abbreviations of ribonucleoside 5′-phosphates			
Base	Mono-	Di-	Tri-
Adenine	AMP	ADP	ATP
Guanine	GMP	GDP	GTP
Cytosine	CMP	CDP	CTP
Uracil	UMP	UDP	UTP

Nucleotides Carry Chemical Energy in Cells

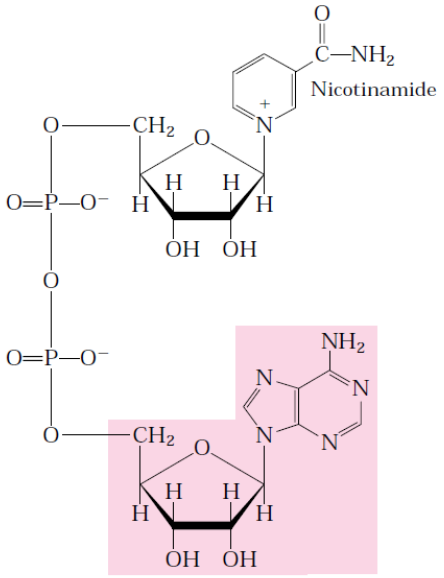
- The energy released by hydrolysis of ATP and the other nucleoside triphosphates is accounted for by the structure of the triphosphate group.
- The bond between the ribose and the phosphate is an **ester linkage**.
- The α-β and β-γ linkages are **phosphoanhydrides**.
- Hydrolysis of the ester linkage yields about 14 kJ/mol under standard conditions, whereas hydrolysis of each anhydride bond yields about 30 kJ/mol.
- ATP hydrolysis often plays an important thermodynamic role in biosynthesis. When coupled to a reaction with a positive free-energy change, ATP hydrolysis shifts the equilibrium of the overall process to favor product formation.

Adenine Nucleotides Are Components of Many Enzyme Cofactors

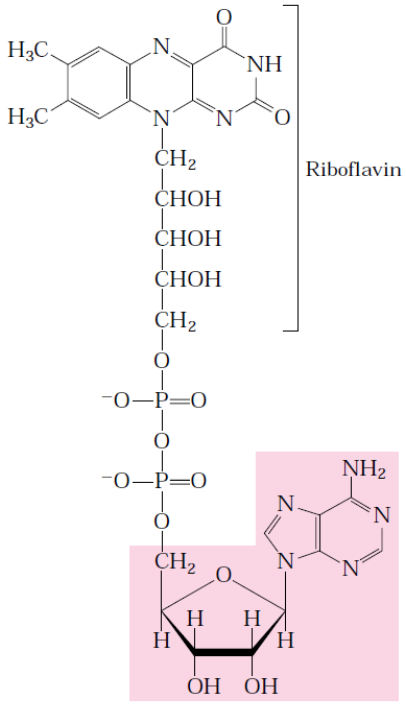
- A variety of enzyme cofactors serving a wide range of chemical functions include adenosine as part of their structure.
- They are unrelated structurally except for the presence of adenosine.
- In none of these cofactors does the adenosine portion participate directly in the primary function, but removal of adenosine generally results in a drastic reduction of cofactor activities.



Coenzyme A



Nicotinamide adenine dinucleotide (NAD^+)



Flavin adenine dinucleotide (FAD)

Some Nucleotides Are Regulatory Molecules

- Cells respond to their environment by taking cues from hormones or other external chemical signals.
- The interaction of these extracellular chemical signals ("**first messengers**") with receptors on the cell surface often leads to the production of **second messengers** inside the cell, which in turn leads to adaptive changes in the cell interior.
- Often, the second messenger is a nucleotide.
- One of the most common is **adenosine 3,5-cyclic monophosphate (cyclic AMP**, or **cAMP)**, formed from ATP.

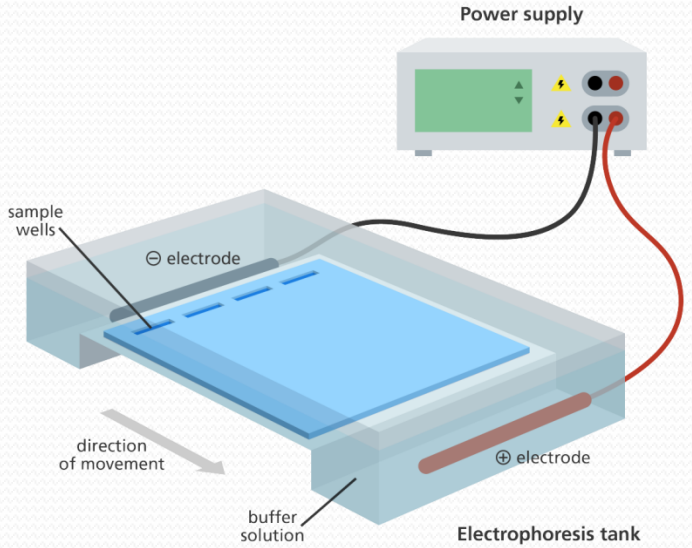
Why adenosine?

- Why is adenosine, rather than some other large molecule, used in these structures? The answer here may involve a form of evolutionary economy.
- Adenosine is certainly not unique in the amount of potential binding energy it can contribute. **The importance of adenosine probably lies not so much in some special chemical characteristic but in the evolutionary advantage of using one compound for multiple roles.**
- Once ATP became the universal source of chemical energy, systems developed to synthesize ATP in greater abundance than the other nucleotides; because it is abundant, it becomes the logical choice for incorporation into a wide variety of structures.
- The economy extends to protein structure. A single protein domain that binds adenosine can be used in a wide variety of enzymes. Such a domain, called a **nucleotide-binding fold,** is found in many enzymes that bind ATP and nucleotide cofactors.

Gel Electrophoresis

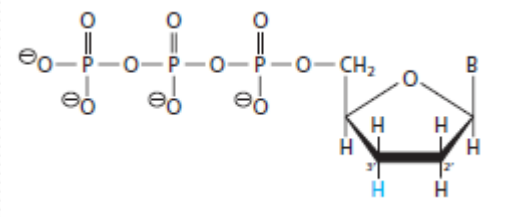
- Gel electrophoresis is a laboratory method used to separate mixtures of DNA, RNA, or proteins according to molecular size. In gel electrophoresis, the molecules to be separated are pushed by an electrical field through a gel that contains small pores. Because DNA and RNA are negatively charged molecules, they will be pulled toward the positively charged end of the gel.
- DNA molecules can be seperated by gel electorphoresis using agarose gel.
- The molecules travel through the pores in the gel at a speed that is inversely related to their lengths.
- Small DNA molecule will travel a greater distance through the gel than will a larger DNA molecule.

DNA Gel Electrophoresis

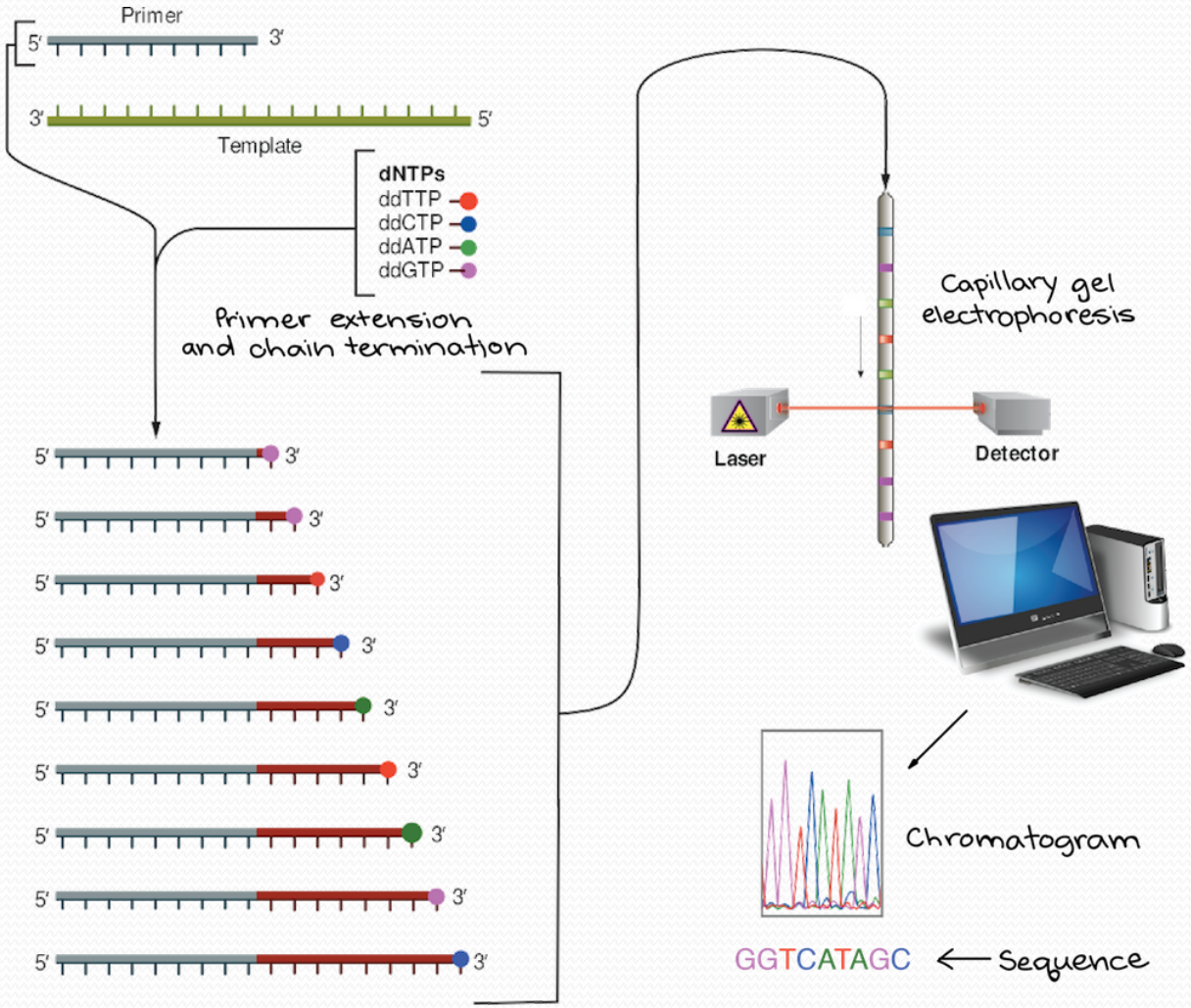


DNA Sequencing

- In 1976 Frederick Sanger developed a method for sequencing DNA enzymatically.
- Sanger was awarded his second Nobel Prize for this achievement (he received his first Nobel Prize for developing a method for sequencing proteins).
- The Sanger sequencing method uses -dideoxynucleoside triphosphates
- (ddNTPs), which differ from the deoxyribonucleotide substrates of DNA synthesis by lacking a -hydroxyl group.
- The dideoxyribonucleotides, which can serve as substrates for DNA polymerase, are added to the end of the growing chain. Because these nucleotides lack a -hydroxyl group, subsequent nucleotide additions cannot take place and incorporation of a dideoxynucleotide terminates the growth of the DNA chain.



ddNTP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GGTCATAGC
Sequence