BIO 311C Spring 2010

Lecture 36 – Wednesday 28 Apr.

Synthesis of a Polypeptide Chain



The coded information of only one class of RNA, called messenger RNA (mRNA), is used to specify the sequence of amino acids in polypeptide chains.

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In prokaryotic cells:

the primary transcripts of mRNA can be utilized for translation immediately after transcription, and may permit the start of translation well before their transcription is complete. Thus, the mRNA is not processed before it is expressed.



From textbook, Fig. 17.24. p. 347

In the nucleus of eukaryotic cells:

The primary transcript must first be processed, then must exit through nuclear pores, in order to facilitate translation in the cytoplasmic matrix. Thus, transcription and translation are separated in space and time.



From textbook Fig. 17.3, p. 329

Note: Transcription in eukaryotic organelles (mitochondria and plastids) is very similar to that in prokaryotic cells. It does not require post-transcriptional processing before translation can begin.

Eukaryotic Cell



In the nucleus of eukaryotic cells:

Several nucleotide sequences within mRNA primary transcripts mark the transcripts for post-transcriptional processing.



primary transcript destined to become an mRNA molecule

Prokaryote cells use the same start and stop codons as do eukaryotic cells.

Addition of a Modified-G Cap to the 5' End, and a poly-A Tail to the 3' End, a pre-mRNA Primary Transcript of a Eukaryotic Cell



"UTR" means "untranslated region" of the mRNA molecule.

Most eukaryotic pre-mRNA primary transcripts contain one or more introns that are spliced out of the transcript before it is transported out the nucleus.



Textbook Fig. 17.10, p. 335

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Exons are the portions of the RNA molecule that are not removed as introns. Exons are joined end-to-end when the introns are removed.

The numbers along the transcript shown above refer to the numerical order of the nucleotides to be expressed during translation, after all introns have been removed. Thus, the processed mRNA shown above has a 146-nucleotide sequence used as information to synthesize a polypeptide chain.

The word intron refers to a region of a gene which specifies a corresponding region of an RNA molecule that is excised, and also refers to the region of excised RNA.

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The Use of Spliceosomes to Remove Introns From Molecules of RNA

Textbook Fig. 17.11, p. 335



A spliceosome is a molecular machine that contains small molecules of RNA (snRNA = small nuclear RNAs) that bind to polypeptide chains in forming snRNPs (small nuclear ribonucleoproteins). Several snRNPs bind to other proteins in forming the spliceosome.

The snRNAs are RNA transcripts about 150 nucleotides long that remain in the nucleus. They represent a class of RNA molecules that are completely distinct from mRNA.

Spliceosomes are enzymes that remove introns from mRNA, then spice together the remaining exons, to form a functional mRNA.

Individual domains of functional proteins often correspond to individual exons.



Processed mRNA, when attached to an appropriate small protein, is allowed to pass through the nuclear pores.



nucleus

Nuclear pores are complex structures that are restrict access to most large molecules. They allow temporary access only when the nuclear pore complex recognizes a molecule that is intended to pass through. Ribosomes are the chemical machines that synthesize polypeptide chains in cells.



Ribosomes are typically shown as a single unit, but they occur as two separate subunits except during the time they are involved in synthesizing a polypeptide chain.

Each subunit of a ribosome is composed to two different classes of molecules: protein and RNA. The RNA that is used as a structural component of ribosomes, called rRNA, does not contain coded information for the primary structure of polypeptide chains.

Steps in the Synthesis, Assembly and Translocation of Ribosome Subunits

- a. RNA molecules for <u>assembly</u> into ribosome subunits are <u>transcribed</u> in the nucleolus of eukaryotic cells.
- b. Proteins for <u>assembly</u> of ribosomes are <u>synthesized</u> in the cytoplasmic matrix and <u>translocated</u> through nuclear pores to the nucleolus.
- c. RNA and proteins are <u>assembled</u> into ribosomal subunits in the nucleolus.
- d. Fully assembled ribosomal subunits are <u>translocated</u> through nuclear pores from the nucleus to the cytoplasmic matrix.



tRNAs are a class of relatively small RNA molecules (approximately 80 nucleotides long) that are transcribed from DNA like other kinds of RNA. They have a characteristic secondary and tertiary structures.



tRNA secondary structure

tRNA tertiary structure

Pre-tRNA primary transcripts are post-transcriptionally processed by modifying the chemical structure of a few specific nitrogen bases in order to convert them into distinct functional tRNA molecules.

tRNA molecules function by:

- a. covalently bonding to an amino acid at its 3' end,
- b. bonding via non-covalent bonds to a specific site on a ribosome.
- c. hydrogen bonding a three-nucleotide sequence (called an anticodon) on the t-RNA with a complementary three-nucleotide sequence (a codon) on a molecule of mRNA that is attached to a ribosome.



Tertiary structure of tRNA

Textbook illustration of a molecule of tRNA

Activating an Amino Acid (= Charging a tRNA)



Textbook fig. 17.15, p. 338

The cell produces a distinct synthetase for each different tRNA.

Each different synthetase covalently bonds to only one kind of tRNA and only one kind of amino acid, covalently bonding them together.

After the tRNA and amino acid are covelently bonded together, the tRNA is said to be "charged" and the amino acid is said to be "activated". Equations for Activating an Amino Acid (= Charging a tRNA) In this example the name of the amino acid to be activated is alanine (ala)

(1.) ala + tRNA_{ala} + ATP
$$\xrightarrow{synthetase_{ala}} tRNA_{ala} + AMP + P-P_i$$

(2.) P-P_i + H₂O $\xrightarrow{synthetase_{ala}} 2P_i$
(Sum) ala + tRNA_{ala} + ATP + H₂O $\xrightarrow{synthetase_{ala}} tRNA_{ala} + AMP + 2P_i$

Thus, 2 high-energy phosphate bonds are hydrolyzed for each amino acid that is activated.

Note: pyrophosphate is shown here as $P-P_i$. Sometimes it is abbreviated as $P \sim P$ to illustrate its "high-energy" bond. Either expression for pyrophosphate is correct.

Illustration of a charged tRNA molecule, showing it:

- a. covalently bound to an amino acid
- b. adapted to the correct site on a molecule of mRNA



This alignment is facilitated when all components are bound to a ribosome (not shown here).

Application of the Genetic Code and Base-Pairing Rules



Direction of anticodon

You should be able to use a table showing the genetic code and apply the base-pairing rules for nucleotides in order to determine:

- a. the mRNA codon to which this tRNA molecule aligns,
- b. the amino acid that is bound to the amino acid attachment site.
- c. the 3-nucleotide sequence of the plus and minus strands of the DNA that produces the mRNA codon.

You should also know which is the 5' end and which is the 3'end of each polynucleotide.



Correspondence of Information Content in Various Kinds of Information Molecules



Direction of transcription and translation

Correspondence of Nucleotides and Amino acids During Translation



Illustration of a ribosome in the midst of synthesis of a polypeptide chain



with respect to mRNA with attached tRNA

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The initiation of Synthesis of a Polypeptide Chain

Textbook Fig. 17.17, p. 340



Sequence of events:

- 1. mRNA binds to a ribosomal small subunit with its <u>start</u> codon in properly aligned position.
- 2. tRNA_{met} that is bound to the amino acid "met" aligns with the start codon.
- 3. the ribosomal large subunit binds to the unit, holding the mRNA between the two subunits.

Sequence of Events During the Insertion of Each Amino Acid into the Growing Polypeptide Chain

Textbook Fig. 17.18, p. 341



Termination of the Synthesis of a Polypeptide Chain



Sequence of events in polypeptide chain termination

- 1. The ribosome encounters a stop codon on mRNA which places a release factor where a t-RNA would otherwise sit.
- 2. The ribosome hydrolyses and releases the polypeptide chain from tRNA, and also releases the tRNA to which it was attached
- 3. The ribosomal subunits come apart, releasing the mRNA