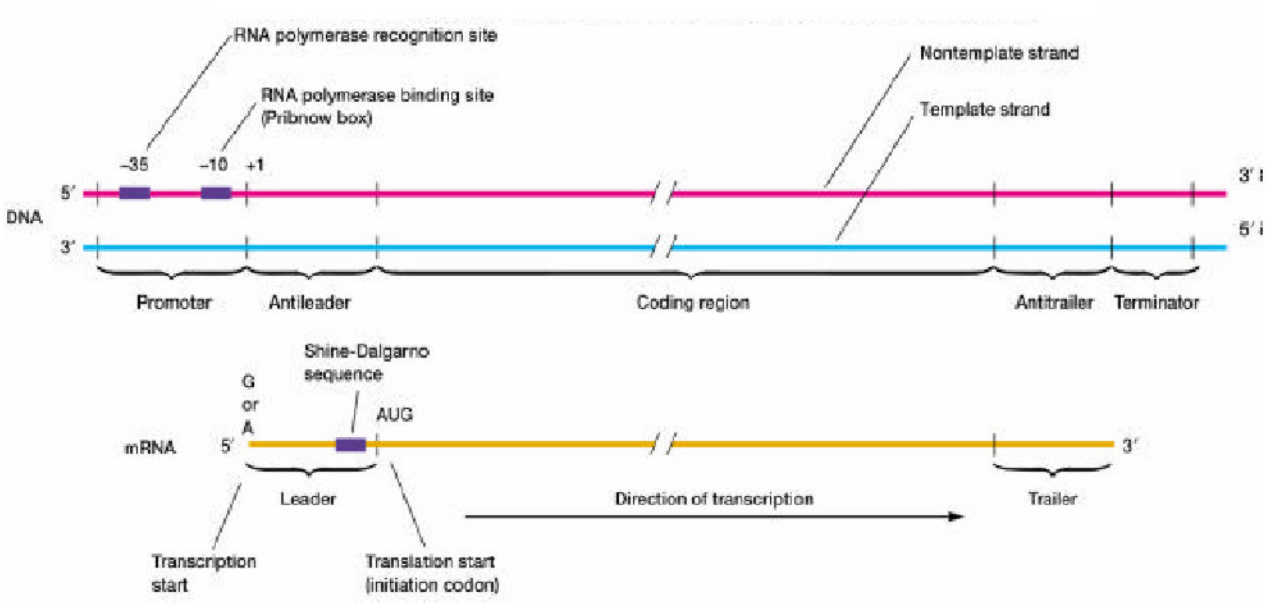

RNA polymerase recognition site
RNA polymerase binding site
(Pribnow box)
Nontemplate strand
Template strand
–35
–10
+1
5′
3′
DNA
3′
5′
Promoter
Antileader
Coding region
Antitrailer
Terminator
Shine-Dalgarno
sequence
G
or
A
AUG
mRNA
5′
3′
Leader
Direction of transcription
Trailer
Transcription
start
Translation start
(initiation codon)

mRNA synthesis.

Can you name four difference between DNA & RNA

DNA	RNA
Double strand	single strand
Thymine	Uracil
Deoxyribose	ribose
DNA Polymerase	RNA Polymerase

What are three processes of transcription

- Initiation of mRNA at the promoter site
- mRNA elongation along the DNA
- mRNA elongation termination

At what levels does RNA act at?

1) Genetic level
   mRNA

2) Functional level
   tRNA
   rRNA

How is a gene identified on a DNA chromosome?

Promoter sequences.

What is promoter site on a DNA molecule?

The promoter is a DNA binding site for RNA Polymerase

What is the role of the sigma factor during transcription

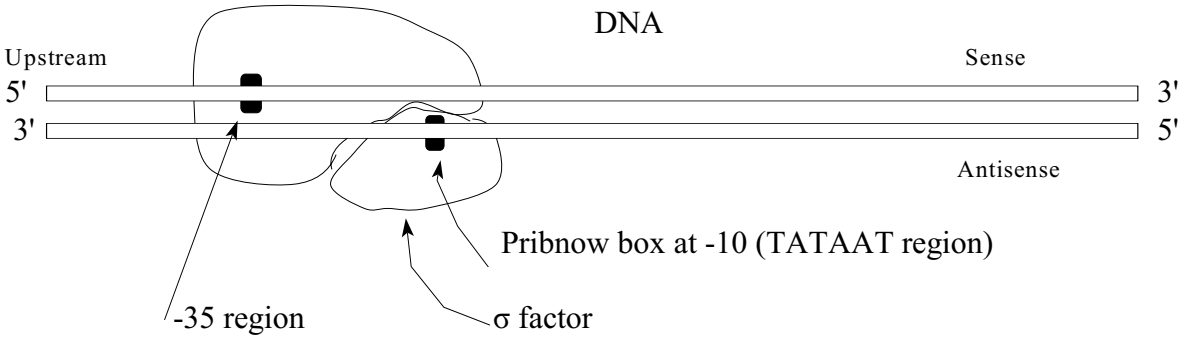
The sigma factor aids in RNA polymerase binding and & decoding the DNA. The sigma factor is release after a small section of mRNA is transcribed.

Where do sigma factors arise from?

The DNA codes for sigma factor
There are 7 know sigma factors for *E.coli*
And, there are 17 identified sigma factors for *Bacillus subtilis*

The template stand 3' to 5' DNA strand codes for mRNA. The strand is considered the antisense strand.

The non-template 5' to 3' DNA strand is considered the sense strand. This strand likes very much like the mRNA except there is "T" for "U."



Nucleotide sequence rich in TTGACG

Other promoters use a CAAT box and a GC box between -40 and -100 nucleotide sequences

How is mRNA transcription terminated? Two proposed methods:

mRNA transcription termination is accomplished when a specific sequence of DNA contains inverted repeats with a control non-repeating segments

Intrinsic terminator

This site is rich in GC followed by AT sequences

DNA

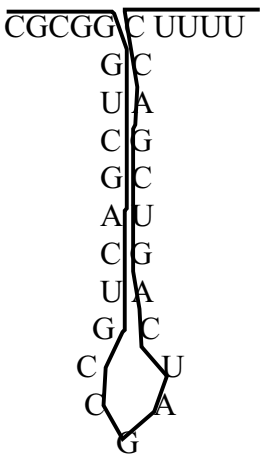
TGCG GGTGCACTG CCGAT CAGTCGACC TTTT Sense strand

ACGC CCACGTGAC GGCTA GTCAGCTGG AAAA Antisense (template) strand

Transcription of template strand

mRNA

UGCG GGUGCACUG CCGAU CAGUCGACC UUUU



The mRNA fold to form a secondary structure

Stem-loop in RNA immediately up stream from a run of uracils leads to termination of transcript. Loop interferes with RNA polymerase activity causing to stop transcbing the DNA strand.

Another factor sometimes used by bacteria is a protein complex called the Rho factor (used in *E. coli*). Rho factor binds tightly to RNA and moves down the chain to RNA polymerase//DNA complex.

Rho factor causes RNA//DNA complex to disassociate.

What is a ploy cistronic mRNA?

A ploy cistronic mRNA is a series of genes coded onto one single mRNA strand. An example of this type of mRNA can be found in the *E.coli* lactose operon system.

Transcription

Transcription is the process that takes place as DNA is used as a template to create RNA. This is how the information stored in DNA gets passed on to RNA. The enzyme that helps this takes place is called RNA polymerase. RNA polymerase uses one strand of double stranded DNA as a template to form single stranded RNA.

Transcription takes place in three stages - initiation, elongation and termination.

Initiation of Transcription

Initiation begins as RNA polymerase recognizes and binds to certain regions of DNA called promoters. The RNA polymerase finds promoters by sliding along the DNA without having to unwind the two strands of DNA from each other.

After the RNA polymerase finds a promoter it begins to unwind the two strands of DNA in that region. It is necessary for the DNA to be unwound at this point so that the RNA polymerase can get closer to the individual nucleotides. A segment of DNA of around 17 base pairs (bp) stays unwound throughout the transcription process.

The strand of the double stranded DNA that the polymerase binds to as it moves along is called the template or sometimes the antisense strand. The other strand is called the coding or sense strand. The new chain being formed will be identical to the coding strand, because of the way the bases pair up.

Nucleotides that are before the place where transcription actually starts are numbered with negative numbers. The nucleotide located exactly where transcription starts is numbered +1.

Next, two NTP's that can pair with the nucleotides in the +1 and +2 positions of the DNA strand, bind to the RNA polymerase. NTP's are any of four ribonucleoside triphosphates - ATP (adenine triphosphate), GTP (guanine triphosphate), UTP (uracil triphosphate) or CTP (cytosine triphosphate). In RNA, Uracil (U) replaces thymine and pairs with A (adenine). So, since C can only pair with G and A with U, if the nucleotides in positions +1 and +2 are G and A, then the ribonucleoside triphosphates CTP and UTP will bind to the RNA polymerase. A phosphodiester bond then forms between these two ribonucleotides.

All ribonucleoside triphosphates have three phosphate groups attached to them. The phosphodiester bond forms when the OH (an oxygen and hydrogen molecule known as a hydroxyl group) on the 3′ carbon of one ribonucleoside triphosphate attacks the innermost

In eucaryotic transcription three different RNA polymerase enzymes are involved:

RNA polymerase I and III are involved in the transcription of ribosomal and transfer RNA, while RNA polymerase II is mainly involved with the transcription of mRNA (messenger RNA). Eucaryotic RNA polymerases are very large with multiple subunits. Although they are somewhat similar to the procaryotic RNA polymerase, they are more complex.

Promoters

The two most important promoters in bacteria are the Pribnow Box and the -35 region. The Pribnow Box is located about 10 nucleotides from where the actual transcription begins. The sequence of the nucleotides in the Pribnow Box is TATAAT (thymine, adenine, thymine, adenine, adenine, thymine). The -35 region is located 35 nucleotides before the transcription start place. Its nucleotide sequence is TTGACA (thymine, thymine, guanine, adenine, cytosine, adenine).

The two promoters do not have to have exactly the same sequence as listed above. However, the more similar they are to the sequences above, the stronger the promoter they will be. Transcription from strong promoters can start as frequently as every two seconds and as little as every 10 seconds in weak promoters, in E. coli.

One of the most important promoters in eucaryotes is called the TATA box (also sometimes called a Hogness box). It is centered about 25 nucleotides before the transcription start site. In yeast it is located somewhere between -30 and -90.

Additional promoters are also usually necessary. A CAAT box and a GC box are also very common and are usually even farther away from the start site (between -40 and -110).

DNA sequences called enhancers also help stimulate transcription in eucaryotes. Enhancers are not well understood yet, but they are known to be able to increase transcription rates even when they are located thousands of base pairs away from the transcription start site.

Instead of the polymerases directly binding to the promoter regions another type of protein, called a transcription factor, does this instead. There many transcription factors involved in eucaryotic transcription. Ones required for most promoters include: TFIIA, TFIIB, TFIID, TFIIE, TFIIF and THIIH.

Capping of eucaryotic RNA

Actual transcription begins the same way in eucaryotes as it does in procaryotes. However, almost immediately, the first ribonucleotide of the chain (the one on the 5′ end) gets modified. The phosphate group on the end of it leaves and the end that is left attacks the innermost phosphate of a GTP molecule and forms a bond with it. This end part is called a cap. Only mRNA is capped, not rRNA (ribosomal RNA) or tRNA (transfer RNA). Caps make mRNA molecules more stable by protecting that end from being degraded by other enzymes.

Differences in termination of transcription

A simple signal in procaryotes is a region of the DNA that has a high percent of G's and C's, followed by an A and T rich region. Since G's and C's pair with each other, a long stretch of them can cause folding of the strand to form what is called a hairpin loop. This

phosphate of the other ribonucleoside triphosphate. This type of an attack is called a nucleophilic attack.

After this first phosphodiester bond forms the elongation process begins.

Elongation of Transcription

In the elongation phase more ribonucleotides are added to the other two, forming a chain. The chain always forms starting from the 5′ end to the 3′ end, meaning that the new ribonucleotides are added to the 3′ end. New ribonucleotides are added to the chain at a rate of 50 per second.

The same base pairing rules still apply during the elongation phase. Each time a C is found in the antisense strand of the template DNA, a G is paired up with it and added to the growing RNA strand. If a T is encountered, a U will be added, etc.

Sometimes mistakes are made and the wrong ribonucleotide will pair up with one of the nucleotides of the DNA strand. This happens at a rate of around 1 mistake in 10,000 - 100,000 ribonucleotides added to the chain.

Termination of Transcription

Just as a strand of DNA being transcribed has promoter regions indicating where transcription should start, it also has stop signals to end the process.

Transcription in Procaryotes and Eucaryotes

There are some important differences between the way procaryotes (bacteria) and eucaryotes (plants, animals, fungi, etc.) carry out transcription. In eucaryotes transcription is usually separated in space and time from translation - transcription takes place in the cell nucleus and translation in the cytoplasm. Procaryotes do not have a cell nucleus and translation begins before transcription even stops.

RNA Polymerase

The RNA polymerase enzyme in bacteria, such as E. coli, is made up of four kinds of subunits. They are:

The whole enzyme, sometimes called the holoenzyme, is made up of 2 of the α (alpha) subunits and one each of β (beta), β (beta prime) and σ (sigma) and is sometimes referred to just as $\alpha_2\beta\beta\sigma$. When the σ subunit is missing it is called the core enzyme and is written as $\alpha_2\beta\beta$.

The β (beta) subunit is involved with forming the phosphodiester bonds and the β (beta prime) subunit is involved with binding to the DNA template. The role of the α (alpha) subunit is not well understood.

There is actually more than one type of σ subunit. The one referred to most of the time and in this article is called σ^{70} (sigma 70) because it has a mass of 70 kd (kilo daltons). σ^{32} is the sigma in RNA polymerase only under certain special conditions.

loop can then interfere with the RNA polymerase activity causing it to stop transcribing the DNA strand.

Sometimes additional factors, such as ρ (rho) are needed to end transcription. ρ terminates transcription by pulling the newly formed ribonucleotide chain away from the transcription site.

Termination of eucaryotic transcription is not well understood yet. Sometimes loops like those in procaryotes form. A cleavage signal, a sequence consisting of AAUAAA, occurs on the newly formed chain. After another enzyme cuts the chain here, yet another enzyme adds about 250 A's to this end of the chain. The significance of this poly A chain is not very well understood.

Go back to:
Introduction, DNA, RNA, proteins,

or forward to:
Translation

Article originally written by All ScienceDen.com on: Nov 05, 2002

Quote of the Month

"Who are we? Where do we come from? Why are we this way and not some other? What does it mean to be human? Are we capable, if need be, of fundamental change, or do the dead hands of forgotten ancestors impel us in some direction, indiscriminately for good or ill, and beyond our control? Can we alter our character? Can we improve our societies? Can we leave our children a world better than the one that was left to us? Can we free them from the demons that torment us and haunt our civilization? In the long run, are we wise enough to know what changes to make? Can we be trusted with our own future?"

- Carl Sagan, Shadows of Forgotten Ancestors (p.4)

Chapter 7

Protein Synthesis

Go through the various sites and review DNA synthesis

Go to:

http://www.accessexcellence.org/RC/VL/GG/dna_replicating.html

Simple drawing of Protein synthesis

Go to:

http://www.accessexcellence.org/RC/VL/GG/protein_synthesis.html

Know the genetic code for the synthesis of polypeptides.

Go to:
http://molbio.info.nih.gov/molbio/gcode.html

This last web site is the genetic code which you can also find in text book.

Chapter 8
Protein Synthesis

I. What is the role of proteins in a cellular system

A. Proteins are used for maintenance, growth and development

II. How are proteins made : TRANSCRIPTION and TRANSLATION

A. DNA

1. Instructions are coded in nucleotides consisting of :

A - adenine T - thymine G - guanine C - cytosine

2. Twisted double stranded DNA bases pair in :

A - T ; C - G

3. In DNA replication base pairs unwind to serve as templates
assembly of new complementary strand

4. Gene - a linear stretch of DNA nucleotide sequence

a. Coding for an assembly of amino acids that forms a polypeptide chain

5. The path for gene expression has two steps :

a. *Transcription* - *m*RNA strand coded from DNA strand in a 5´ - 3´ direction

b. *Translation* - *m*RNA moved from nucleus to cytoplasm proteins are synthesized in the ribosome

B. Three kinds of RNA play a part in protein synthesis

1. *m*RNA : Single strand of information coded from DNA

2. *r*RNA : Ribosome RNA functions as a catalytic site for acid assembly into a protein

3. *t*RNA : Transfer RNA which bring the correct amino acid to the ribosome

III. TRANSCRIPTION OF DNA TO RNA

A. RNA assembly

1. Three ways RNA differs from DNA (a comparison and contrast)

a. RNA is single strand DNA is double strand

b. RNA uses Uracil DNA uses Thymine

c. RNA uses ribose sugar DNA uses deoxyribose sugar

B. RNA transcription differs from DNA replication

a. A single strand of RNA is synthesized from DNA

b. RNA polymerase catalyzes the synthesis of *m*RNA

1. DNA replication uses DNA polymerase

c. *m*RNA uses single region of a single strand of DNA is as a template

IV. *m*RNA to PROTEINS

A. Genetic code

1. Every three base pairs (***triplets***) of *m*RNA specifies an amino acid to be included in a growing polypeptide chain

a. *m*RNA bases pairs are referred to as ***CODONS***

b. 61 base pairs code for 20 amino acids and 3 base pairs serve to ***stop protein synthesis : (UAA, UAG, UGA)***

c. **AUG** (specifies methionine) is the "**start**" codon

B. The roles of *t*RNA & *m*RNA in protein synthesis

1. Each of the 20 amino acids must attach to a specific *t*RNA

a. Each *t*RNA has specific triplet base pair (*ANTI-CODON)* specific for the *m*RNA triplet base pair

2. After *m*RNA arrives in cytoplasm an anticodon of *t*RNA binds to the correct codon sequence of the *m*RNA

a. The first two base pairs of anticodon must pair with the codon by the rules of A with U & G with C

ATG and AUG denote sequences of DNA and RNA respectively that are the start codon or initiation codon encoding the amino acid methionine (Met) in eukaryotes and a modified Met (fMet) in prokaryotes.

The principle called Central dogma of molecular biology describes the process of translation of a gene to a protein. Basically specific sequences of DNA act as a template to synthesize mRNA in a process termed "transcription" in the nucleus. This mRNA is exported from the nucleus into the cytoplasm of the cell and acts as a template to synthesize protein in a process called "translation."

Three nucleotide bases code for one amino acid in the genetic code. Usually the first three bases of the coding sequence(CDS) of mRNA to be translated into protein are AUG (or ATG in DNA). AUG encodes for methionine, and therefore the first amino acid of many proteins is methionine. The start codon is almost always preceded by an untranslated region 5' UTR.

Very rarely in higher organisms (eukaryotes) non AUG start codons are used.

In addition to AUG, alternative start codons, mainly GUG and UUG are used in prokaryotes. For example E. coli uses 77% ATG (AUG), 14% GTG (GUG), 8% TTG (UUG) and a few others. As observed in completely sequenced E coli genomes .

b. The third base pair has some latitude in pairing resulting in condition of *WOBBLE* effect

c. Transcription takes place within a ribosome structure

1) The ribosome is composed of *r*RNA and proteins which work together during protein synthesis

C. STAGES OF TRANSLATION

1. (1) Initiation; (2) Elongation; (3) Termination

2. Initiation

a. A complex is formed between *t*RNA the small ribosomal subunit, *m*RNA and the large ribosomal subunit

1) Initiation step begins with initiator *t*RNA (a *t*RNA containing methionine amino acid)

2) Initiator *t*RNA binds to 'P' site of small ribosomal subunit

3) Scanning of *m*RNA allows recognition of codon AUG

4) Large ribosomal subunit binds to small ribosomal subunit

5) Specific *t*RNA for *m*RNA (anticodon to codon) binds to 'A' site of small ribosomal subunit

6) Reaction catalyzed between amino and carboxyl group resulting in peptide bond

3. Elongation

a. A series of *t*RNAs delivers amino acids in sequence by codon anti-codon matching forming peptide bonds

4. Termination

a. Stop codon reached

b. Polypeptide chain is released into cytoplasm

5. Several ribosomes (POLYSOME) may be moving along the same *m*RNA simultaneously

V. MUTATION AND PROTEIN SYNTHESIS

A. Changes in the genetic struction of DNA

1. Changes in the protein structure should result

B. Gene mutation results from:

1. Bases being added, deleted, or replaced

2. Mutations are rare and may result from mutagens such as:

a. Viruses, ultraviolet light (UV), & chemicals

3. Spontaneous mutations

a. DNA template frameshift

b. Transposable elements of DNA “jumping” to a new location on the DNA