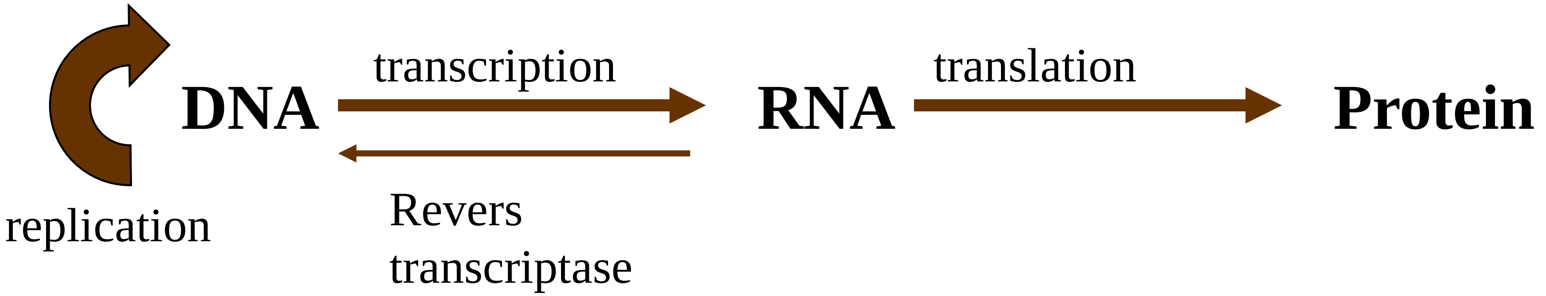
DNA replication

The central dogma of molecular biology



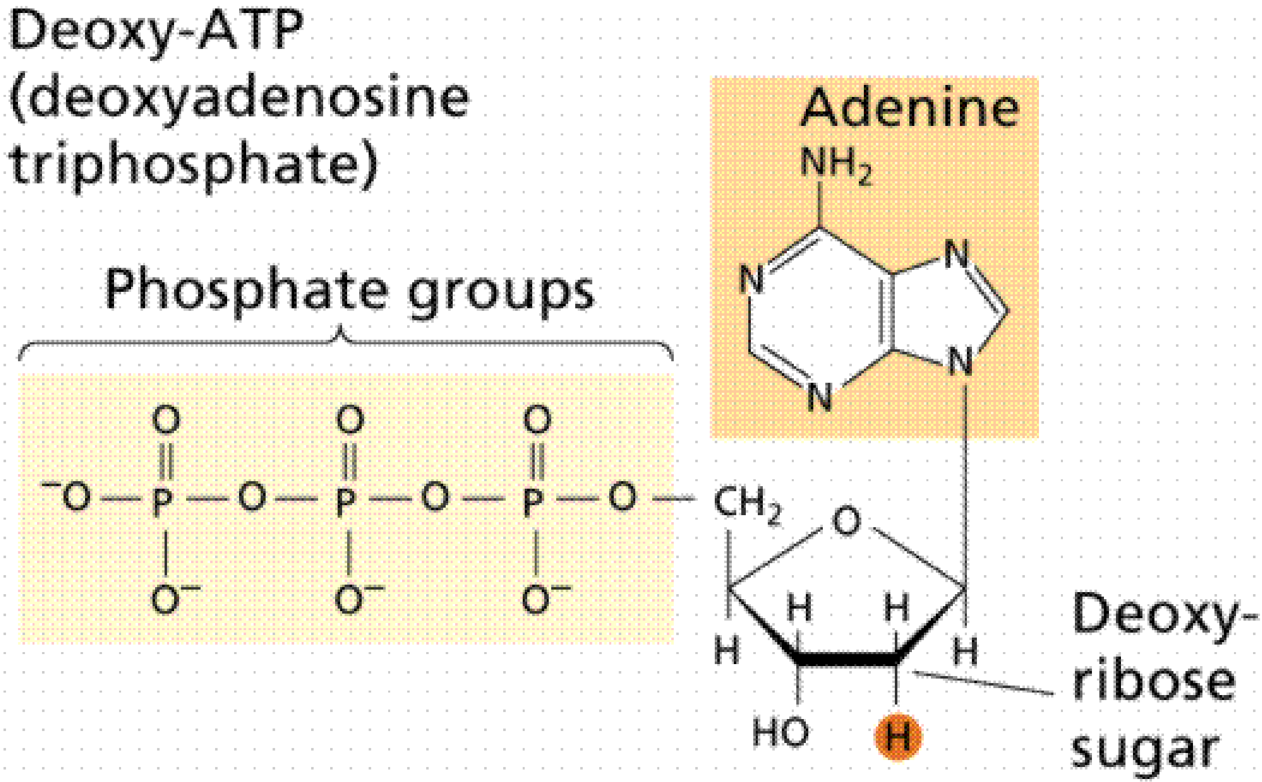
The information stored by DNA:

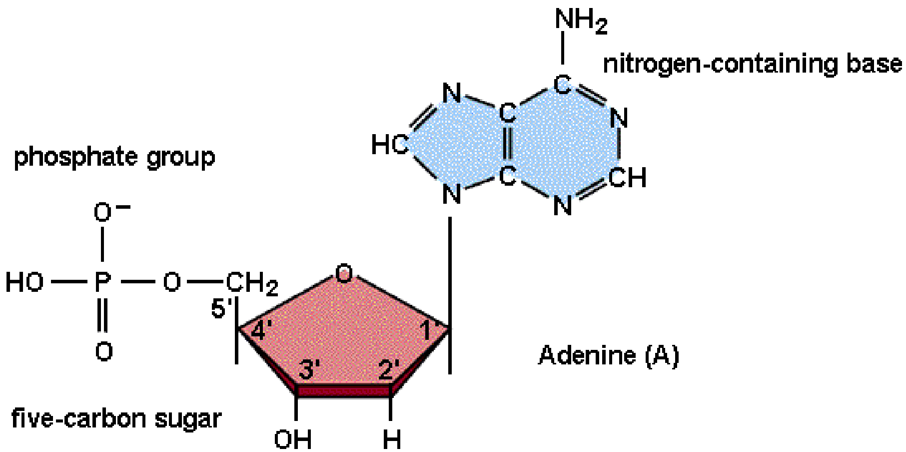
- protein structure
- the regulation of protein synthesis

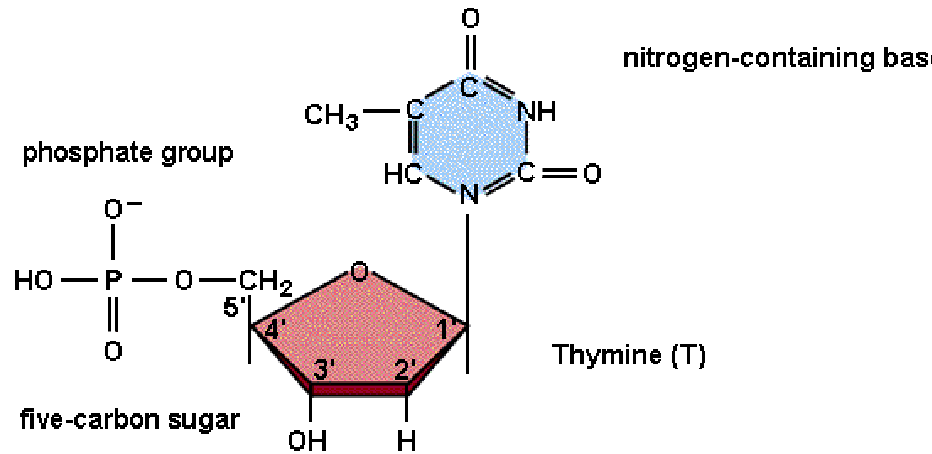
Nucleic acids: polimers made out of nuleotid monomers

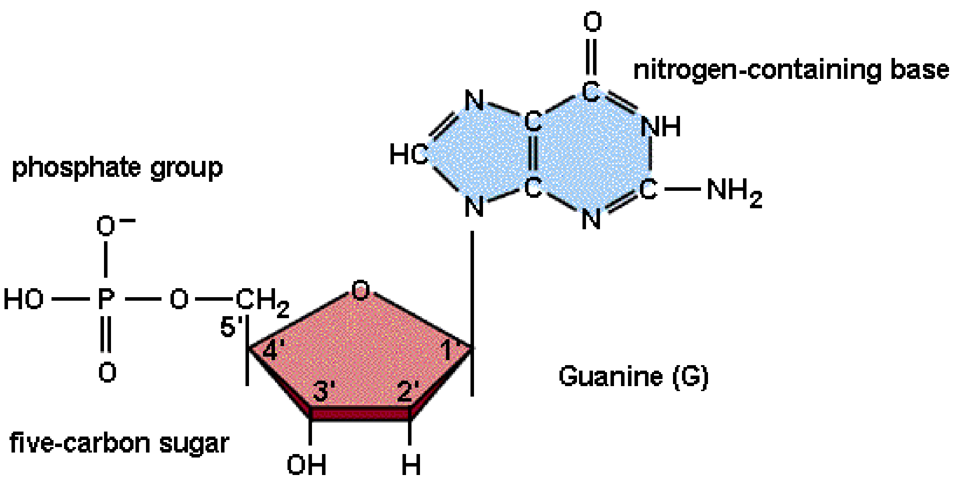
RNA: adenine, guanine, cytosine, uracil bases and ribose

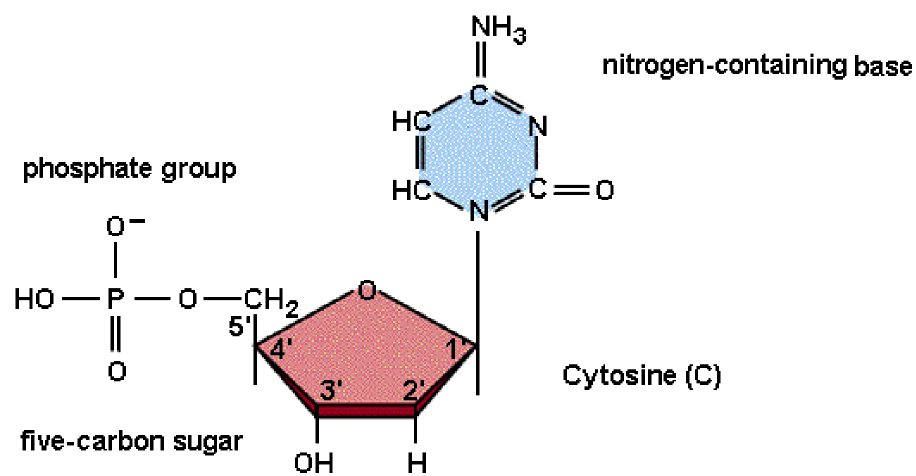
DNS: adenine, guanine, cytosine, thymine beses and deoxy-riboses

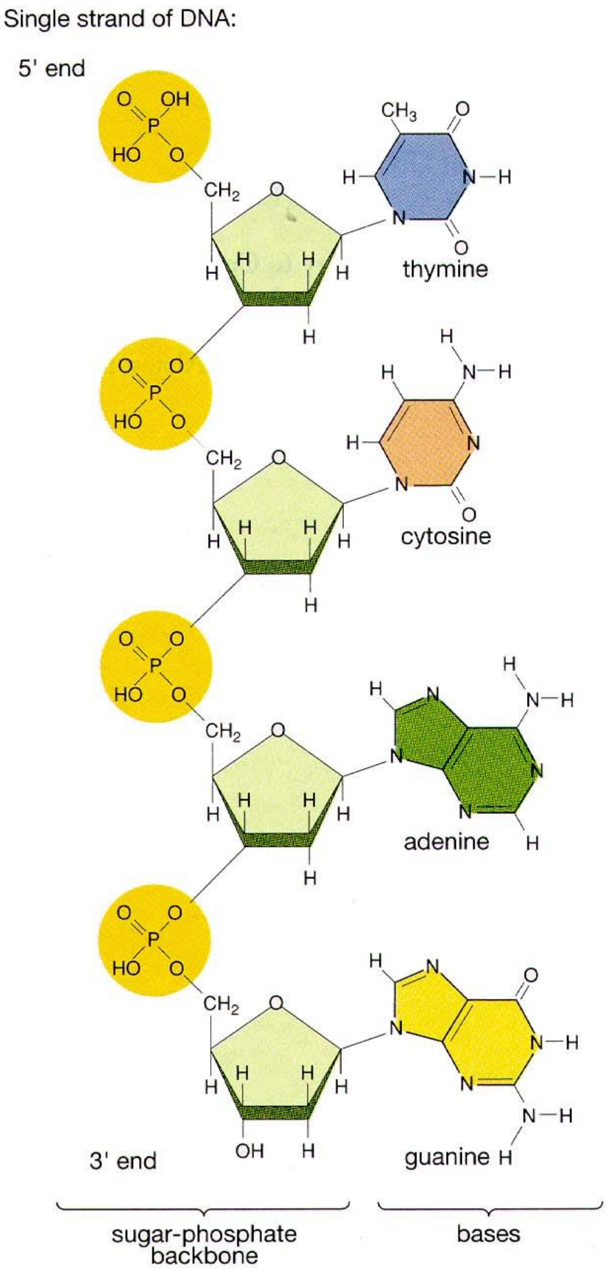

Deoxy-ATP
(deoxyadenosine
triphosphate)
Phosphate groups
Adenine
NH2
N
N
N
N
⁻O
P
O
O
P
O
O
P
O
O
O
O⁻
O⁻
O⁻
CH2
O
H
H
H
H
HO
H
Deoxy-
ribose
sugar


nitrogen-containing base
phosphate group
five-carbon sugar
Adenine (A)


nitrogen-containing base
phosphate group
five-carbon sugar
Thymine (T)

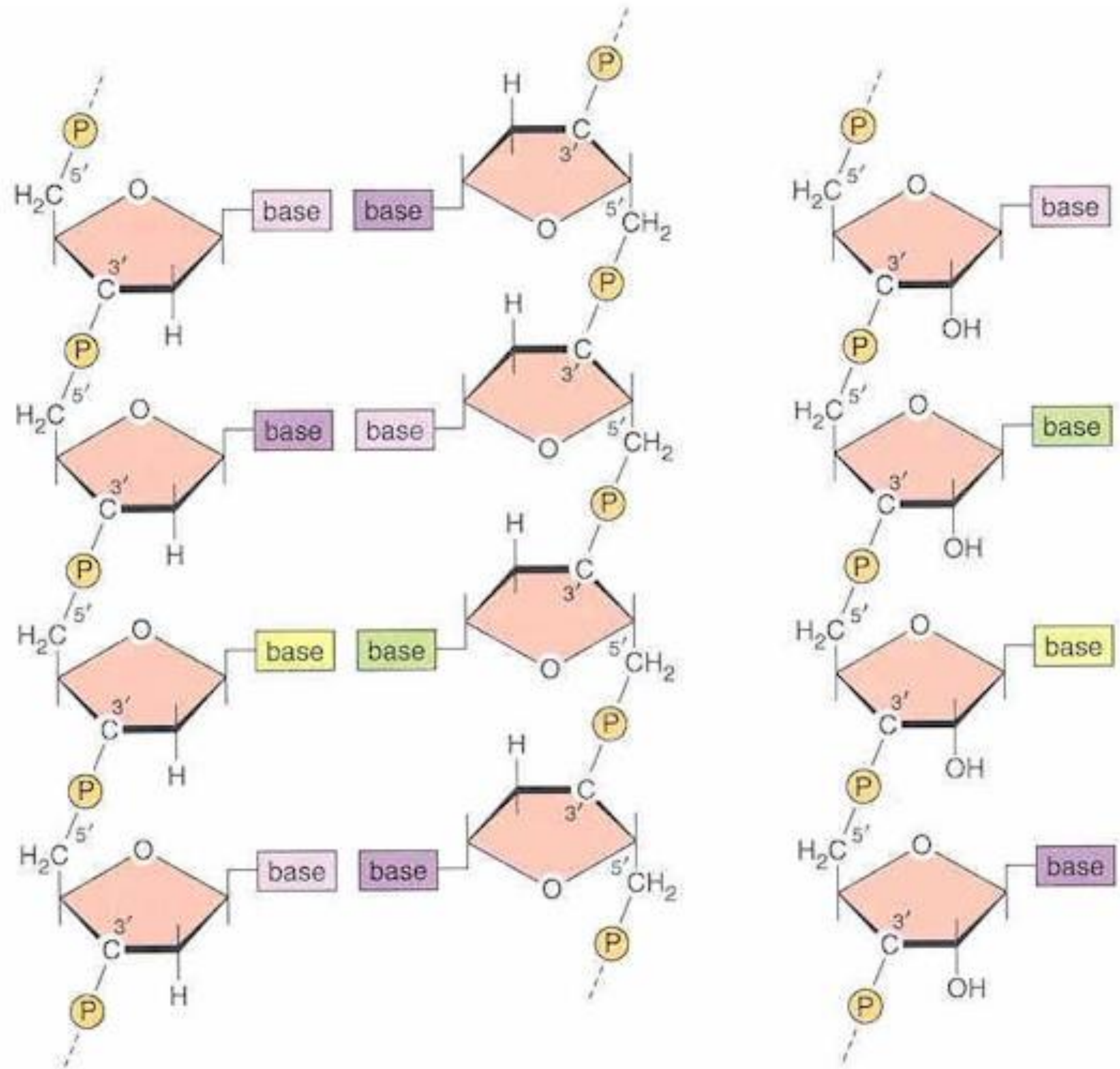

nitrogen-containing base
phosphate group
five-carbon sugar
Guanine (G)


nitrogen-containing base
phosphate group
five-carbon sugar
Cytosine (C)

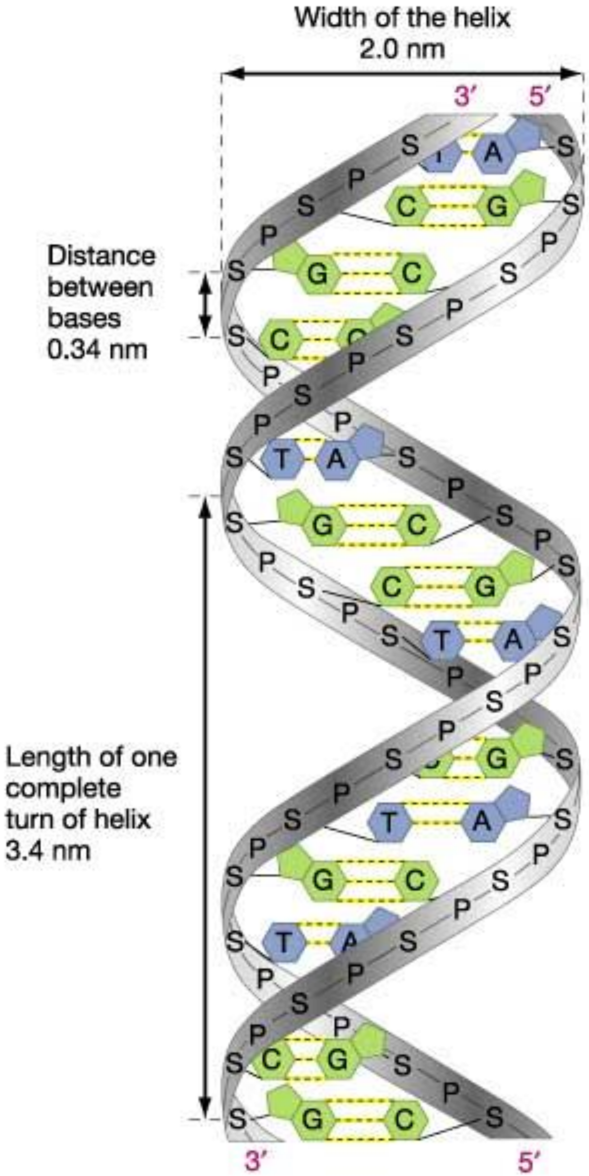


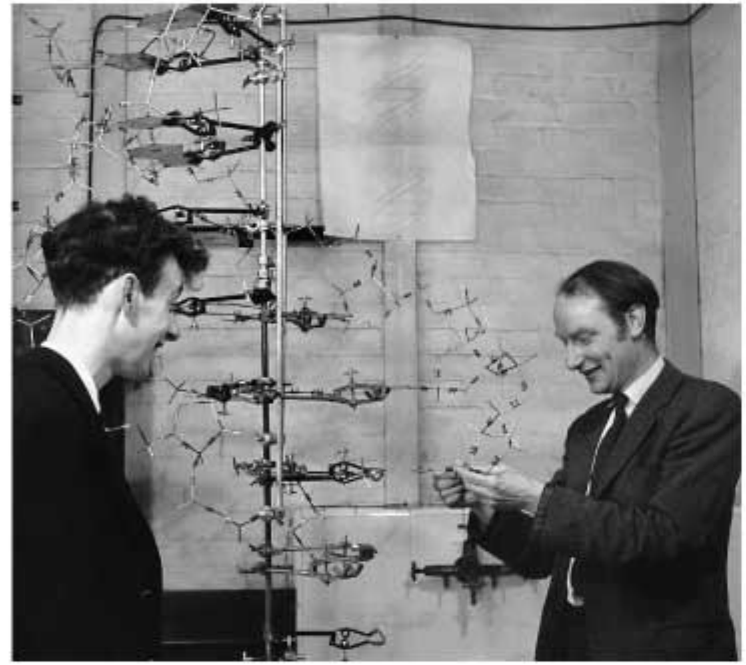
Polimer backbone: bridges between ribonucleotide (RNA), or deoxyribonucleotide units (DNA).

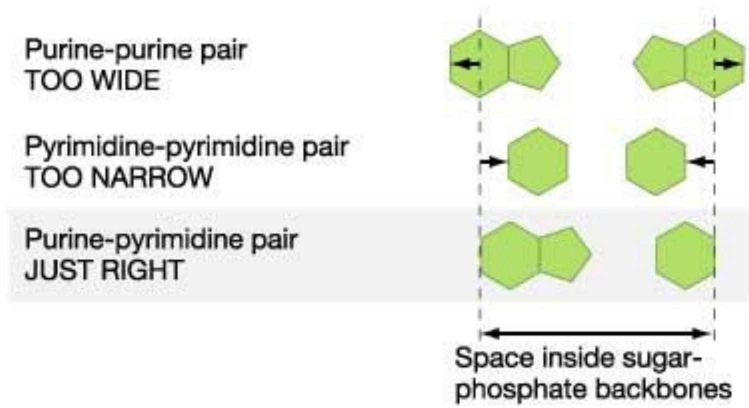
Information: the sequence of deoxyribonucleotides



a DNA b RNA


Width of the helix
2.0 nm
3′
5′
Distance between bases 0.34 nm
Length of one complete turn of helix 3.4 nm
3′
5′

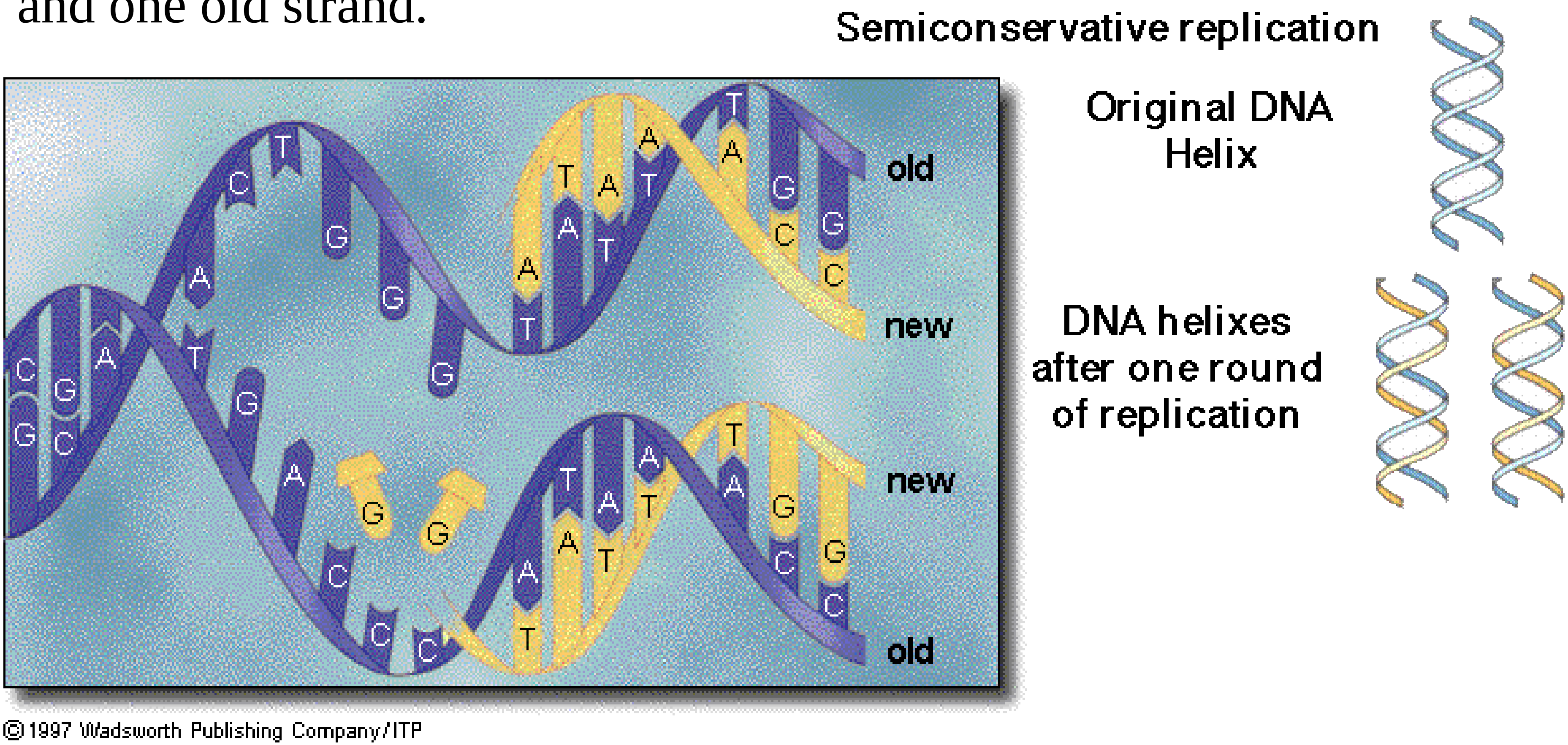



Purine-purine pair
TOO WIDE
Pyrimidine-pyrimidine pair
TOO NARROW
Purine-pyrimidine pair
JUST RIGHT
Space inside sugar-phosphate backbones

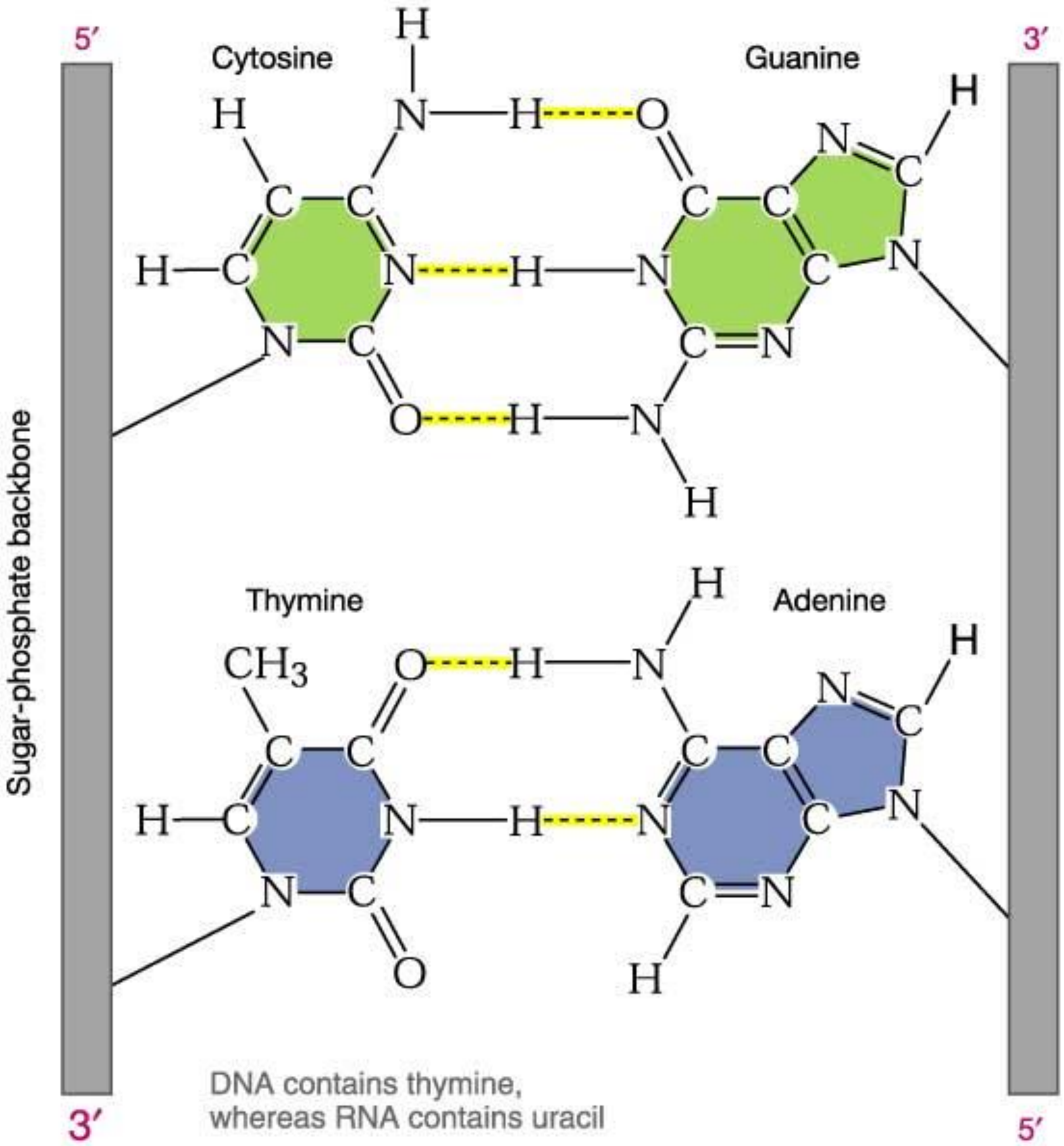
DNA replication must occur before a cell can produce two genetically identical daughter cells.

The replication of DNA is semiconservative

Each DNA strand serves as a template for the synthesis of a new strand, producing two new DNA molecules, each with one new strand and one old strand.



The hydrogen bonds between complementary bases and the common geometry of the standard A=T and G≡C base pairs provide the correct pairing



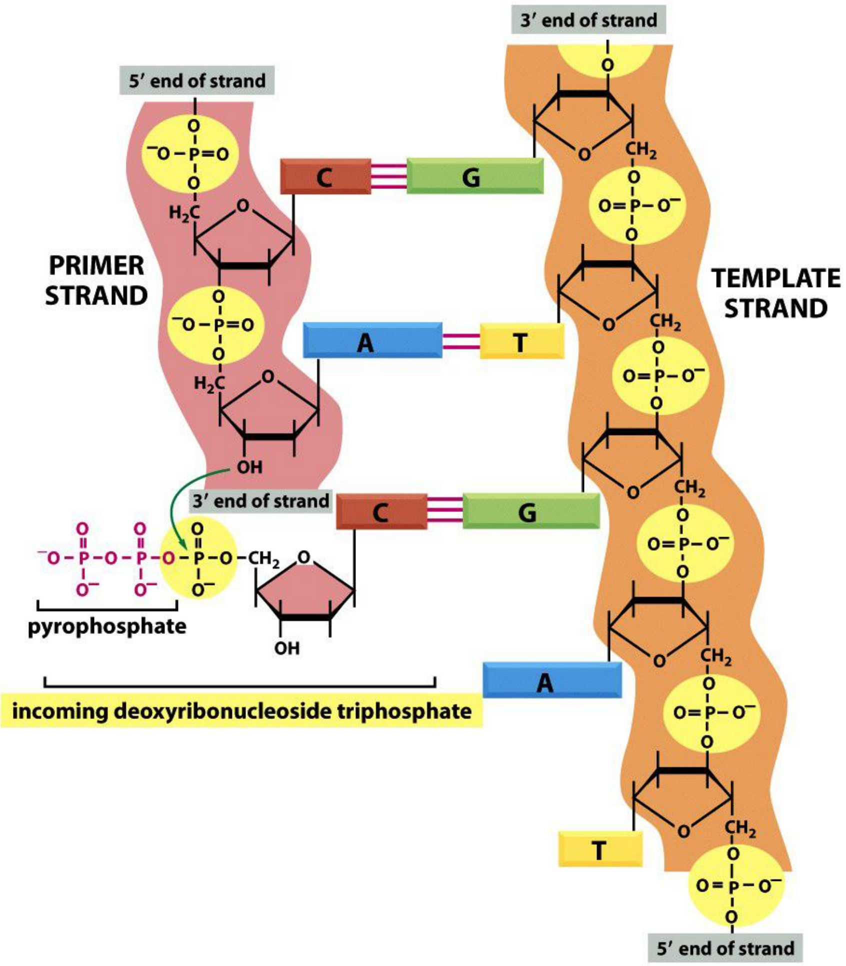
DNA polymerization has two requirements:

1. **Template**
2. **Primer**

The primer is a strand segment (complementary to the template) with a free 3’-hydroxyl group

All DNA polymerases can only add nucleotides to a preexisting strand

Many primers are oligonucleotides of RNA.



The replication of DNA in prokaryotes

The enzymes of the process

DNA polimerase I: the first known enzyme DNA polimerase, consist of one polipeptide chain, 3 different activity:

- synthetic activity
- correction 3’-5’ exonuclease
- correction 5’-3’ exonuclease activity

The main function of DNA polymerase I is repair.

DNA polimerase III: this enzyme responsible for the replication, consist of many subunits, 2 different enzyme activity:

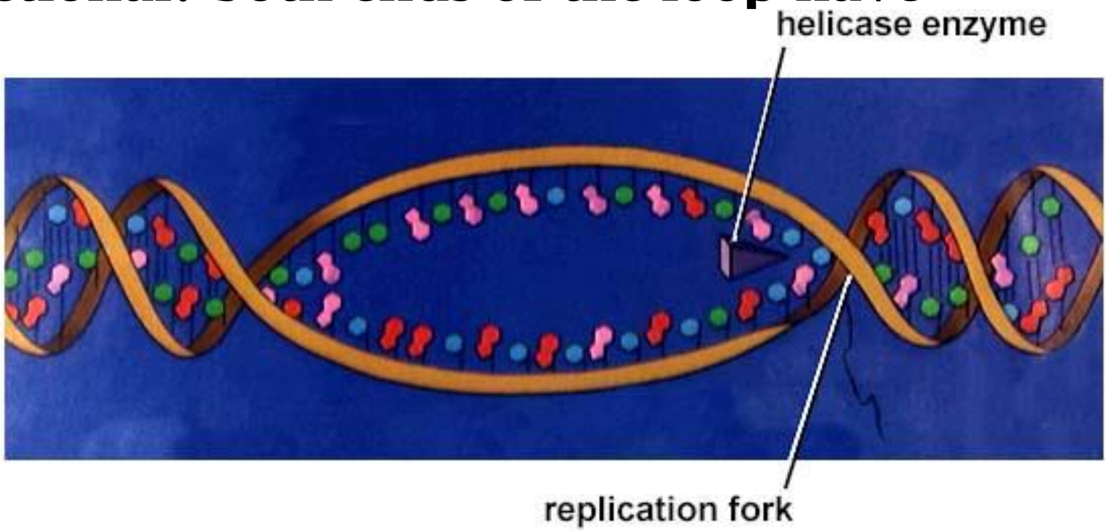
- synthetic activity
- correction 3’-5’ exonuclease activity

DNA polymerase III is the principal replication enzyme

The process of DNA replication

The direction of DNA synthesis: from the 5’ end to the 3’ end.

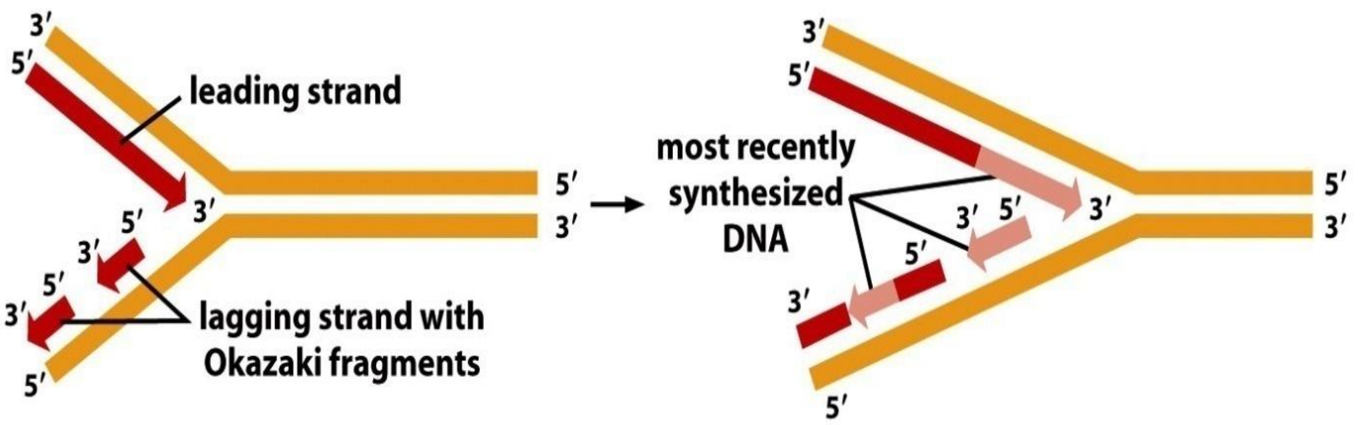
The replication is bidirectional: both ends of the loop have active replication forks



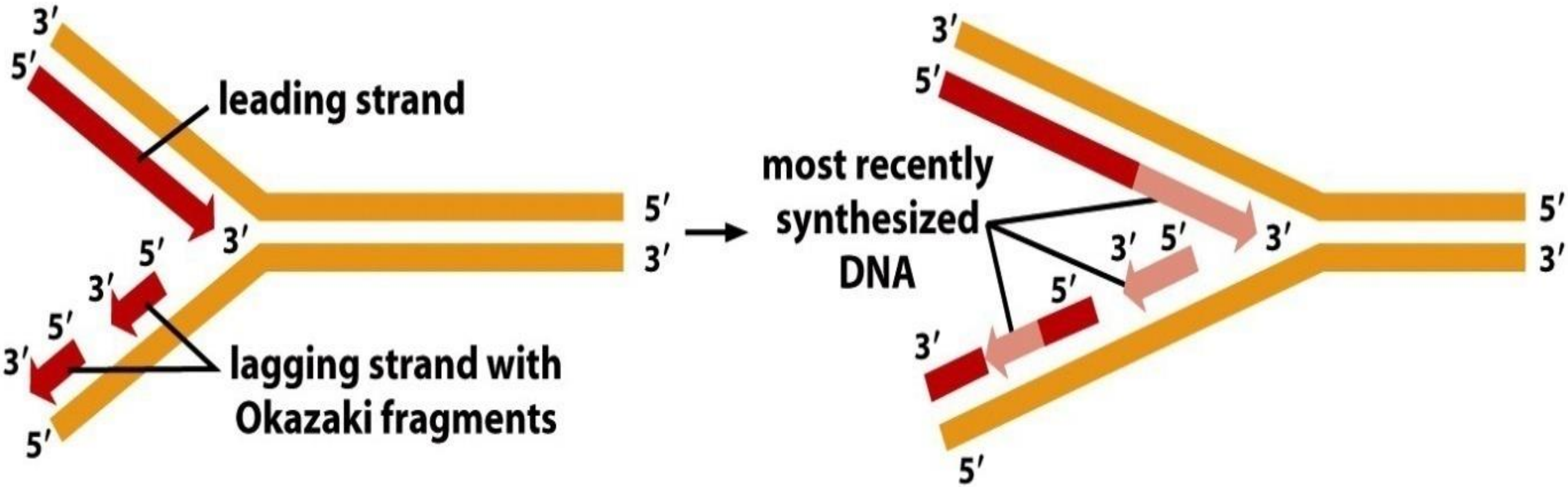
The template DNA strand and the sythesising daughter strand are antiparalel.

synthesis 5’ ⟶ 3’

reading 3’ ⟶ 5’



If both strands were synthesized continuously while the replication fork moved, one strand would have to undergo 3’ → 5’ synthesis



One strand is synthesized continuously and the other discontinuously.
The **leading strand** is **continuously** synthesized in the direction taken by the replication fork.
The other strand, the **lagging strand**, is synthesized **discontinuously** in short pieces

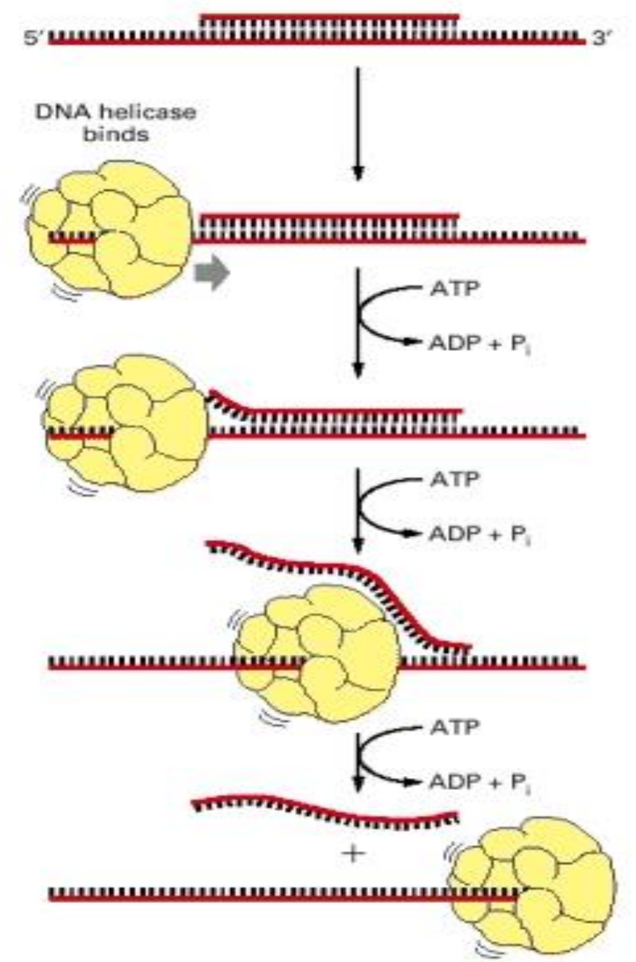
DNA double helix must be opened up ahead of the replication fork

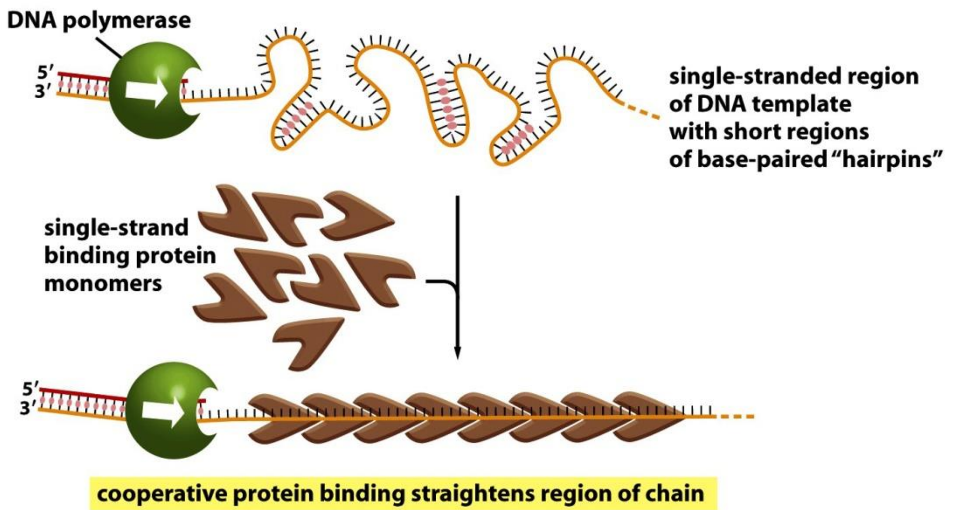
DNA polymerases and DNA primases can copy a DNA double helix only when the template strand has already been exposed by separating it from its complementary strand

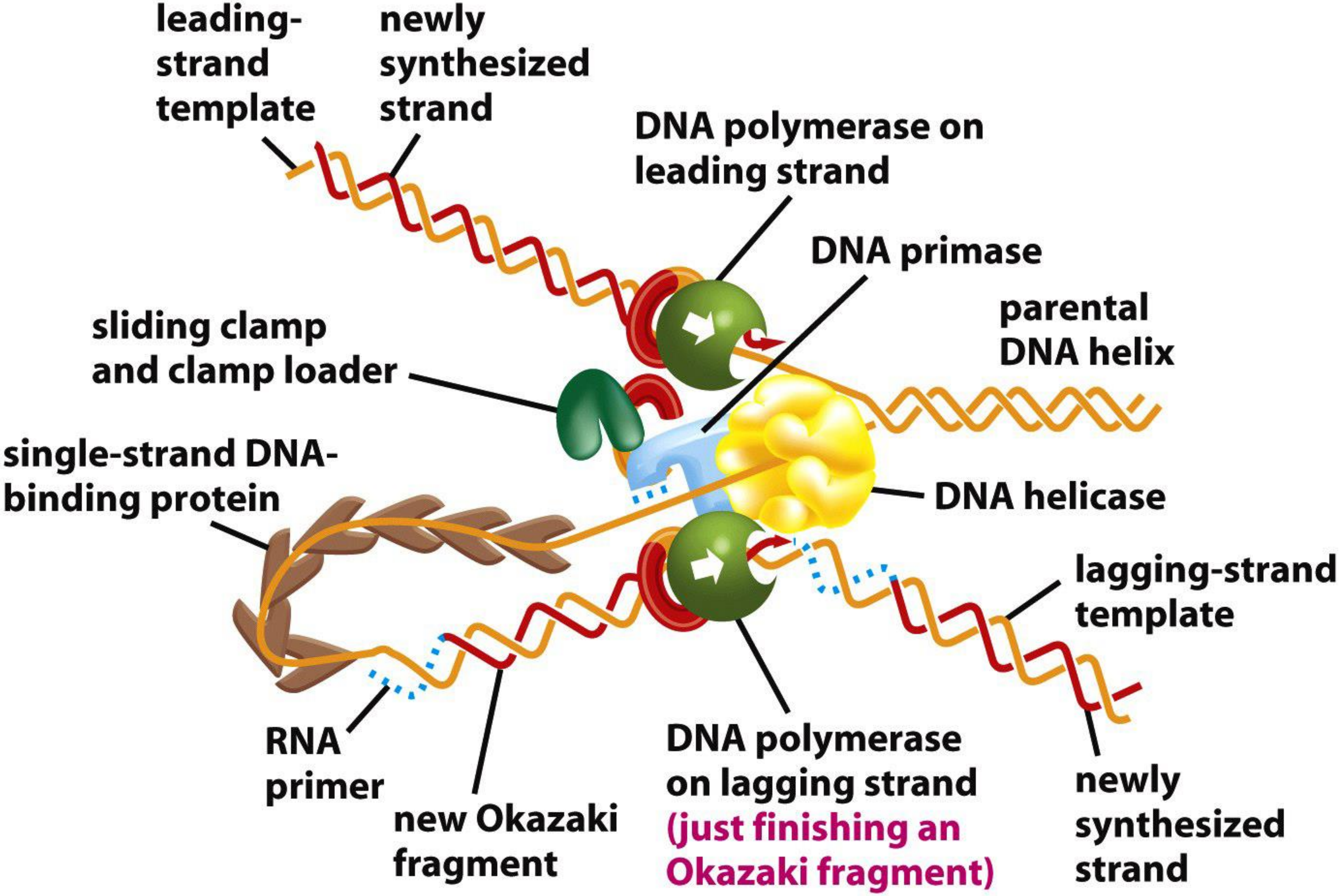
Two types of protein contribute to the opening:

• DNA helicases

• Single-strand DNA-binding proteins: aid helicases by stabilizing the unwound, single-stranded conformation







leading-strand template
newly synthesized strand
DNA polymerase on leading strand
DNA primase
parental DNA helix
sliding clamp and clamp loader
single-strand DNA-binding protein
DNA helicase
lagging-strand template
RNA primer
new Okazaki fragment
DNA polymerase on lagging strand (just finishing an Okazaki fragment)
newly synthesized strand

1. **Leading strand synthesis begins with the synthesis of a short RNA primer at the replication origin by primase.**
2. **Deoxyribonucleotides are added to this primer by a DNA polymerase III complex**
3. **Leading strand synthesis then proceeds continuously, keeping step with the unwinding of DNA at the replication fork.**
4. **Lagging strand synthesis is accomplished in short Okazaki fragments.**
5. **Once an Okazaki fragment has been completed, its RNA primer is removed and replaced with DNA by DNA polymerase I, and the remaining nick is sealed by DNA ligase.**

Eventually, the two replication forks of the circular E. coli chromosome meet at a terminus region.

Proofreading

Replication is very accurate.

The bases opposite each other (in the double DNA helix) should be: complementary bases.

The not complementary bases are dissected by the polimerase enzyme: 3‘-5’ exonuclease activity.

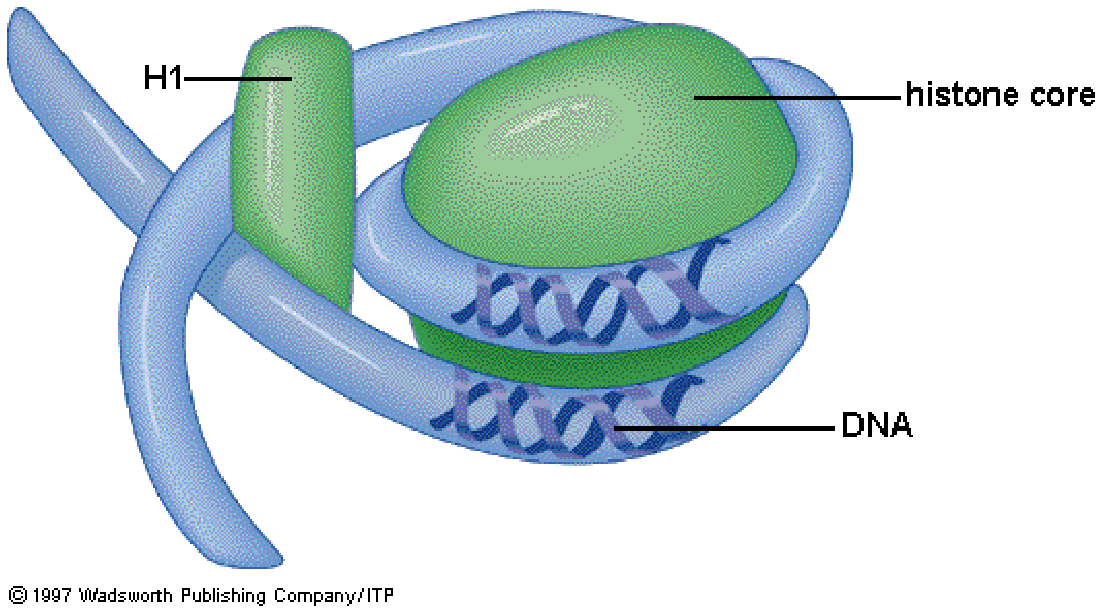
In region of complementary double helixes the 5’-3’ exonuclease activity plays important role in proofreading mechanism.

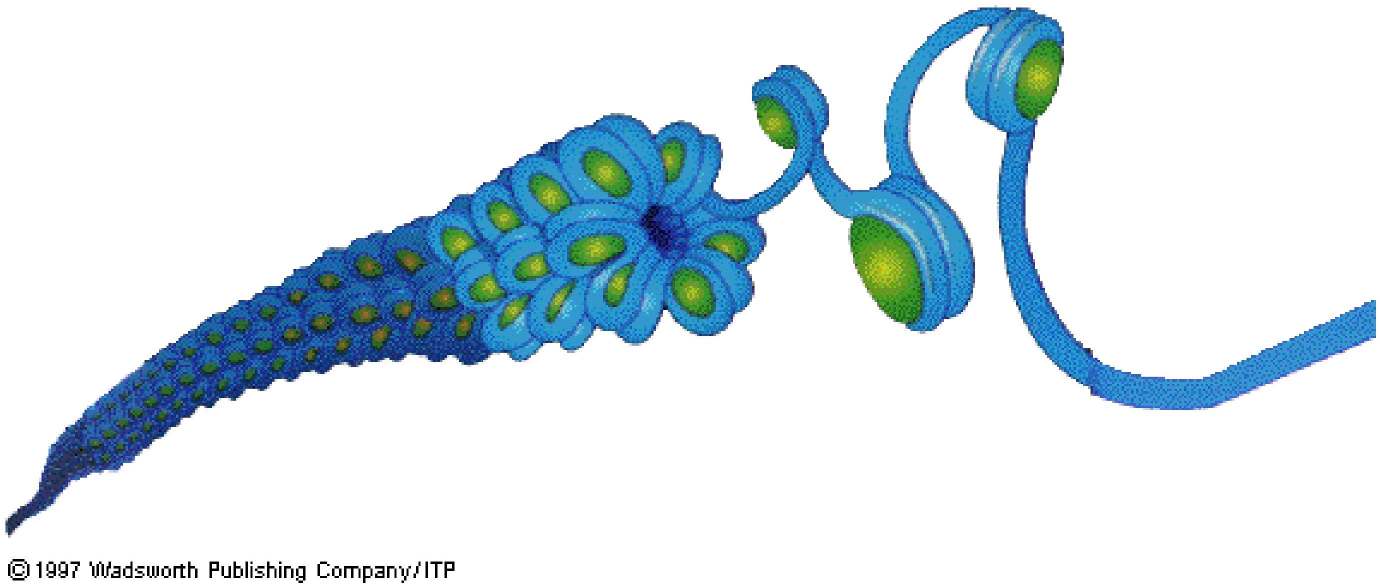
DNA-ligase

Two DNA strands are joined by DNA-ligase enzyme. The energy demand of the reaction is covered by the hydrolysis of NAD in prokaryotes and ATP hydrolysis in eukaryotes.

The organisation of eukaryotic chromosome

nucleosome



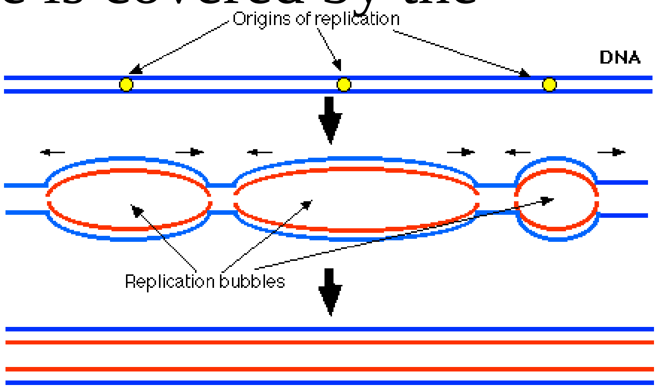


The special features of eukaryotic replication

The replication initiates at many starting ponts along the linear DNA molecule

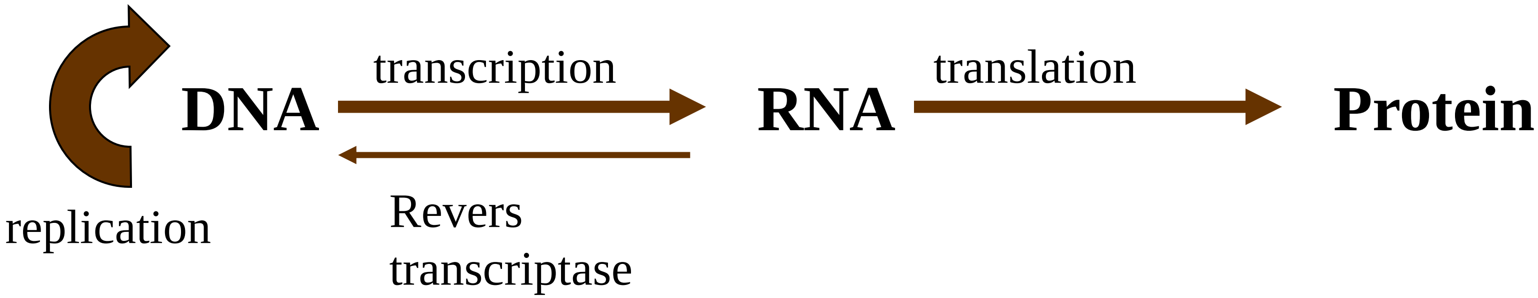
The leading and lagging strand are synthesised by different polimerases

- α-DNA polimerase: lagging strand
- δ-polimerase: leading strand
- the eukaryotic polimerases have not got exonuclease activity
- The energy demand of DNA-ligase is covered by the hydrolysis of ATP.



Transcription: the synthesis of ribonucleic acids

The central dogma of molecular biology

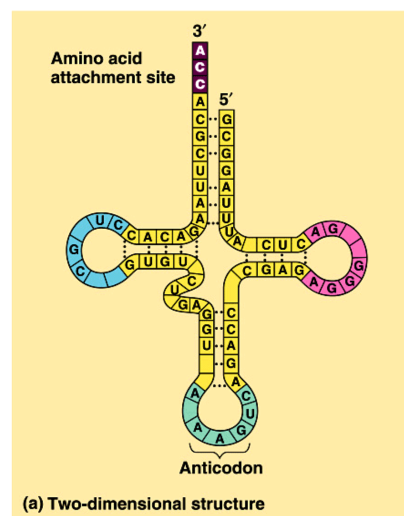


Three major kinds of RNA are produced.

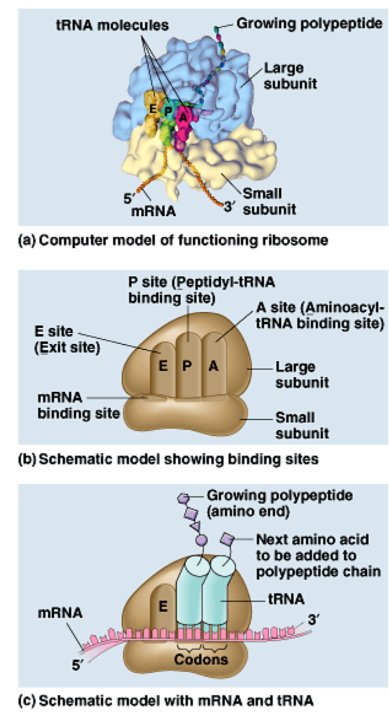
mRNA: carries the genetic information from DNA to the place of protein synthesis (ribosomes).

rRNA: a component of the protein synthesizing machinery (ribosomes).

tRNA: an adaptermolecule, translates the genetic code to amino acids.



(a) Two-dimensional structure



(a) Computer model of functioning ribosome

(b) Schematic model showing binding sites

(c) Schematic model with mRNA and tRNA

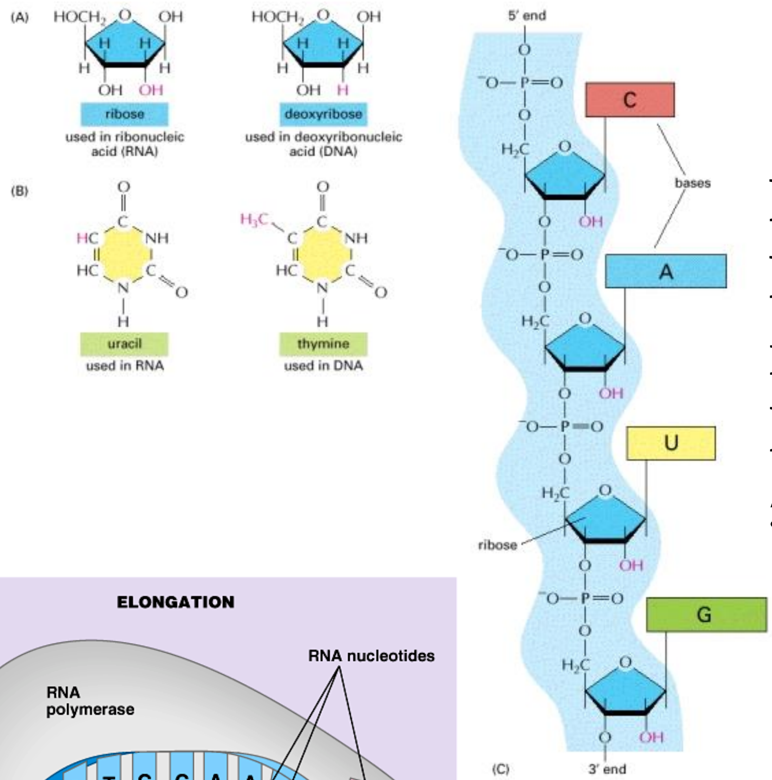
During transcription, an enzyme system converts the genetic information in a segment of **double-stranded DNA into an RNA strand** with a **base sequence complementary** to one of the DNA strands.
Only **particular part of DNA** (genes or groups of genes) **are transcribed**
Specific regulatory sequences mark the beginning and end of the DNA segments to be transcribed and designate which strand in duplex DNA is to be used as the template.

Transcription resembles replication in its fundamental chemical mechanism direction of synthesis, and its use of a template.

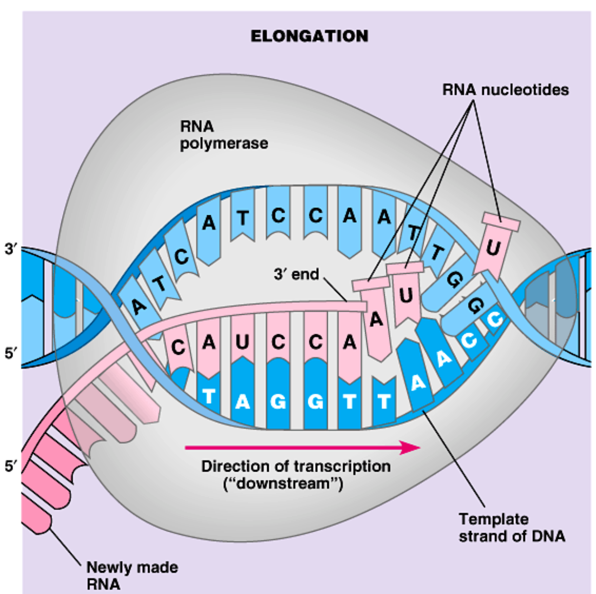
Transcription differs from replication in that it does not require a primer and involves only limited segments of a DNA molecule.

Transcription has three phases, initiation, elongation, and termination.

DNA-dependent RNA polymerase requires a DNA template and all four NTPs of the nucleotide units of RNA.



RNA polymerase elongates an RNA strand by adding ribonucleotide units to the 3'-hydroxyl end, building RNA in the 5' → 3' direction.

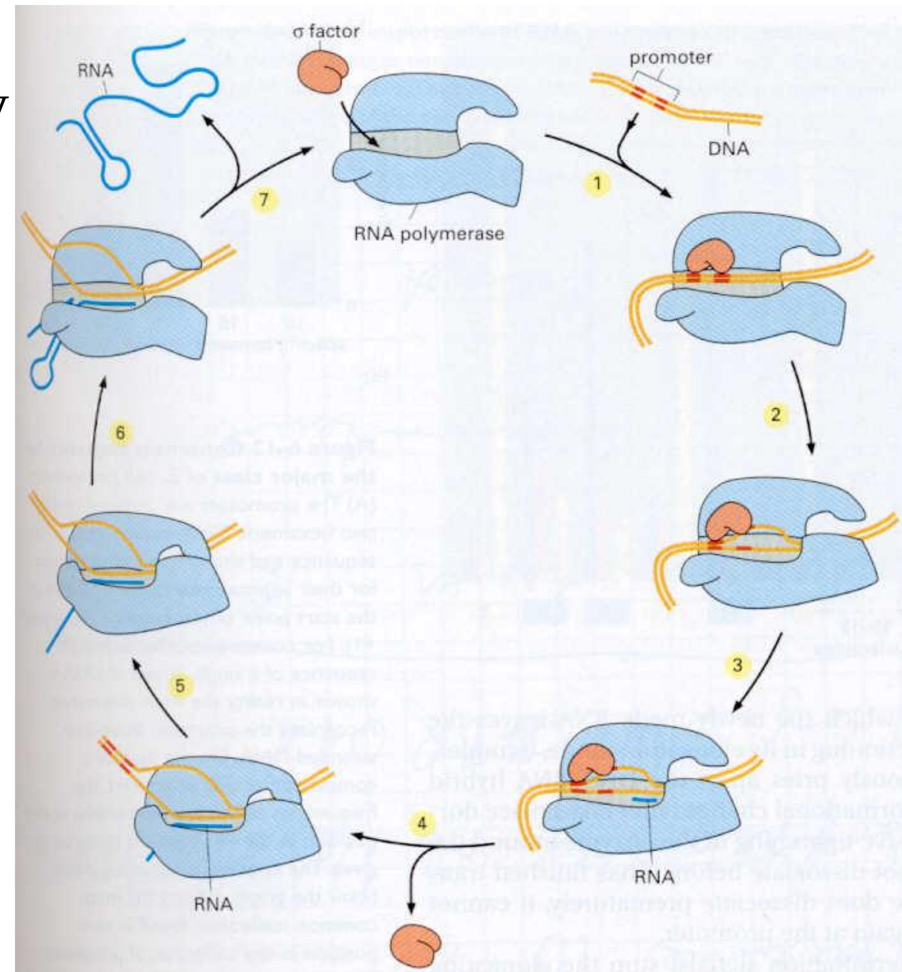


Each nucleotide in the newly formed RNA is selected by base-pairing interactions: U=A, G≡C.

Transcription has three phases, **initiation**, elongation, and termination.

Initiation occurs when RNA polymerase binds at specific DNA sequences called promoters

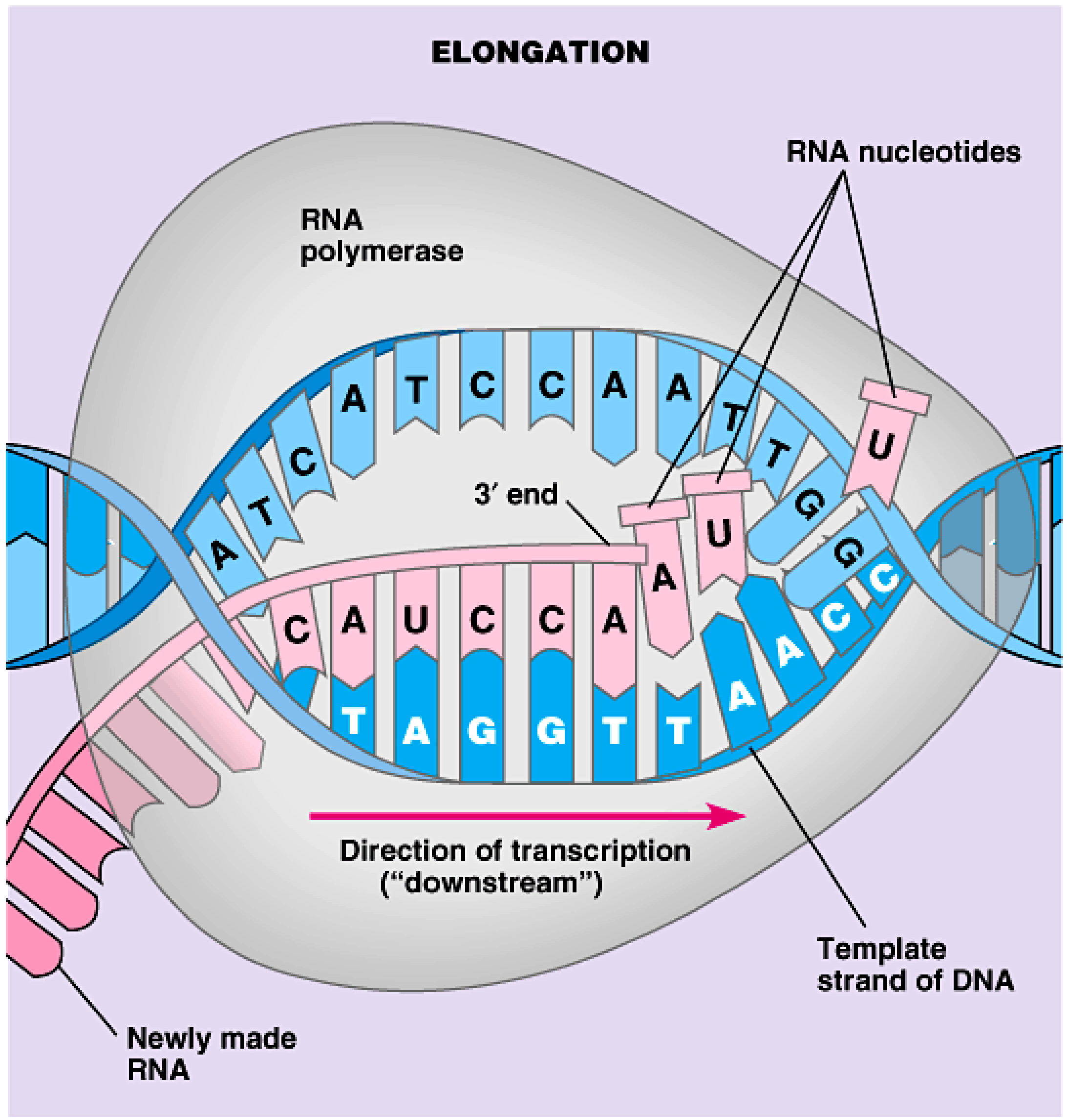
The role of the promoter region in transcription



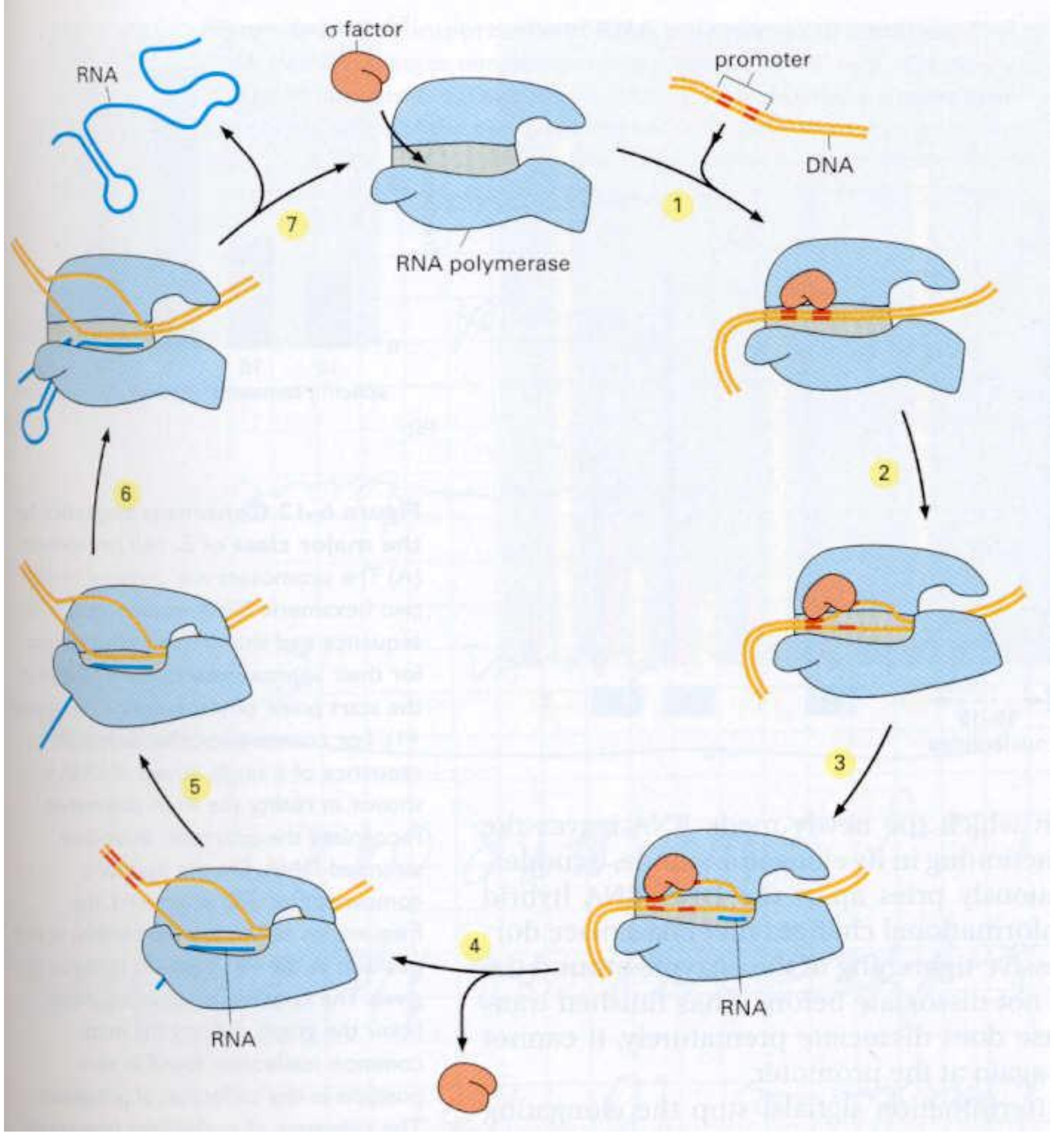
The promoter region is recognised by the σ factor of the RNA polimerase

The DNA duplex must unwind over a short distance, forming a transcription bubble.

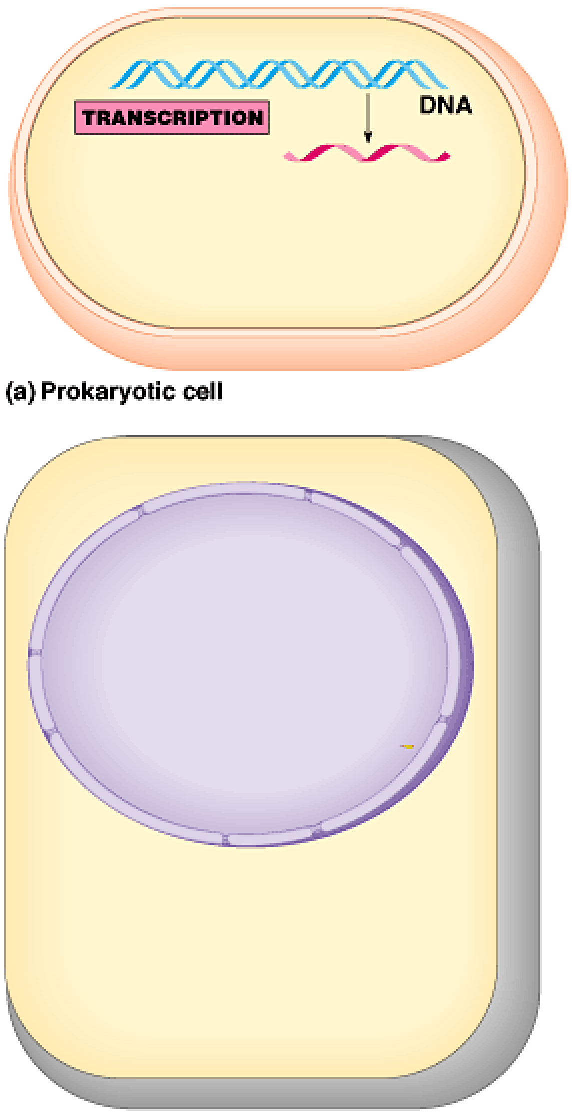
During the elongation phase of transcription, the growing end of the RNA strand forms an 8 bp long hybrid RNA-DNA double helix with the DNA template.


ELONGATION
RNA polymerase
RNA nucleotides
3′
5′
5′
3′ end
A T C A T C C A A T T G G
C A U C C A A U U
T A G G T T A A C C
Direction of transcription ("downstream")
Template strand of DNA
Newly made RNA

When RNA polymerase reaches a terminator sequence, RNA synthesis halts, and the RNA polymerase dissociates from the DNA.

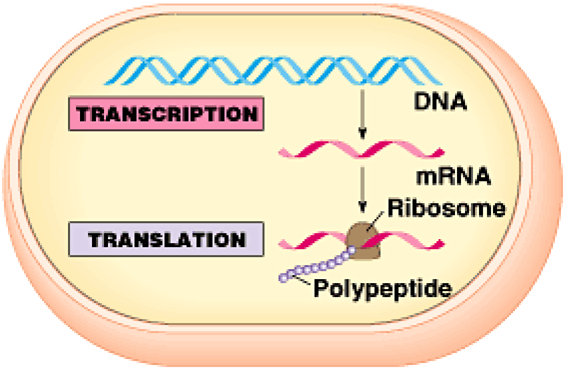


Transcription

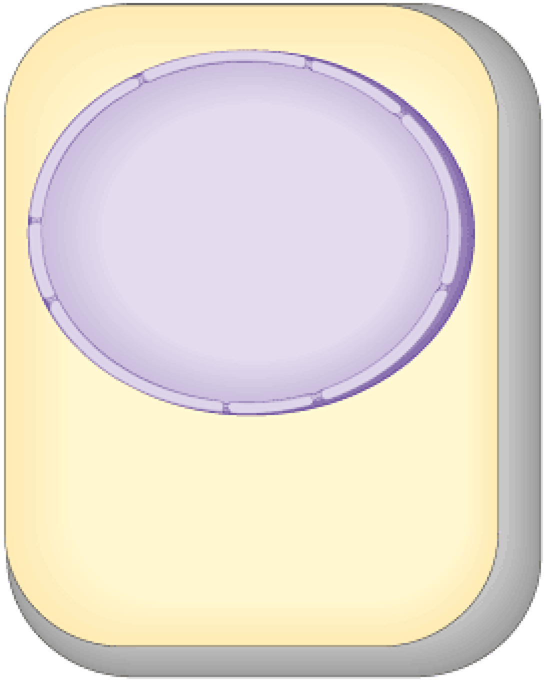


(a) Prokaryotic cell

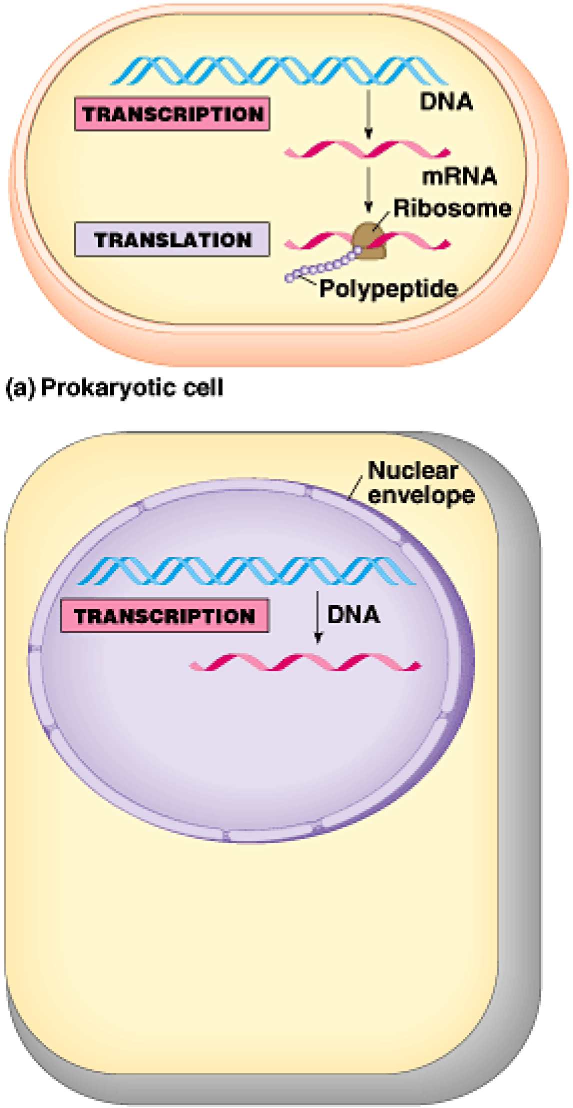
(b) Eukaryotic cell



(a) Prokaryotic cell

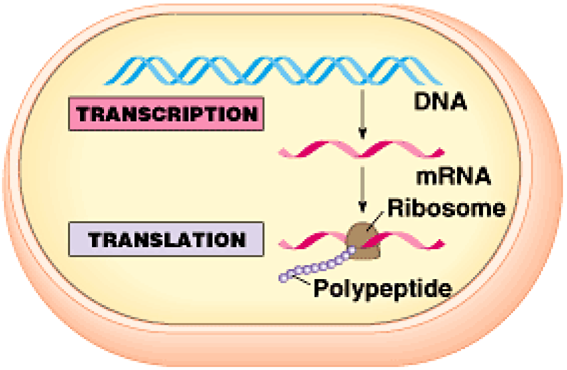


(b) Eukaryotic cell

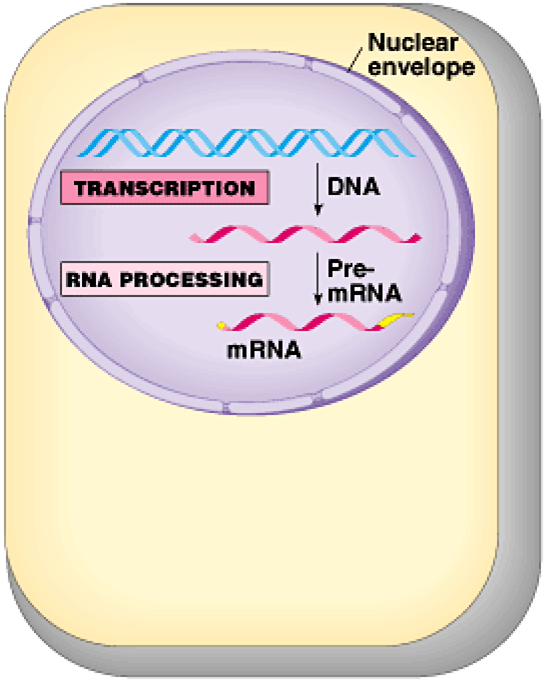


(a) Prokaryotic cell

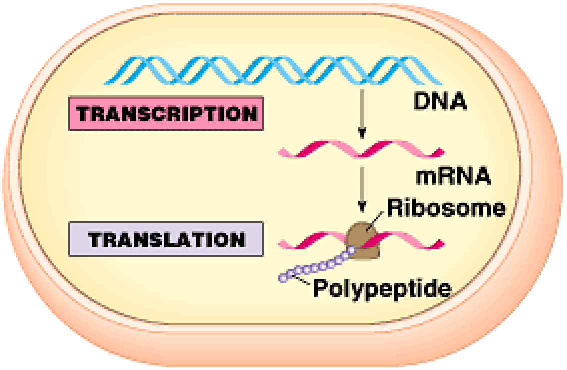
(b) Eukaryotic cell



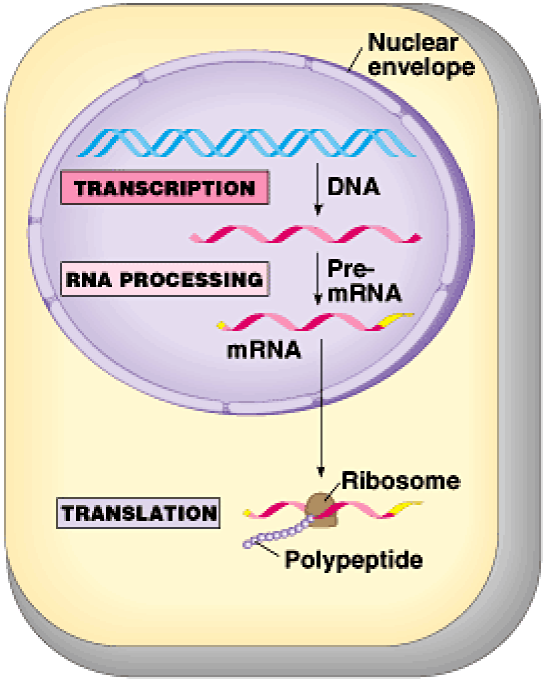
(a) Prokaryotic cell



(b) Eukaryotic cell



(a) Prokaryotic cell



(b) Eukaryotic cell

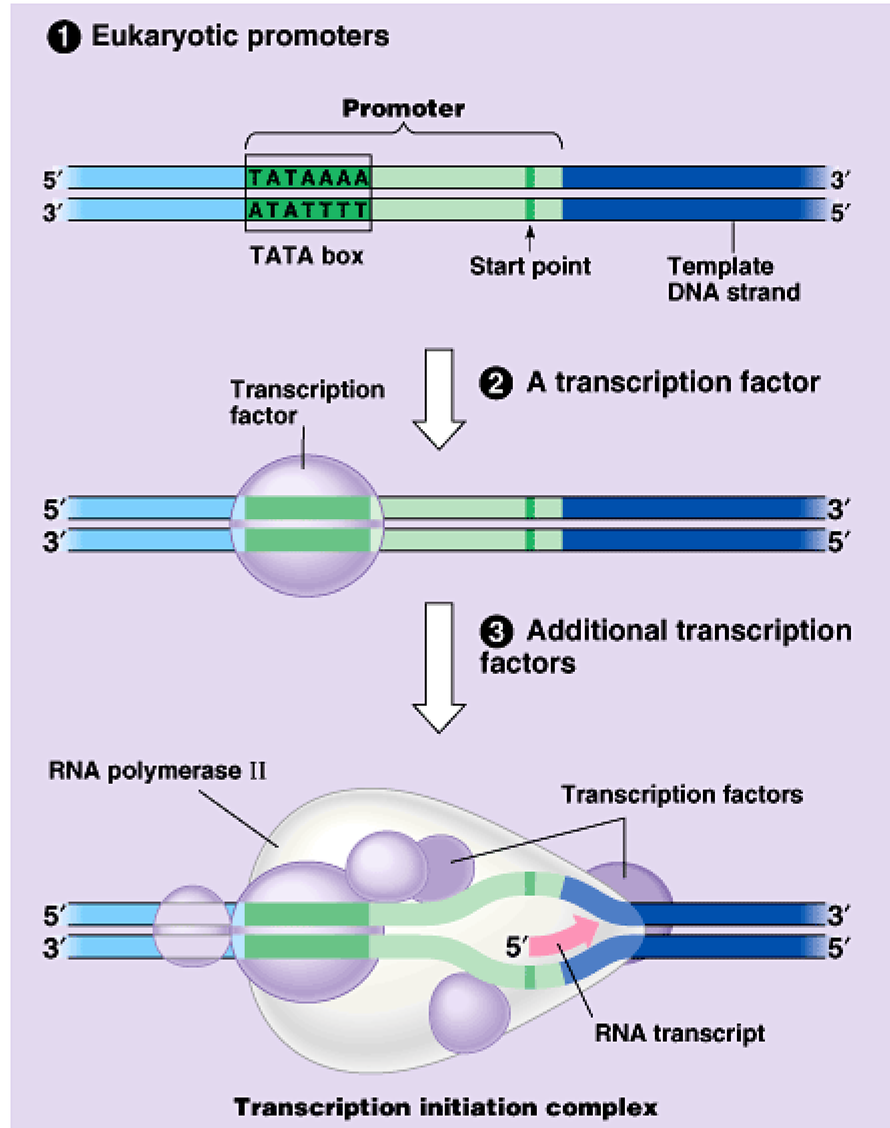
The transcription in a eukaryotic cell is much more complex than that in bacteria.

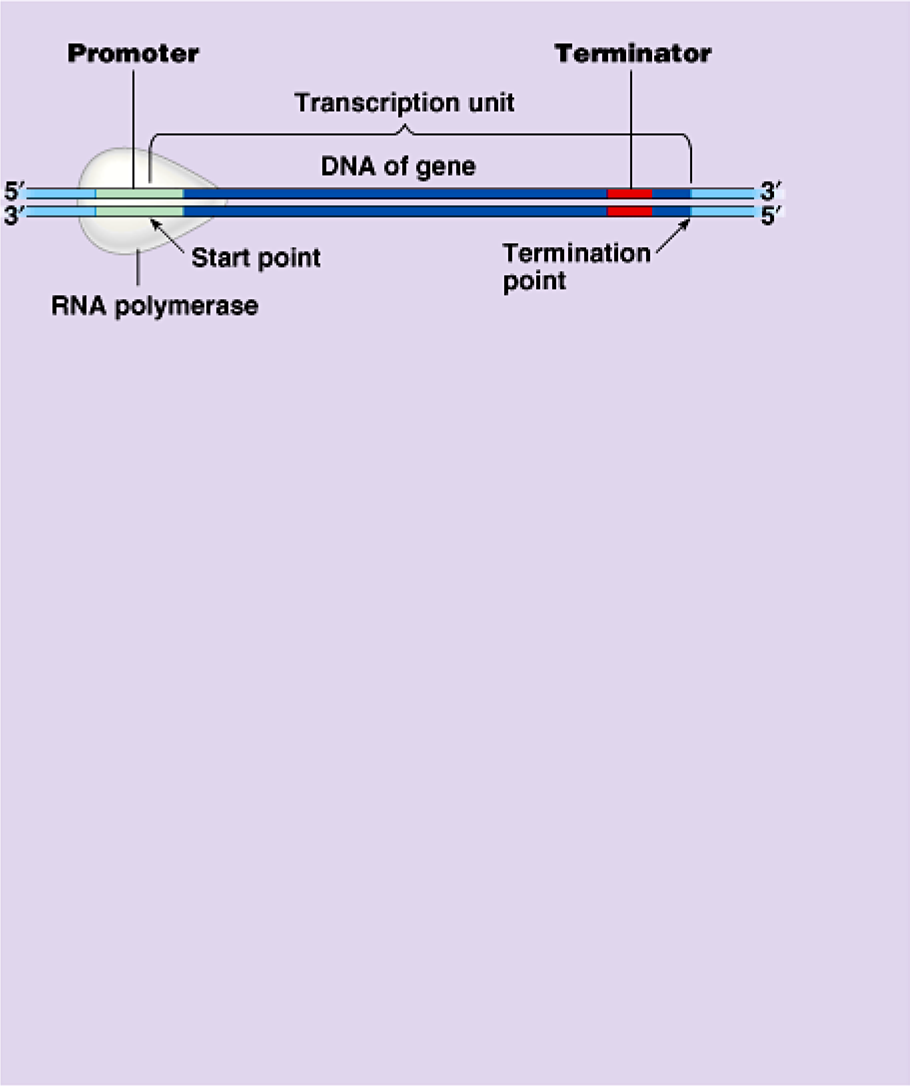
Eukaryotes have three RNA polymerases, designated I, II, and III

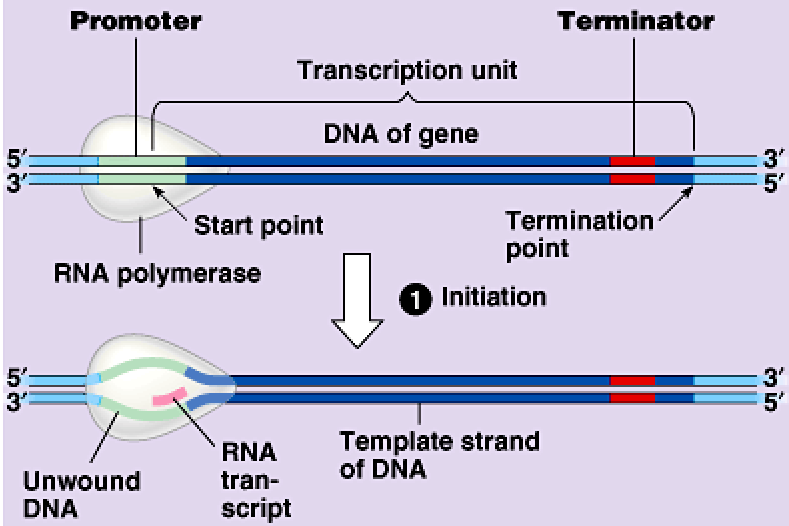
RNA polymerase I is responsible for the synthesis of pre-ribosomal RNA.

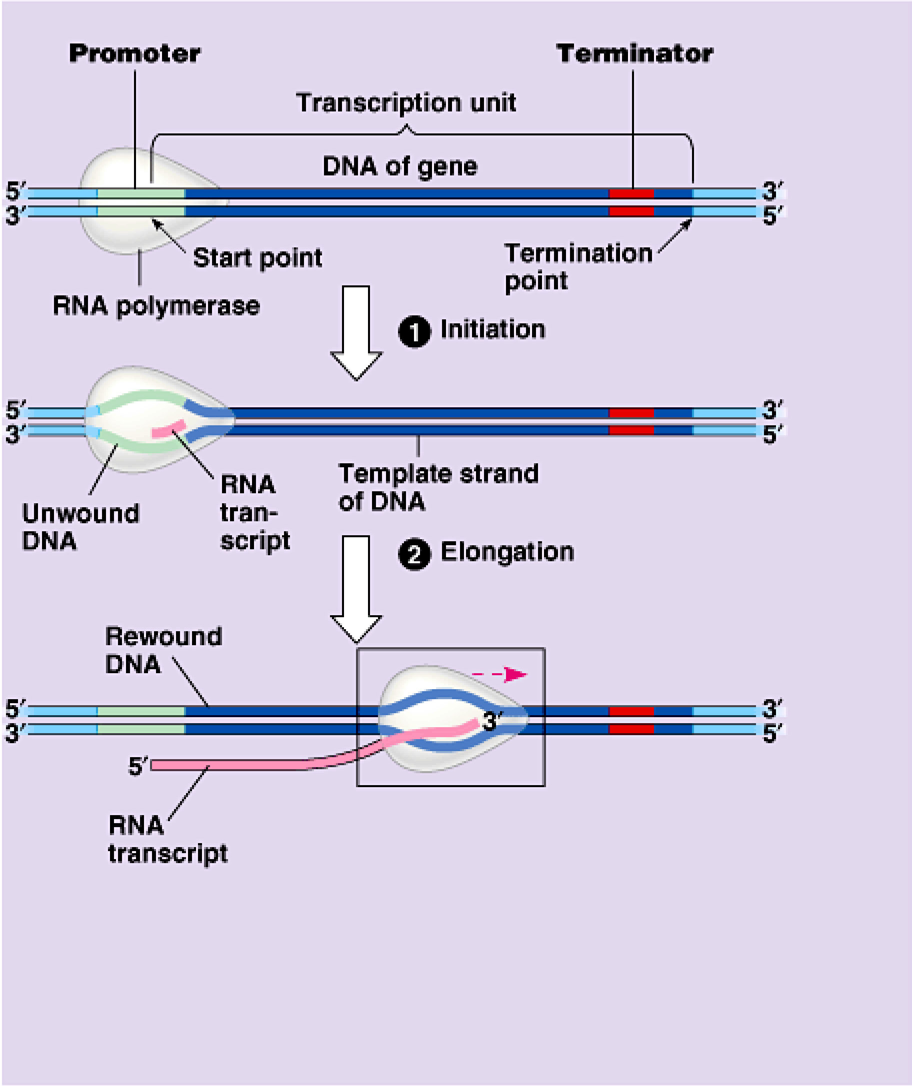
The principal function of RNA polymerase II is synthesis of mRNAs and some specialized RNAs

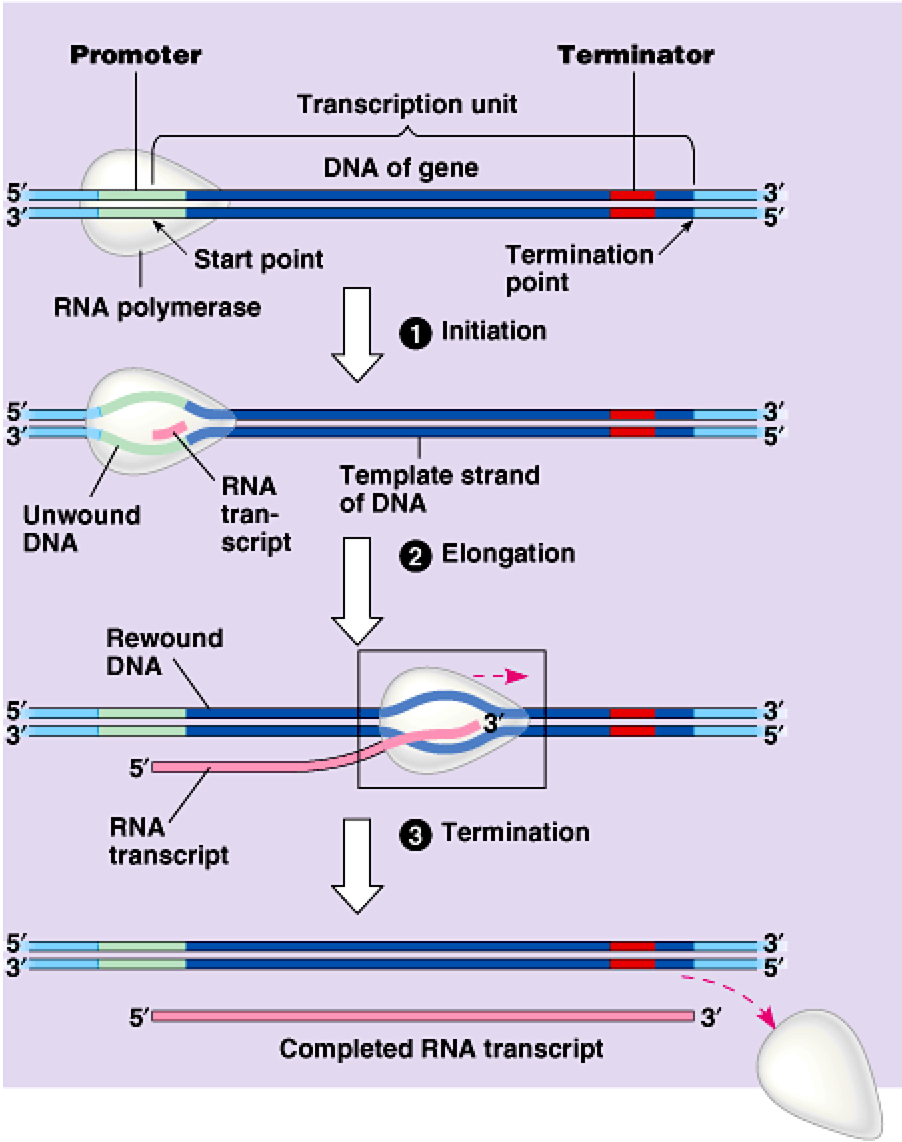
RNA polymerase III makes tRNAs, and some other small specialized RNAs.




Promoter
Terminator
Transcription unit
DNA of gene
5′
3′
3′
5′
Start point
Termination point
RNA polymerase


Promoter
Terminator
Transcription unit
DNA of gene
5′
3′
3′
5′
Start point
Termination point
RNA polymerase
1 Initiation
5′
3′
3′
5′
Unwound DNA
RNA tran- script
Template strand of DNA


Promoter
Terminator
Transcription unit
DNA of gene
5′
3′
3′
5′
Start point
Termination point
RNA polymerase
1 Initiation
5′
3′
3′
5′
Unwound DNA
RNA tran- script
Template strand of DNA
2 Elongation
Rewound DNA
5′
3′
3′
3′
5′
5′
RNA transcript

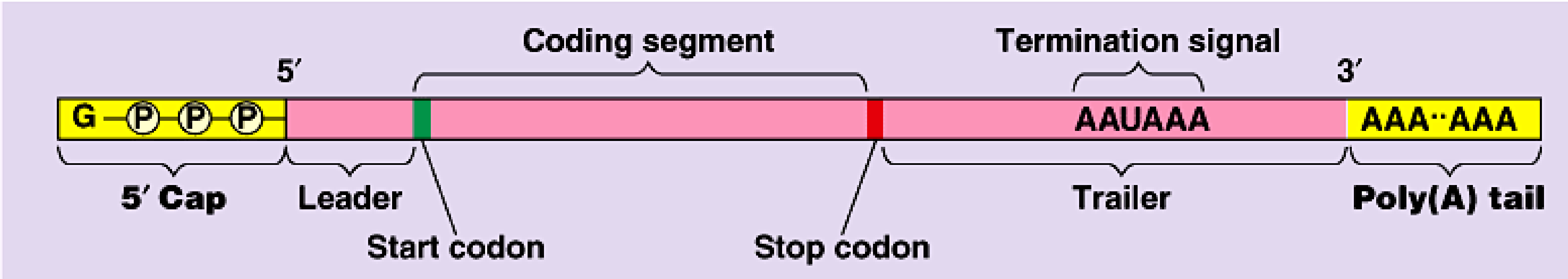


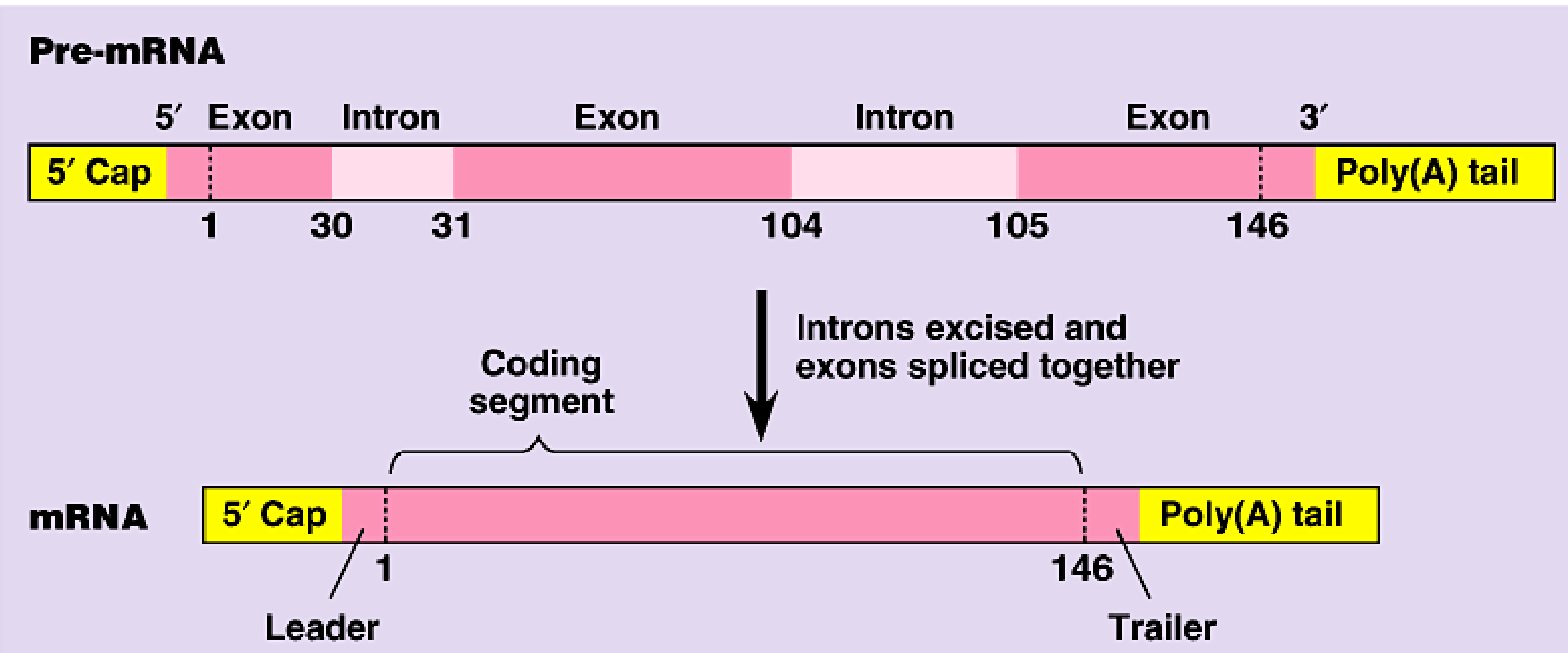
Promoter
Terminator
Transcription unit
DNA of gene
5′
3′
3′
5′
Start point
Termination point
RNA polymerase
1 Initiation
Unwound DNA
RNA tran-script
Template strand of DNA
2 Elongation
Rewound DNA
RNA transcript
3 Termination
Completed RNA transcript

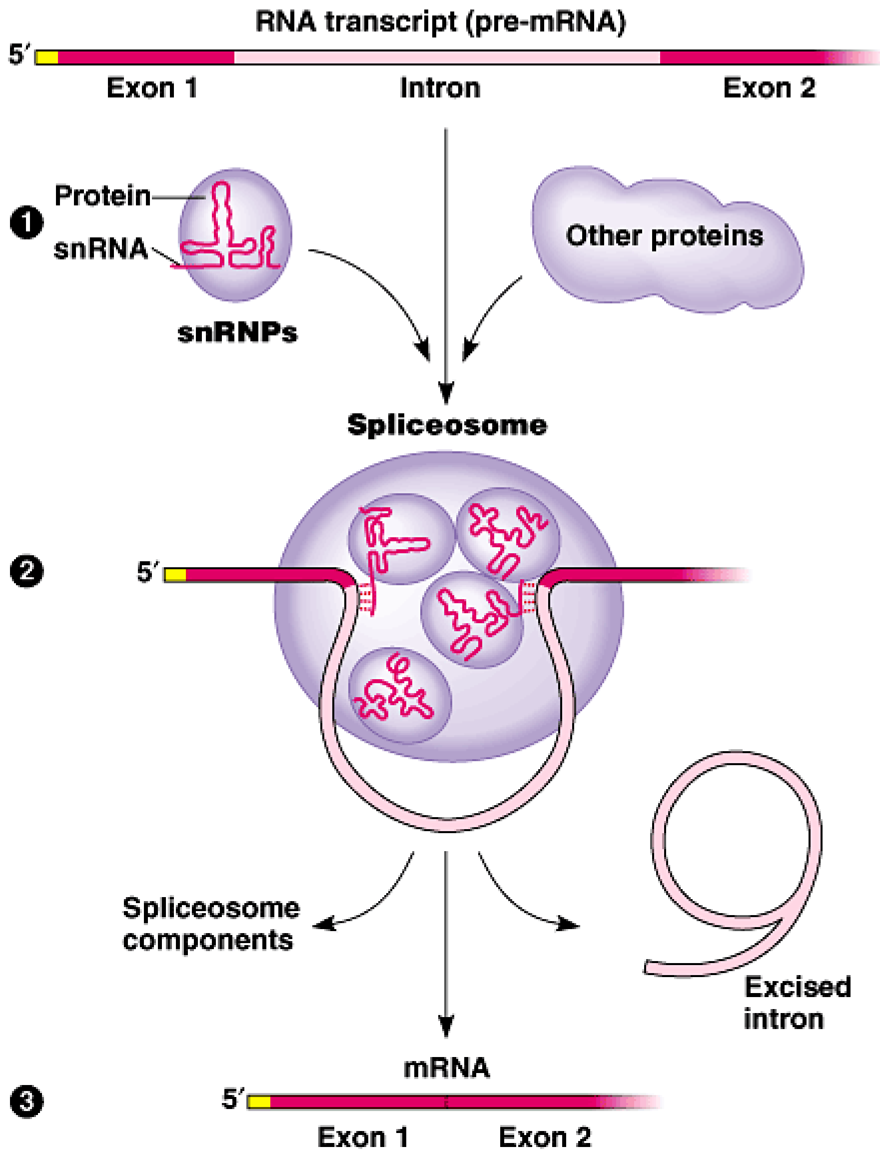
A newly synthesized RNA molecule is call**ed primary transcript.**

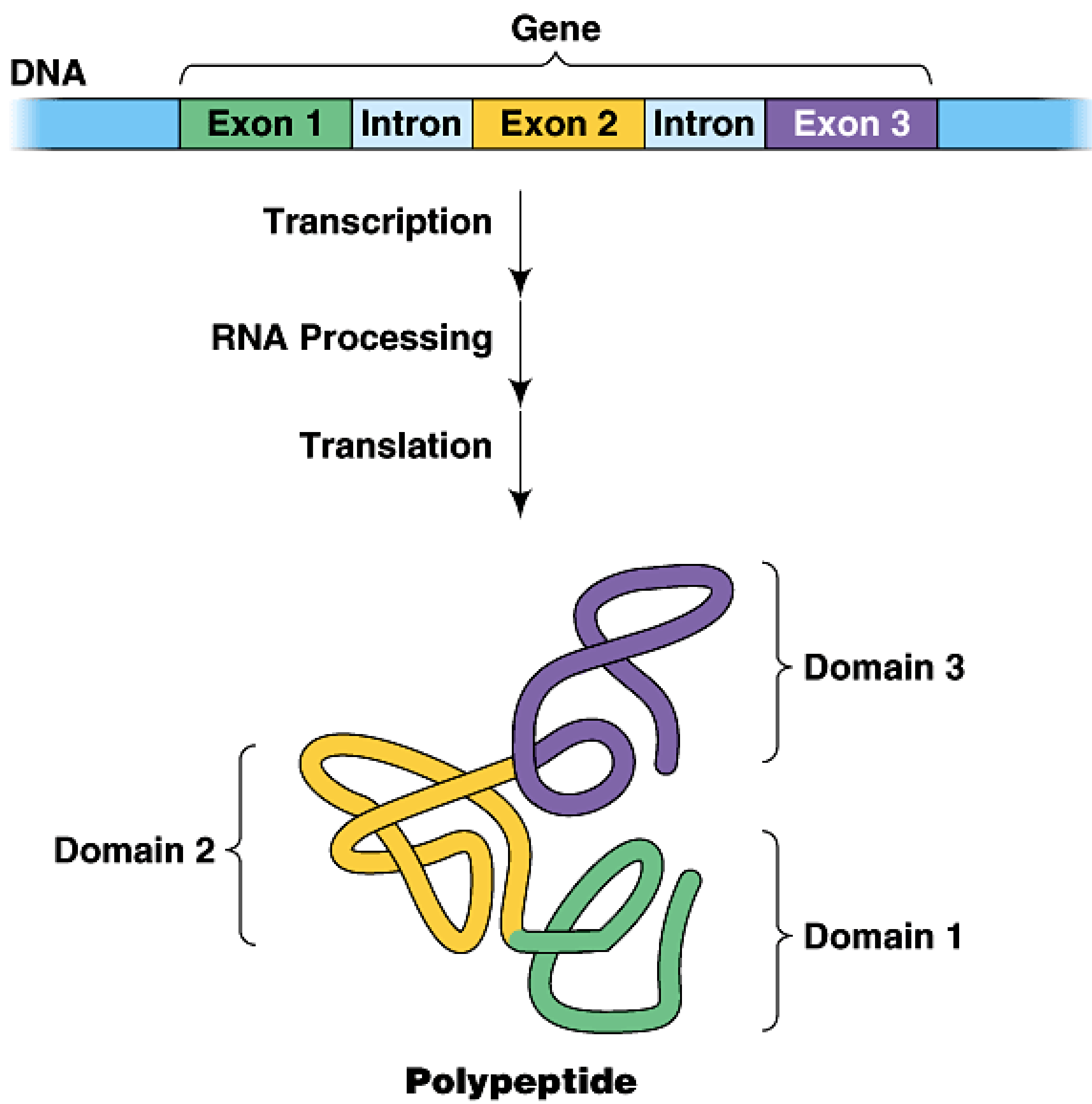
The primary transcript for a eukaryotic mRNA typically contains two types of sequences**: noncoding segments** that break up the coding region are called **introns,** and the **coding segments** are called **exons.**

In a process called **splicing**, the introns are removed from the primary transcript and the exons are joined to form a continuous sequence that defines a functional polypeptide


Coding segment
Termination signal
5′
3′
G –P–P–P
AAUAAA
AAA··AAA
5′ Cap
Leader
Start codon
Stop codon
Trailer
Poly(A) tail


Pre-mRNA
5′
Exon
Intron
Exon
Intron
Exon
3′
5′ Cap
Poly(A) tail
1
30
31
104
105
146
Introns excised and
exons spliced together
Coding
segment
mRNA
5′ Cap
Poly(A) tail
1
146
Leader
Trailer


RNA transcript (pre-mRNA)
5′
Exon 1
Intron
Exon 2
1
Protein
snRNA
snRNPs
Other proteins
Spliceosome
2
5′
Spliceosome components
Excised intron
mRNA
3
5′
Exon 1
Exon 2


Gene
DNA
Exon 1
Intron
Exon 2
Intron
Exon 3
Transcription
RNA Processing
Translation
Domain 3
Domain 2
Domain 1
Polypeptide