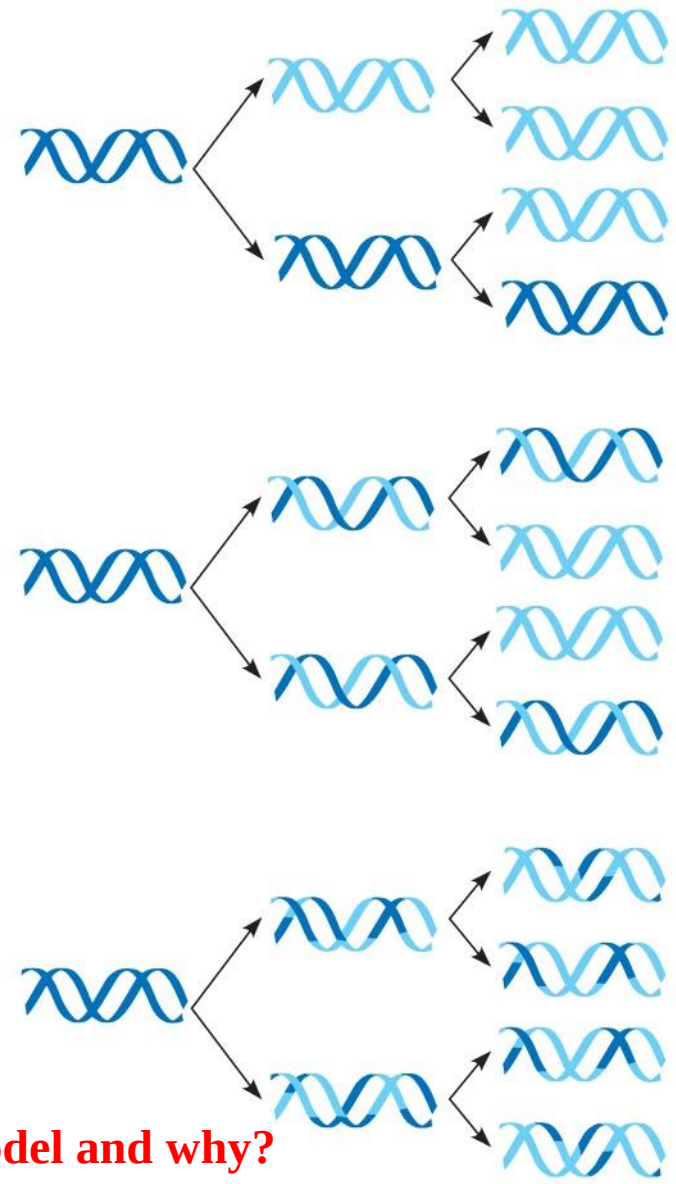
Models for DNA Replication



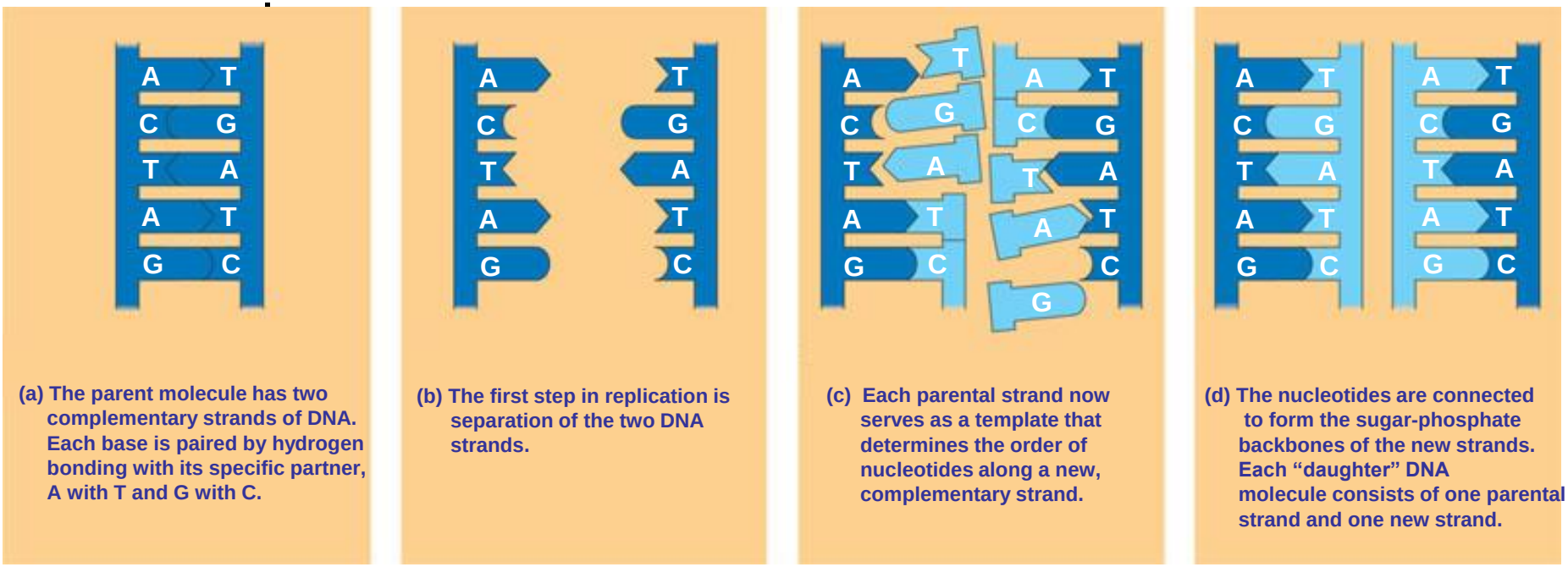
Conservative model. The two parental strands re-associate after acting as templates for new strands, thus restoring the parental double helix.

Semiconservative model. The two strands of the parental molecule separate, and each functions as a template for synthesis of a new, complementary strand.

Dispersive model. Each strand of both daughter molecules contains a mixture of old and newly synthesized DNA.

Which is your favorite model and why?

- In DNA replication
  - The parent molecule unwinds, and two new daughter strands are built based on base-pairing

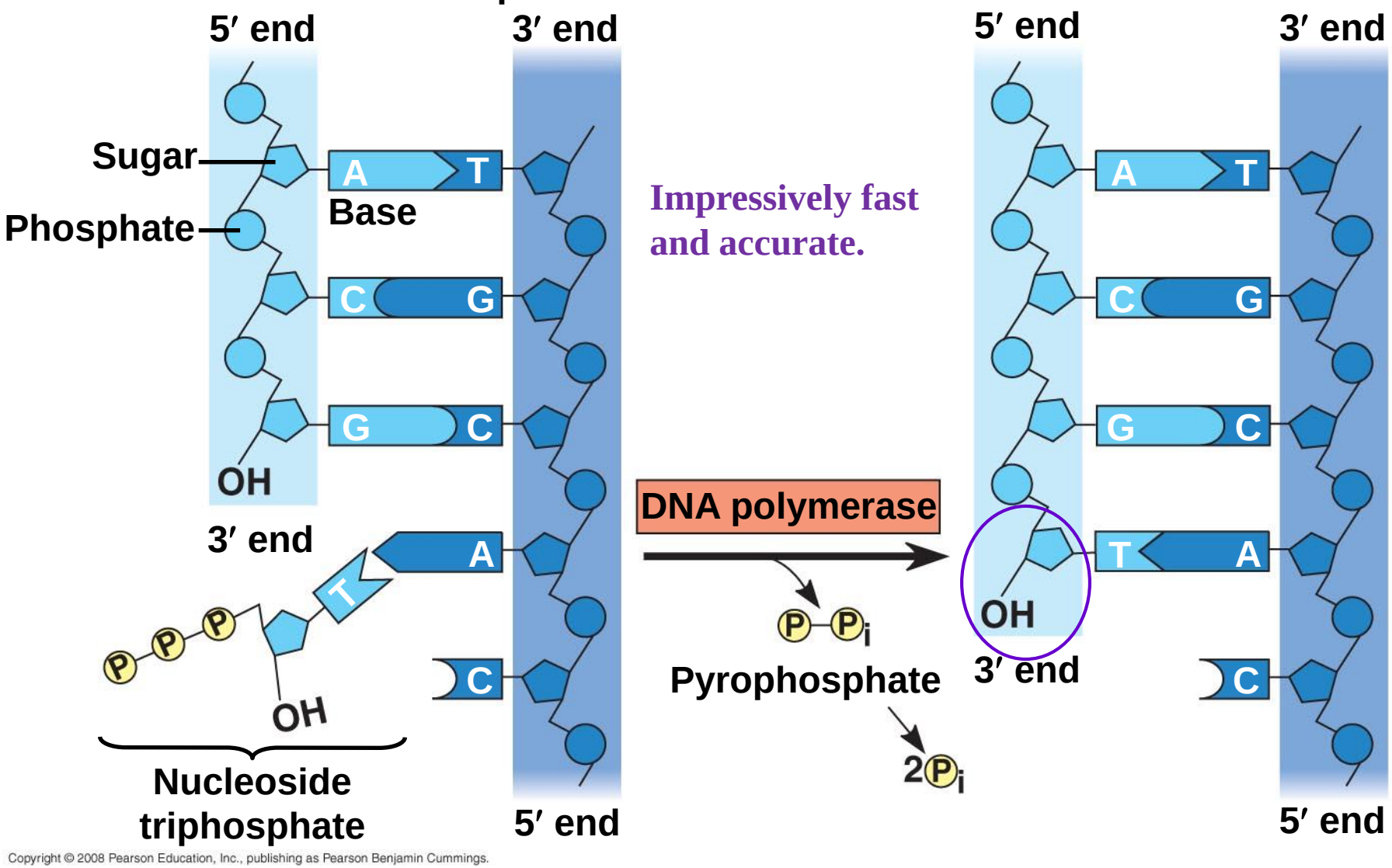


The copying mechanism is analogous to using a photographic negative to make a positive image, which can in turn be used to make another negative, and so on.

Elongation of new DNA at a replication fork

Is catalyzed by enzymes called DNA polymerases, which add nucleotides to the 3′ end of a growing strand

Anabolic and endothermic?



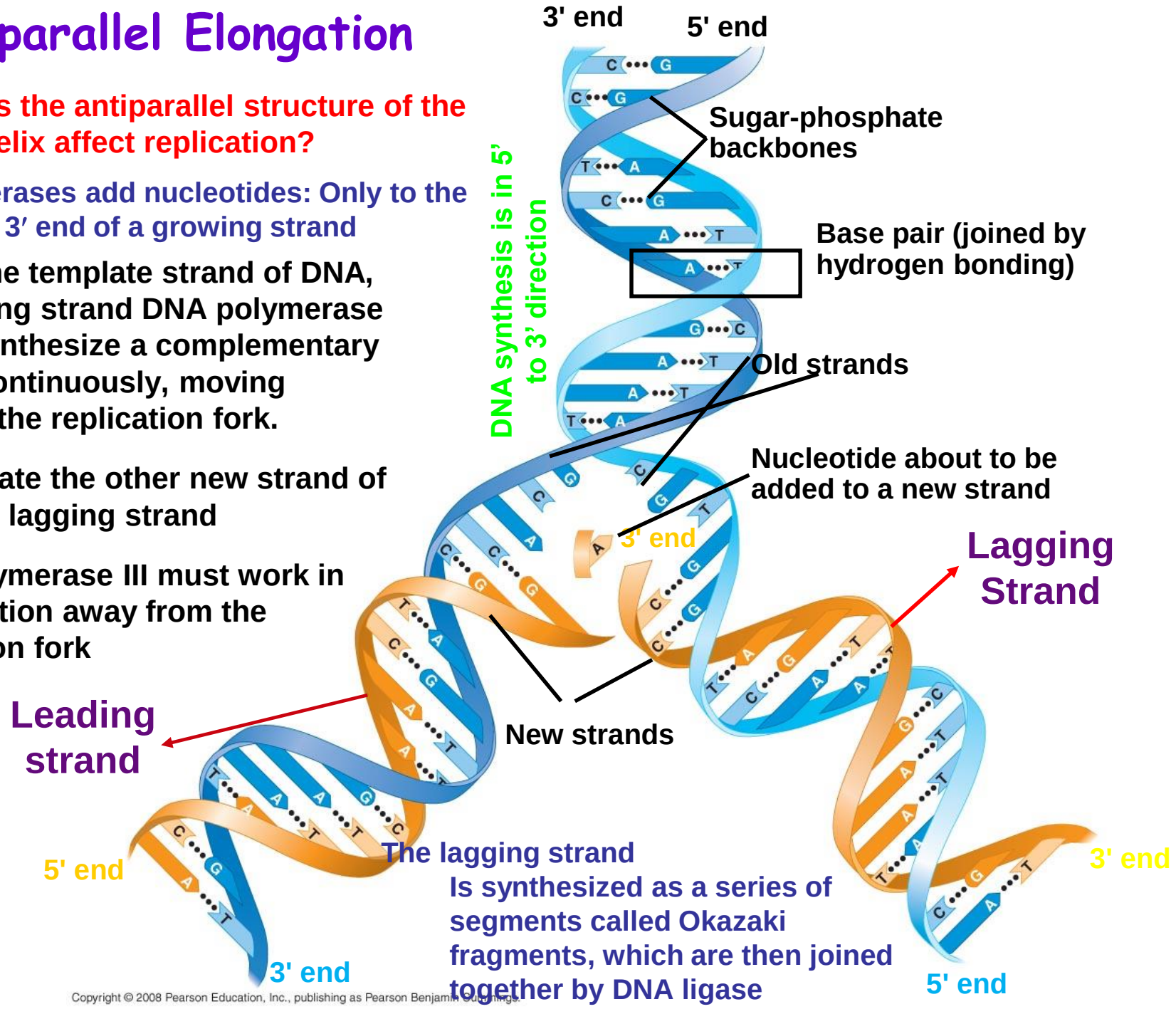
It takes few hours to copy 6 billion base pairs in the human. Equivalent to 1500 books of each book has 2000 pages: Error, 1 per 10^{-8}: Impressively fast and accurate

Antiparallel Elongation

How does the antiparallel structure of the double helix affect replication?

DNA polymerases add nucleotides: Only to the free 3′ end of a growing strand

- Along one template strand of DNA, the leading strand DNA polymerase III can synthesize a complementary strand continuously, moving towards the replication fork.
- To elongate the other new strand of DNA, the lagging strand
- DNA polymerase III must work in the direction away from the replication fork



Proofreading and Repairing DNA

- Estimate that an error occurs in 1 out of 100,000 (10^5) base pairs
- DNA polymerases proofread newly made DNA, replacing any incorrect nucleotides
- After DNA proofreading error ~ 1 in $10^{9\text{-}10}$: Similar to **fixing a word processing error** by using 'delete' key and entering the correct one. DNA polymerases proofread newly made DNA, replacing any incorrect nucleotides
- However error in completed DNA replication is 1 in 10-100 billion (10^{10}-10^{11}) nucleotide

DNA repair

Two types of repair

In mismatch repair of DNA, repair enzymes correct errors in base pairing (replace the incorrect nucleotide)

In nucleotide excision repair, a nuclease cuts out and replaces damaged stretches of DNA

After repair: Error comes down to 1 in $10^{10\text{-}11}$

Defect in Repair machinery

In mismatch repair, dedicated enzymes remove and replace incorrectly paired nucleotides that have resulted from replication errors. Researchers spotlighted the importance of such enzymes when they found that a hereditary defect in one of them is associated with a form of colon cancer. Congenital defects in mismatch repair are known. Apparently, this defect allows cancer causing errors to accumulate in the DNA at a faster rate than normal.

The Nobel Prize in Chemistry 2015 was awarded jointly to Tomas Lindahl, Paul Modrich and Aziz Sancar *"for mechanistic studies of DNA repair"*.

Tomas Lindahl demonstrated that DNA decays at a rate that ought to have made the development of life on Earth impossible. This insight led him to discover a molecular machinery, *base excision repair*, which constantly counteracts the collapse of our DNA.

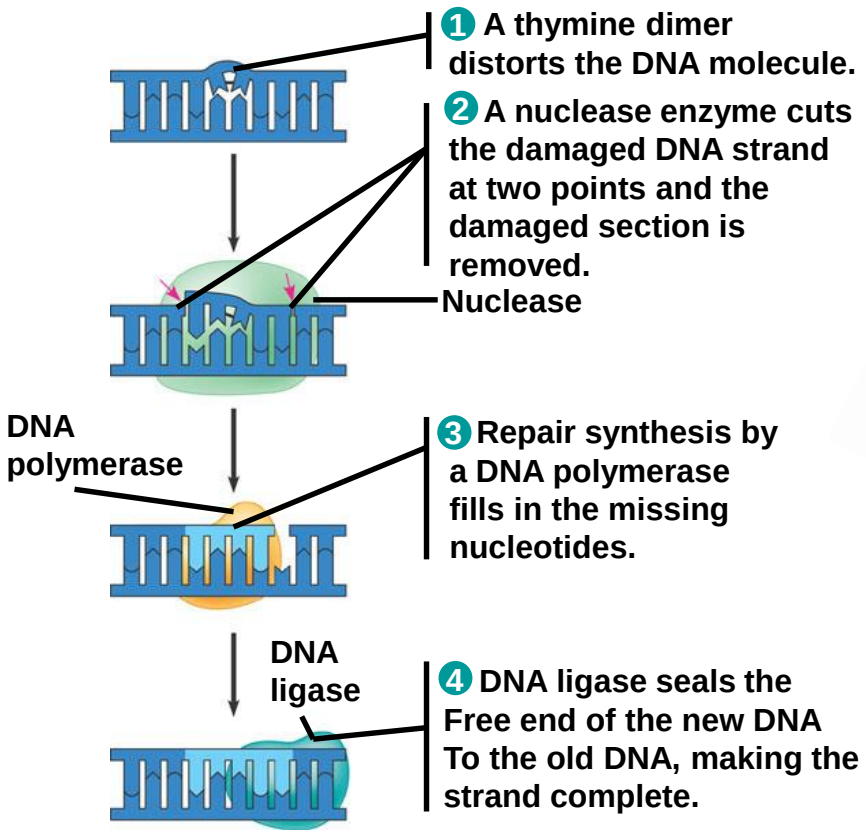
Post-proofreading repair (repair of damaged DNA)

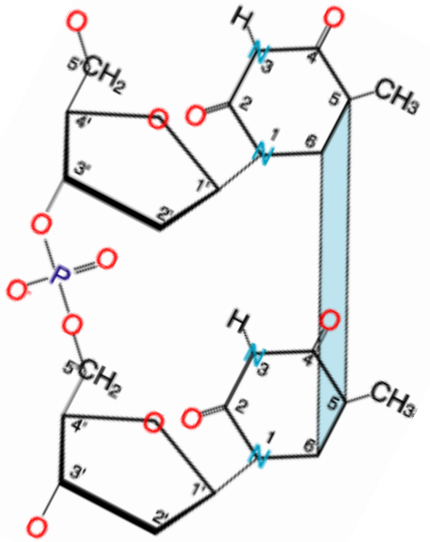
The reason our genetic material does not disintegrate into complete chemical chaos is that a host of molecular systems continuously monitor and repair DNA.

Aziz Sancar has mapped *nucleotide excision repair*, the mechanism that cells use to repair UV damage to DNA.

In nucleotide excision repair

- Enzymes cut out and replace damaged stretches of DNA





Cyclobutane pyrimidine dimer (CPD)

-Xeroderma Pigmenstosa: Children of the night

Why camptothecin and cisplatin are DNA damaging drugs used for cancer treatment?

An eight-year-old girl from Guatemala with xeroderma pigmentosum

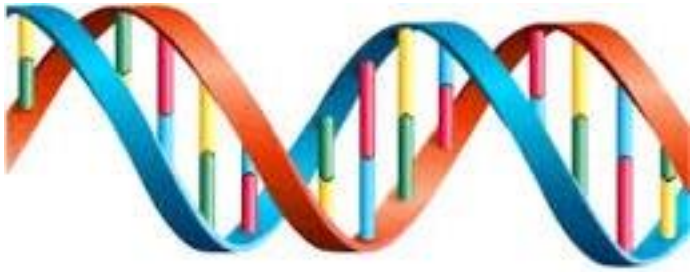
http://en.wikipedia.org/wiki/Xeroderma_pigmentosum

From Gene to Protein

How does the cell use DNA in the form of genes to make a protein?

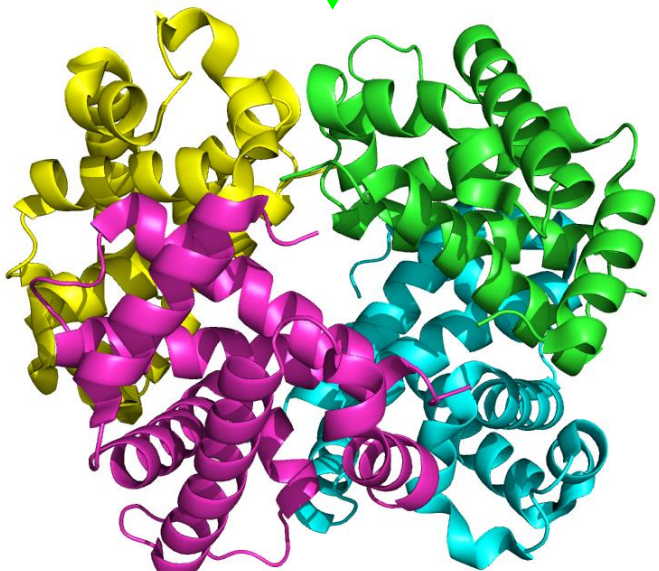
- **The information content of DNA**
  - **Is in the form of specific sequences of nucleotides - genes**
- The DNA inherited by an organism
  - Leads to specific traits by dictating the synthesis of proteins
- **Gene expression** -The process by which DNA directs protein synthesis
  - Transcription
  - Translation

Genes specify proteins via transcription and translation



Transcription

Translation

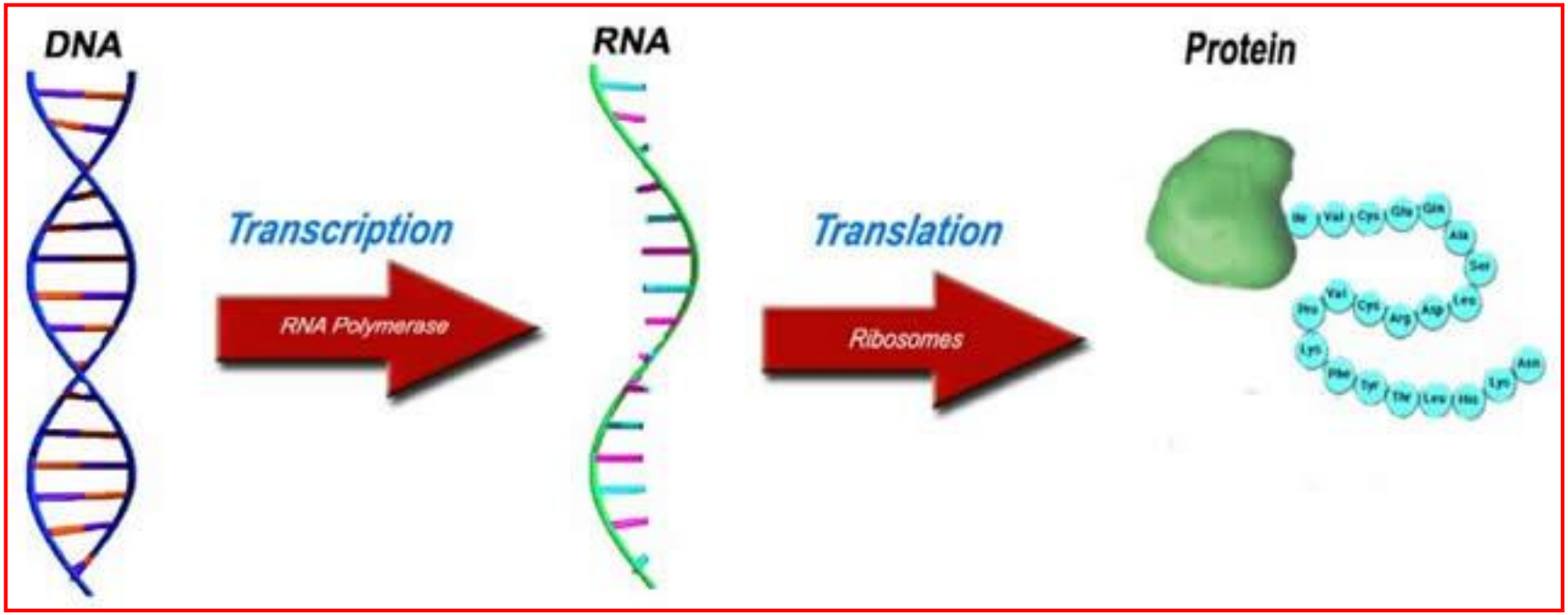


- Transcription
  - Is the synthesis of RNA under the direction of DNA
- Genes provide the instructions for making specific proteins.
- The bridge between DNA and protein synthesis is RNA.
- In DNA or RNA, the four nucleotide monomers act like the letters of the alphabet to communicate information.
- Translation
  - Is the actual synthesis of a polypeptide, which occurs under the direction of mRNA
  - Occurs in ribosomes

- During transcription, a DNA strand provides a template for the synthesis of a complementary RNA strand (mRNA).
- During translation, the information contained in the order of nucleotides in messenger RNA (mRNA) is used to determine the amino acid sequence of a polypeptide.

Central dogma of molecular biology

There are 3 major classes of Biopolymers:
DNA and RNA (both nucleic acids), and protein.



Dogma in molecular biology: **DNA makes RNA makes protein**

How many possible direct transfers of information (including self) that can occur between these (one to one)?

There are 3×3 = 9 conceivable direct transfers of information that can occur between these.

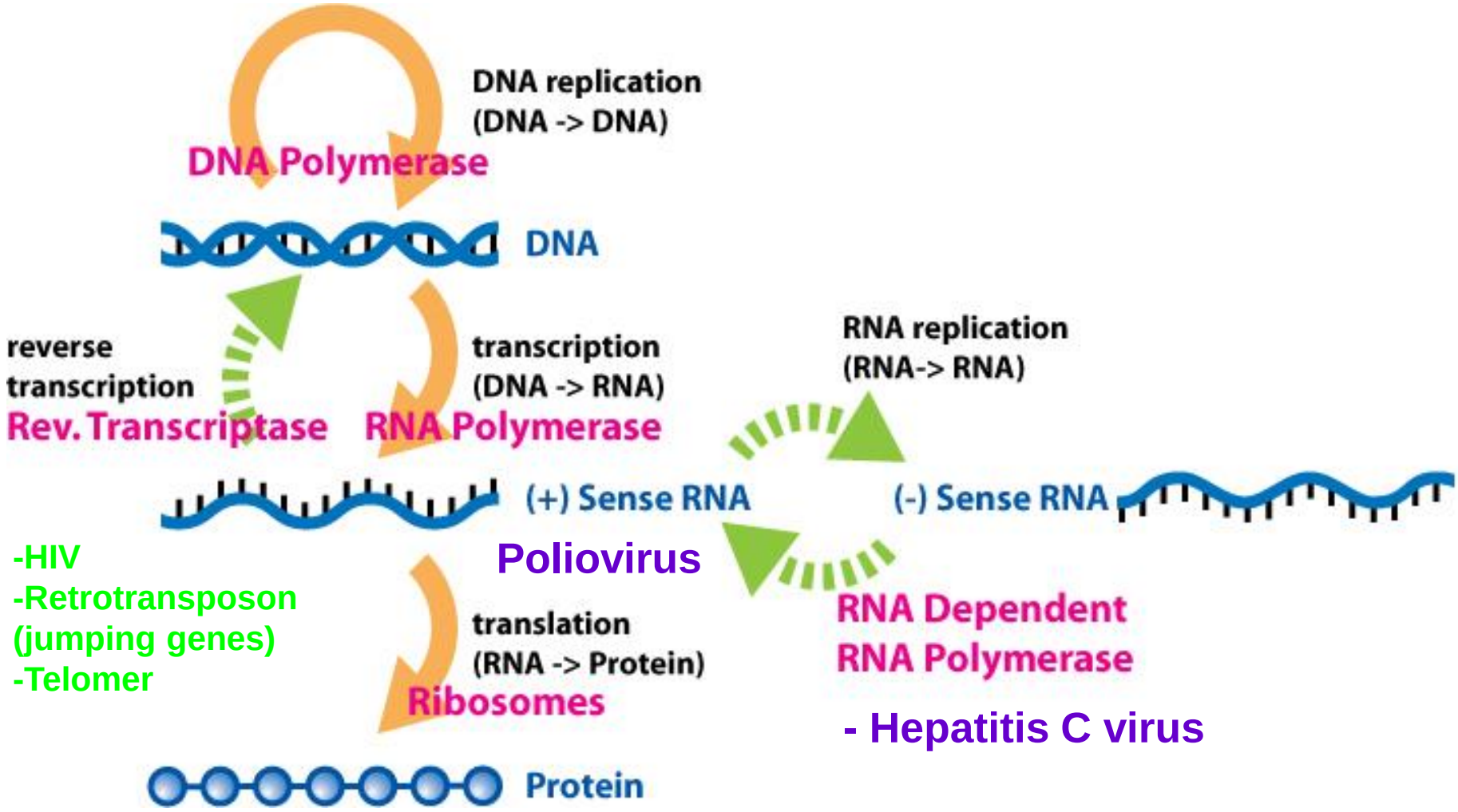
The dogma classes these into 3 groups of 3:

-3 general transfers

-3 special transfers and

-3 unknown transfers

General	Special	Unknown
DNA → DNA	RNA → DNA	protein → DNA
DNA → RNA	RNA → RNA	protein → RNA
RNA → protein	DNA → protein	protein → protein



Jumping gene Barbara McClintock: Nobel Pize in 1983

Elizabeth Blackburn, Carol Greider, and Jack Szostak : 2009 Nobel Prize in Physiology or Medicine: Discovery of how chromosomes are protected by telomeres and the enzyme telomerase.

If genome is single strand, how many rounds of replication is required to produce a copy of it?

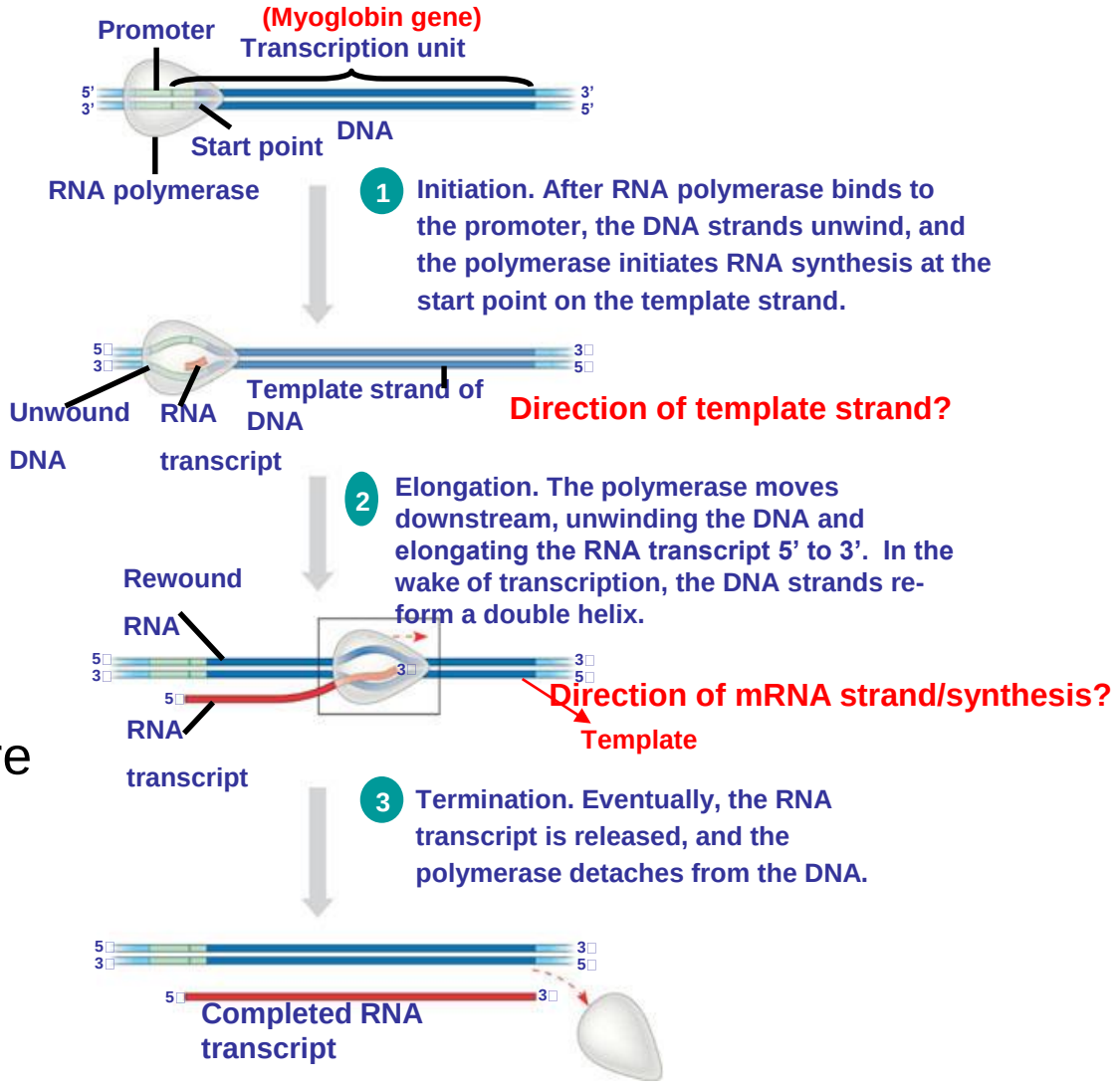
Transcription is the DNA-directed synthesis of RNA

Synthesis of an RNA Transcript

One script to another script of similar language that is DNA to RNA

RNA synthesis

- Is catalyzed by RNA polymerase, which pries (unwinds) the DNA strands apart and hooks together the RNA nucleotides
- Follows the same base-pairing rules as DNA, except that in RNA, uracil substitutes for thymine
- Genes are read 3'->5' creating a 5'->3' RNA molecule.
- The stages of transcription are
  - Initiation
  - Elongation
  - Termination

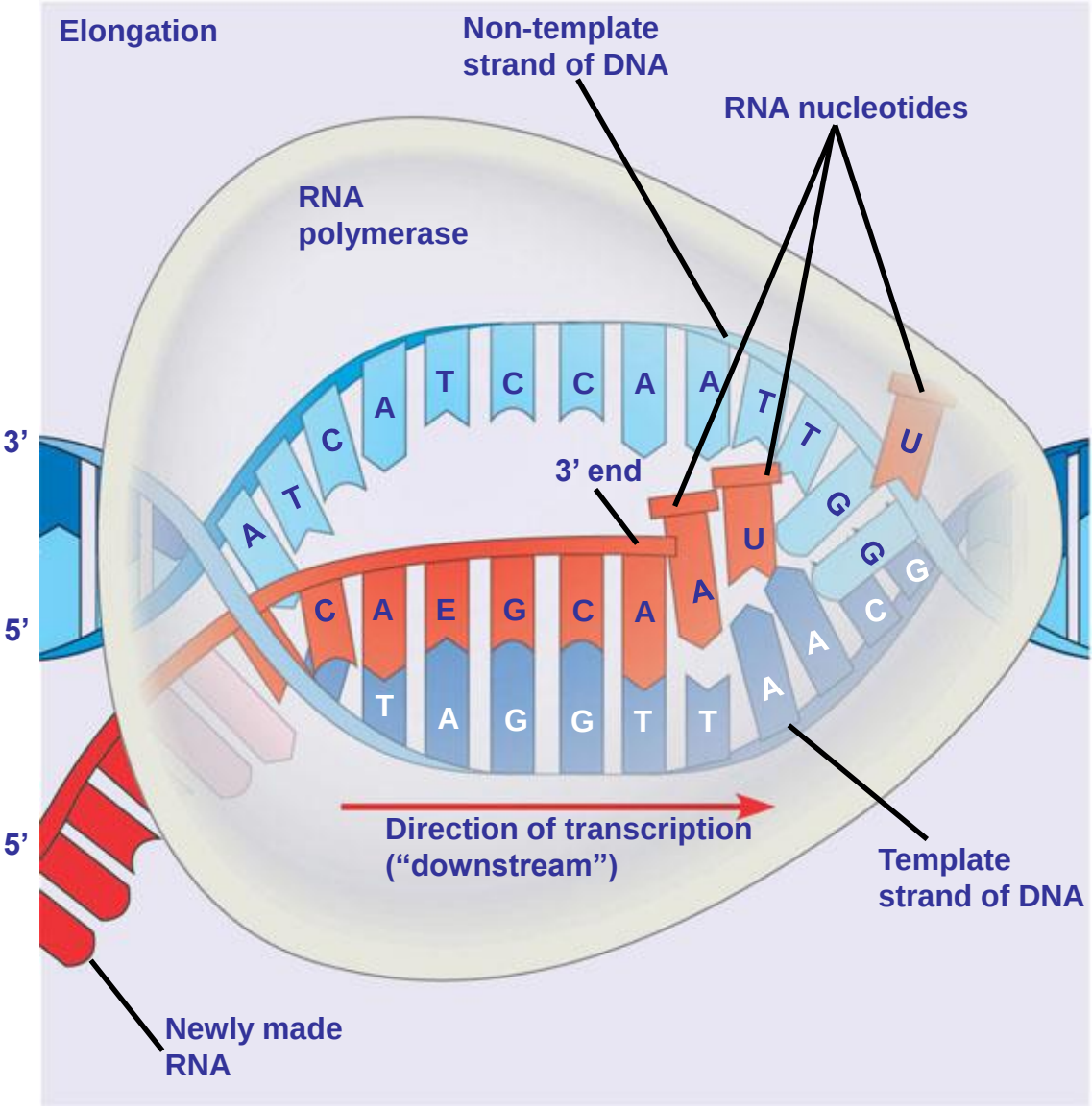


Overview of Transcription

Unlike DNA polymerase it doesn't require primer.

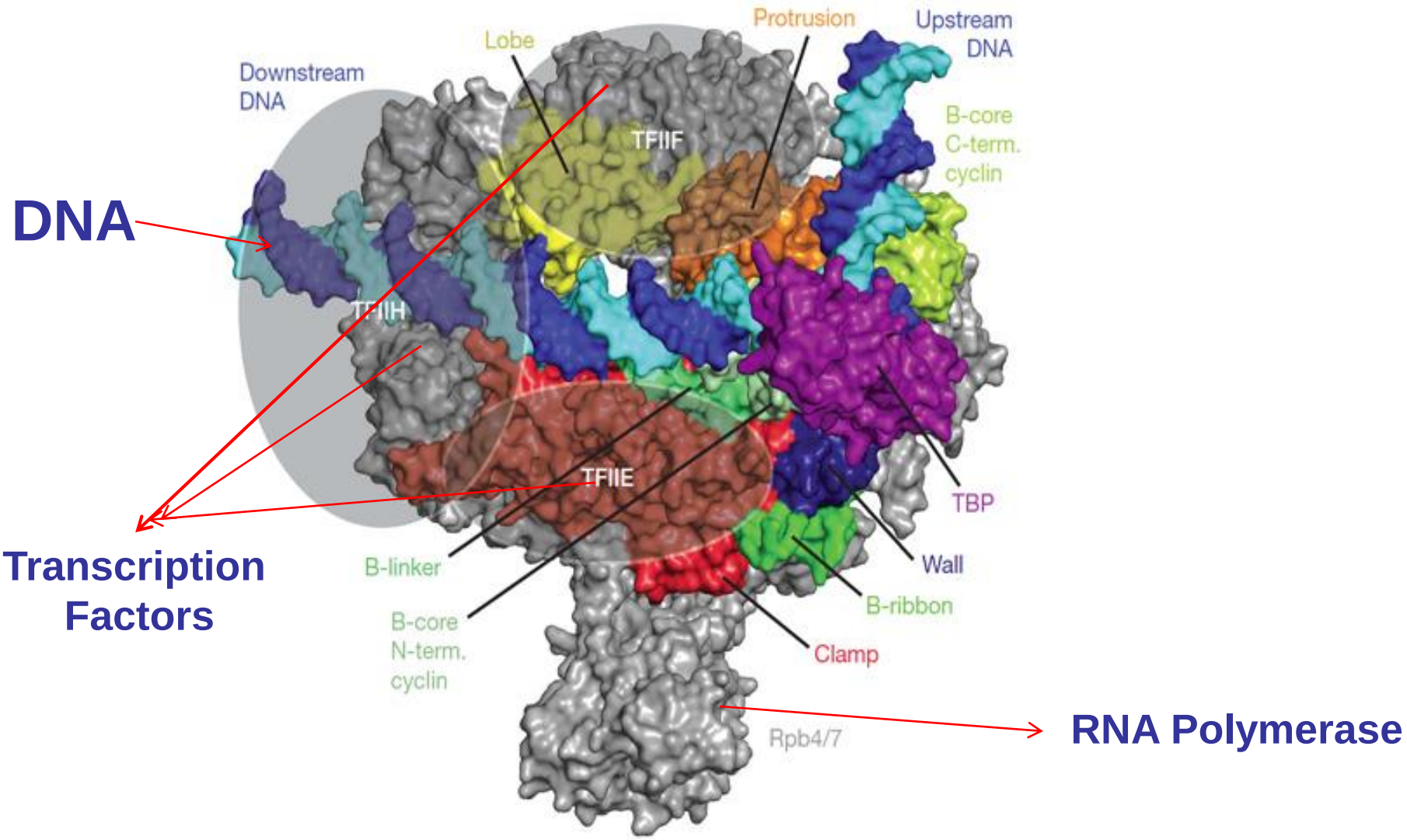
How million copies of proteins are produced from a single gene (scale up)?
Ex: RNA and DNA polymerases, Insulin Hemoglobin etc

- A single gene can be transcribed simultaneously by several RNA polymerases at a time.



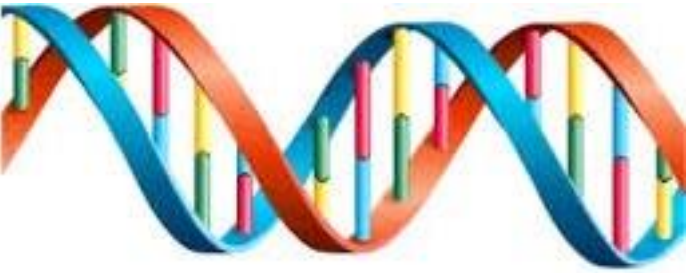
-Difference between Transcription and replication?

-Transcription video



The Nobel Prize in Chemistry 2006: Roger D. Kornberg *"for his studies of the molecular basis of eukaryotic transcription"*.

Genetic Code

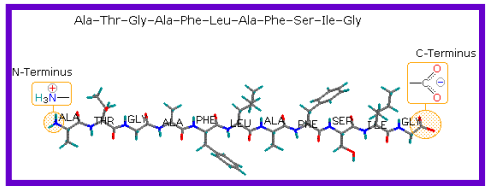


How many bases in gene/mRNA correspond to an amino acid on protein?

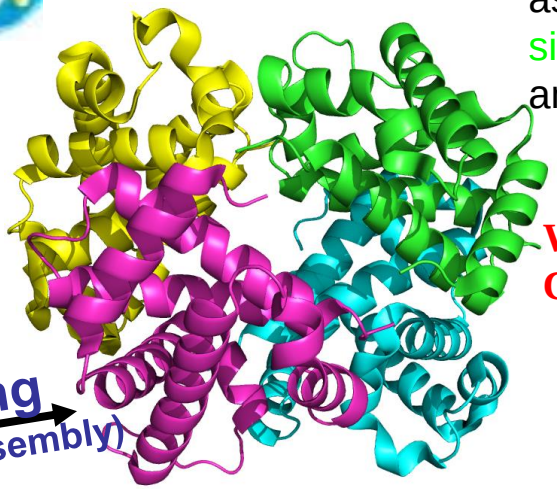
Genetic code (Genetic Information) : Encoded as a sequence of non-overlapping base single/duplet/triplet/quadruplet for one amino acid on protein

Transcription

Translation



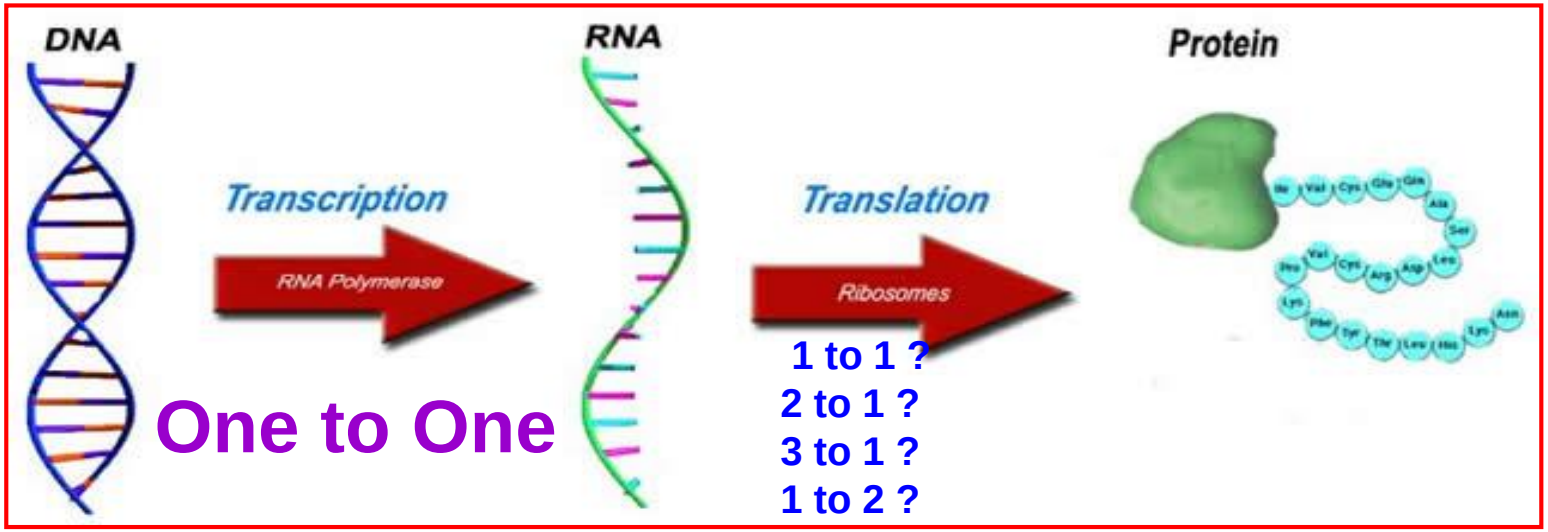
Folding (self assembly)



Why genetic code cannot be language like Chinese?

汉语

Chinese Language



Four (A, T, G and C) bases

Four (U, A, C and G) bases

20 amino acids

The Genetic Code

- How many bases correspond to an amino acid?
  - Can't be 1 for 1, only 4 bases and 20 amino acids.(4^1) (Ex: 'A" cannot specify for more than one amino acid at given position)
  - Can't be 2 bases (AG, GA, UG etc) for 1 amino acid, that would only give 16 possible amino acids.(4^2)
  - **Must be 3 bases = 1 amino acid. (4^3)**
  - **Must not be more than 3 bases?**

Genetic code (Genetic Information): Encoded as a sequence of non-overlapping base **TRIPLETS** called as codons

The genetic instructions for a polypeptide chain are written in DNA as a series of three-nucleotide words.

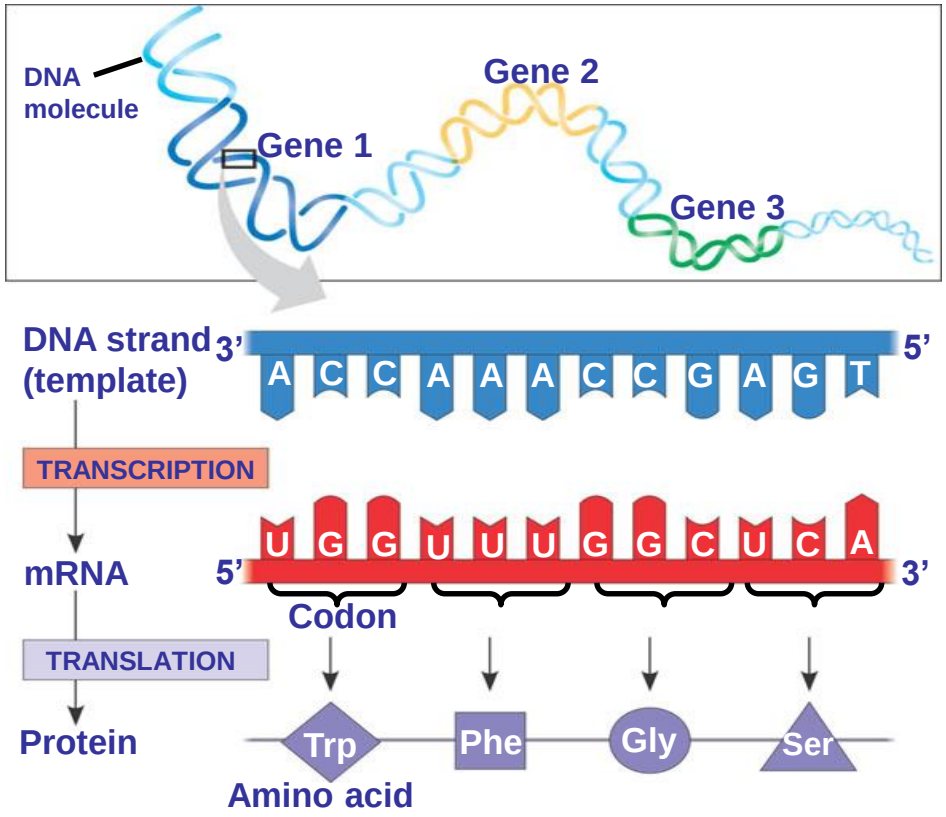
Overview of Transcription and Genetic Code

- During transcription: The gene on the DNA determines the sequence of bases along the length of an mRNA molecule

During transcription, one DNA strand, the template strand, provides a template for ordering the sequence of nucleotides in an RNA transcript.

mRNA molecules are complementary, not (identical) a copy

During translation, blocks of three nucleotides, codons, are decoded into a sequence of amino acids.



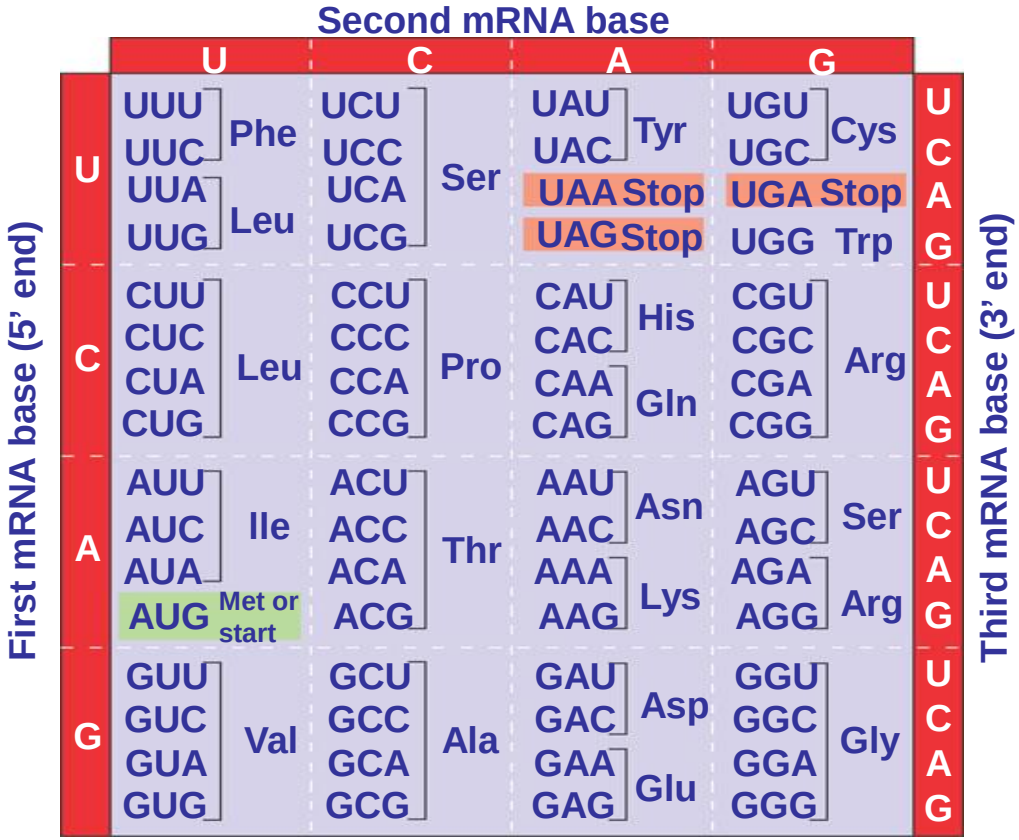
Because codons are base triplets, the number of nucleotides making up a genetic message must be …… times the number of amino acids making up the protein product.

Why DNA cannot be used as template for translation?

Cracking the Code

- A codon in messenger RNA: Witten in 5’ to 3’ direction
  - Is either translated into an amino acid or serves as a translational stop signal

UCU CUC
S L



The Nobel Prize: Physiology or Medicine 1968: Robert W. Holley, Har Gobind Khorana and Marshall W. Nirenberg: *"for their interpretation of the genetic code and its function in protein synthesis"*.