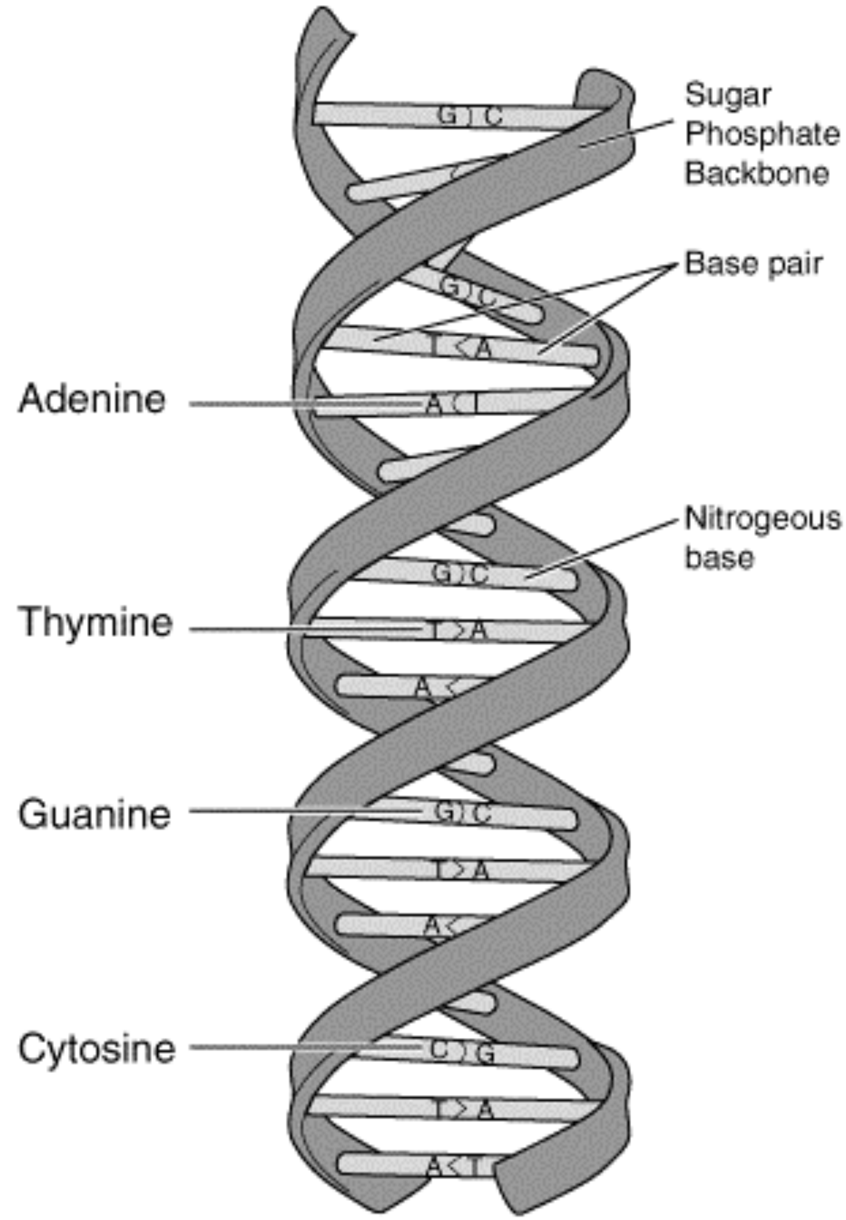
Prokaryotic Transcription

Transcription Basics

- DNA is the genetic material
  - Nucleic acid
  - Capable of self-replication and synthesis of RNA
- RNA is the middle man
  - Nucleic acid
  - Structure and base sequence are determinants of protein synthesis and the transmission of genetic material
- Proteins are crucial for everything!
  - Essential constituents of all living things
  - Examples: enzymes, hormones, antibodies

DNA

- Deoxyribose Nucleic Acid
- DNA exists as double helix
- Each molecule of DNA is composed of a **nucleotide** chain
  - Sugar
  - Phosphate
  - Nitrogenous base (ACTG)
- The two DNA strands stay together by complementary pairing (uses hydrogen bonds)
  - A - T
  - G - C
- DNA is segmented into genes, which are the functional units of heredity



DNA

- In bacteria (prokaryotes), DNA is not separated from the cytoplasm by a nuclear envelope.

- By contrast, in eukaryotes, most of the DNA is located in the cell nucleus.

- The energy-generating organelles known as chloroplasts and mitochondria also carry DNA, as do many viruses.

RNA

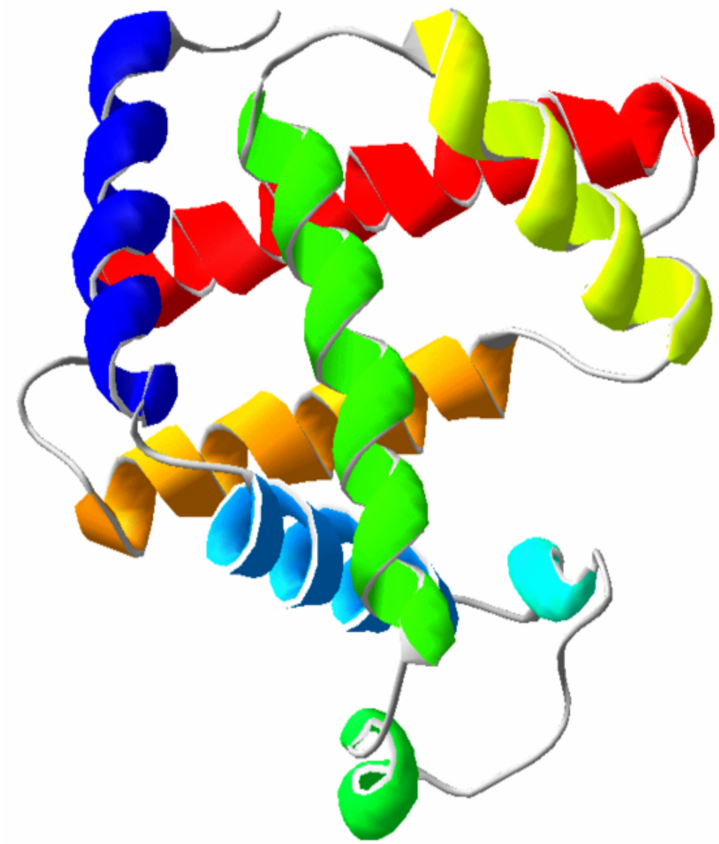
- Ribonucleic Acid
- RNA exists as a single strand, but can be double stranded
- Each molecule of RNA is composed of a **nucleotide** chain
    - Sugar
    - Phosphate
    - Nitrogenous base (ACUG)
    - Why Uracil instead of thymine? Energetically less expensive to make.
- Several forms of RNA exist
    - mRNA
    - tRNA
    - rRNA
    - dsRNA
- Is made in nucleus and resides in cytoplasm

RNA

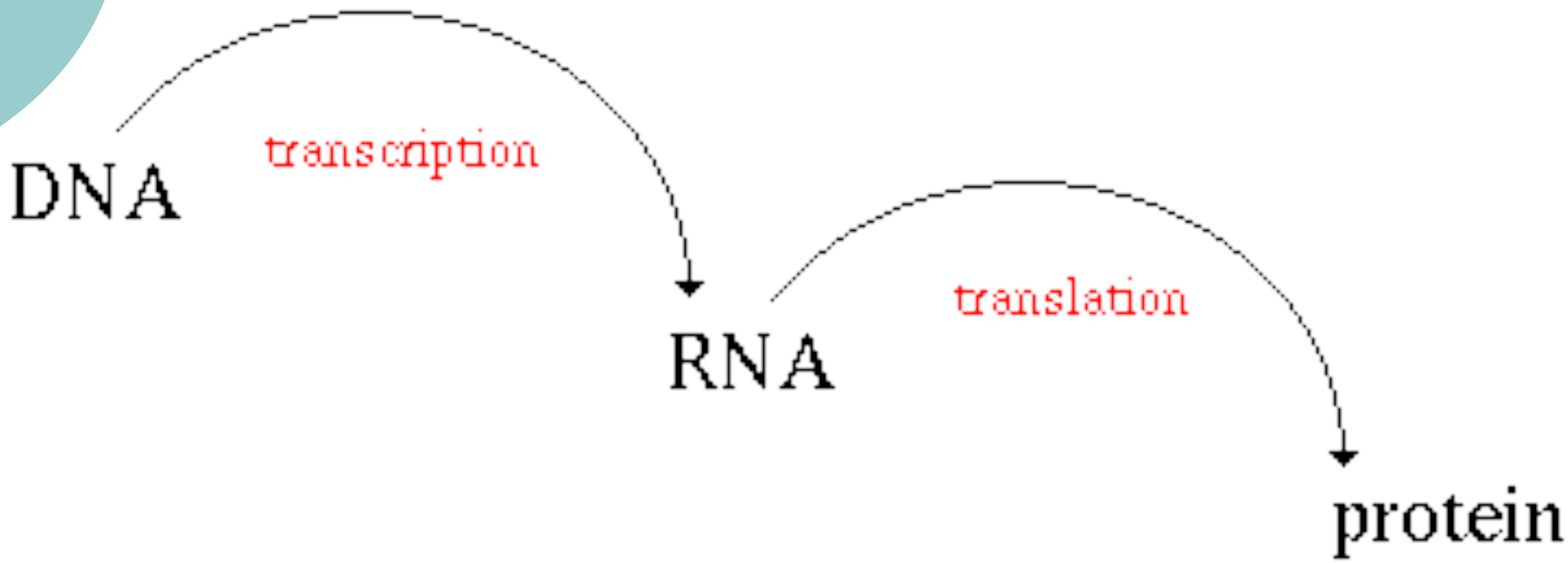
Ribonucleic acid

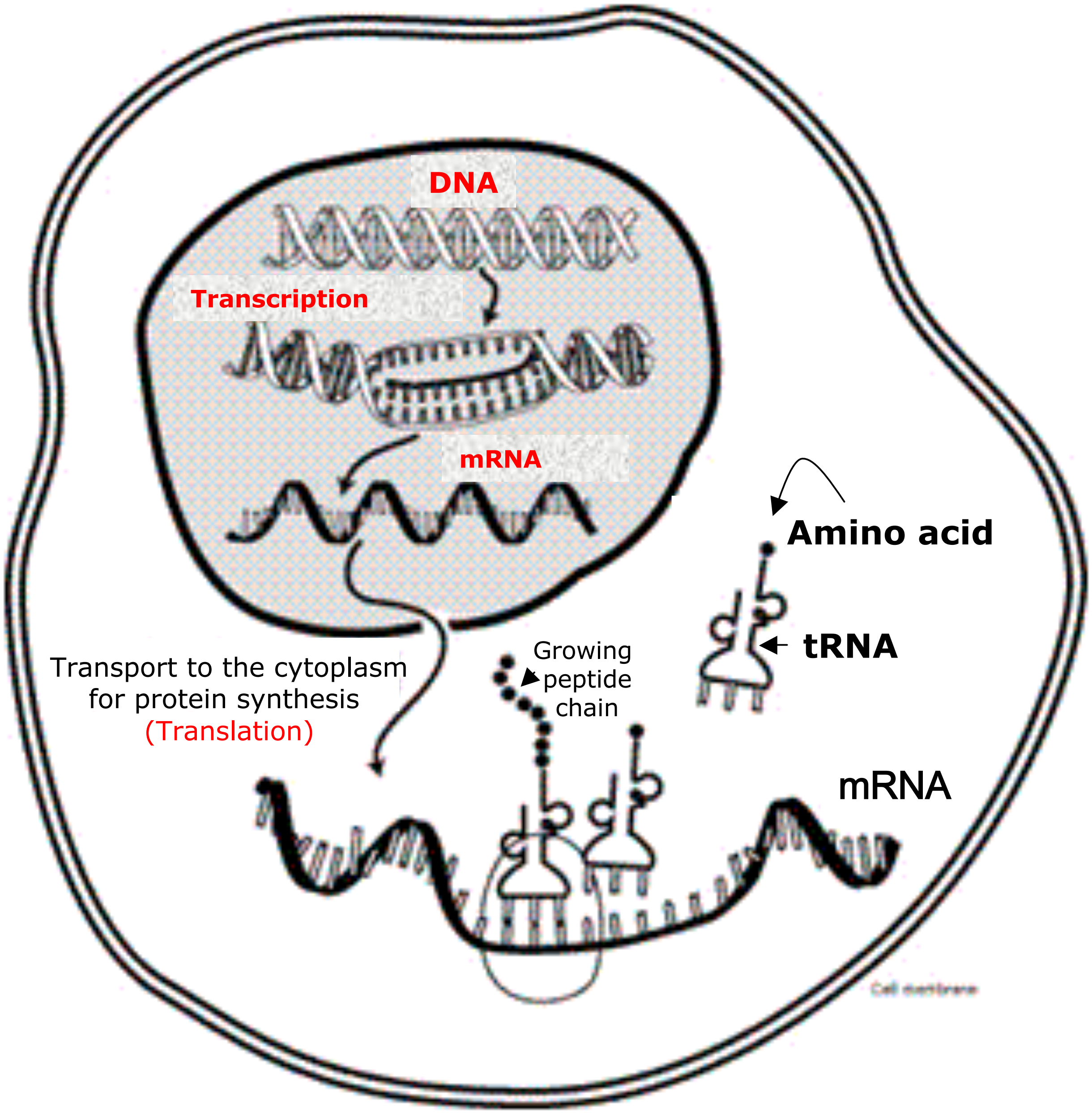
Protein

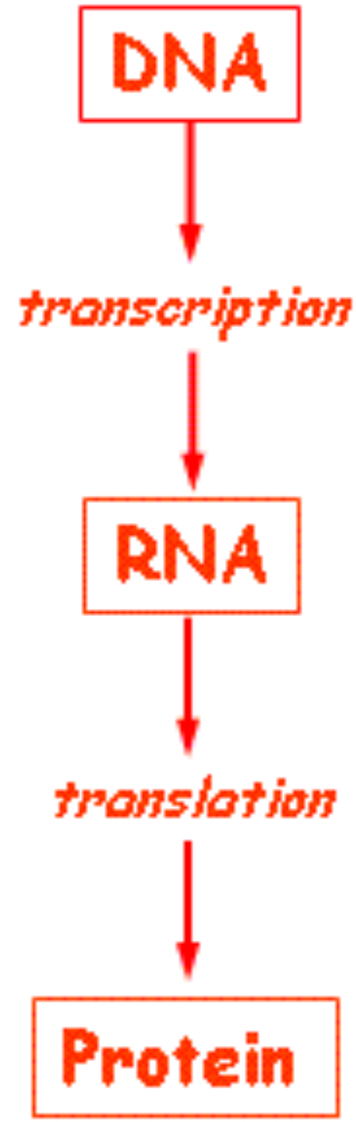
- A linear polymer of amino acids linked together peptide bonds in a specific sequence
- The amino acid chains fold up into 3 dimensional structure
- Protein Structure
  - Primary structure
  - Secondary structure
  - Tertiary structure
  - Quaternary structure
- Essential for the structure and function of all living things and viruses
- Involved in practically every function performed by the cell
- Are found everywhere within the cell and even outside of the cell



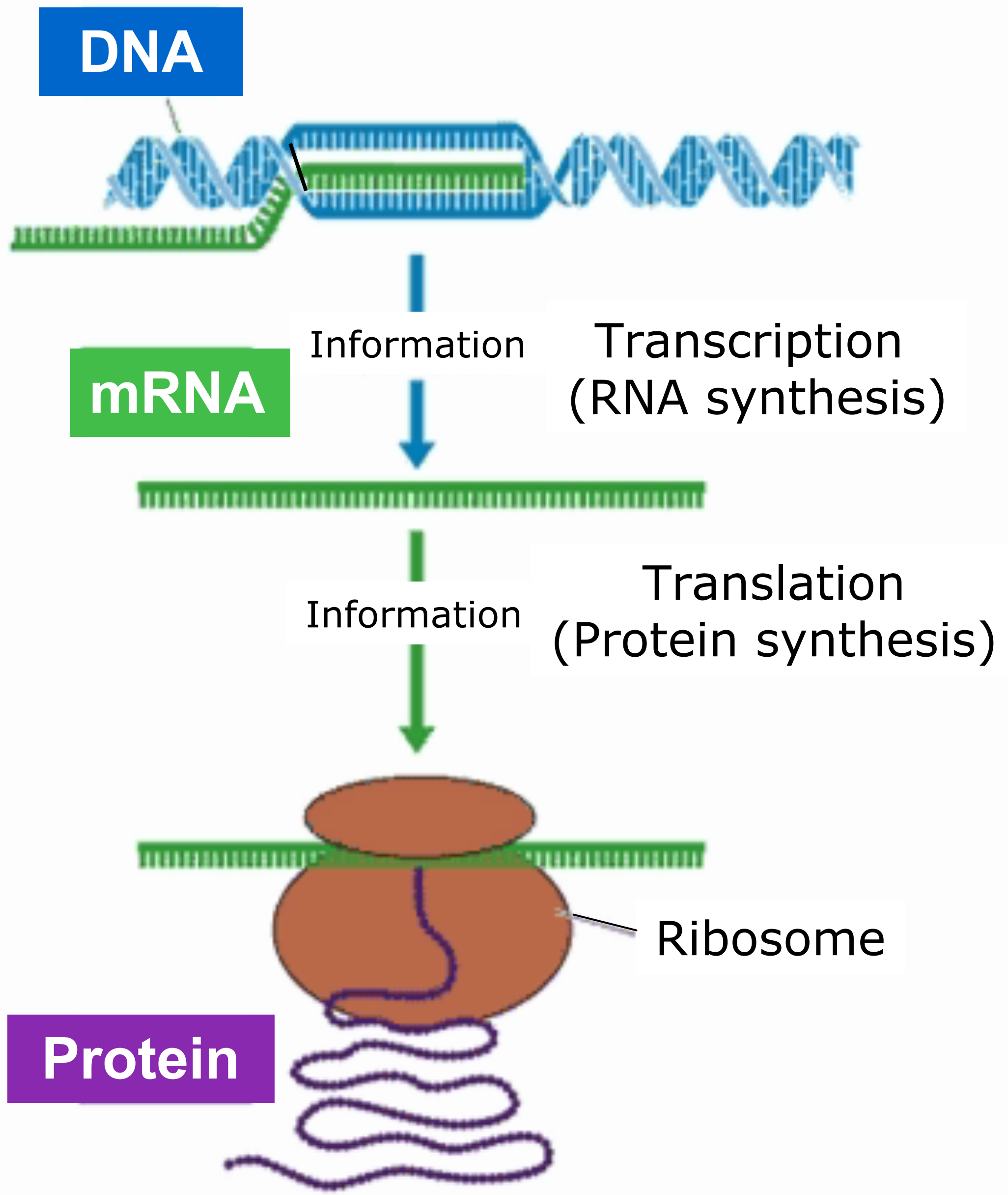
Central Dogma

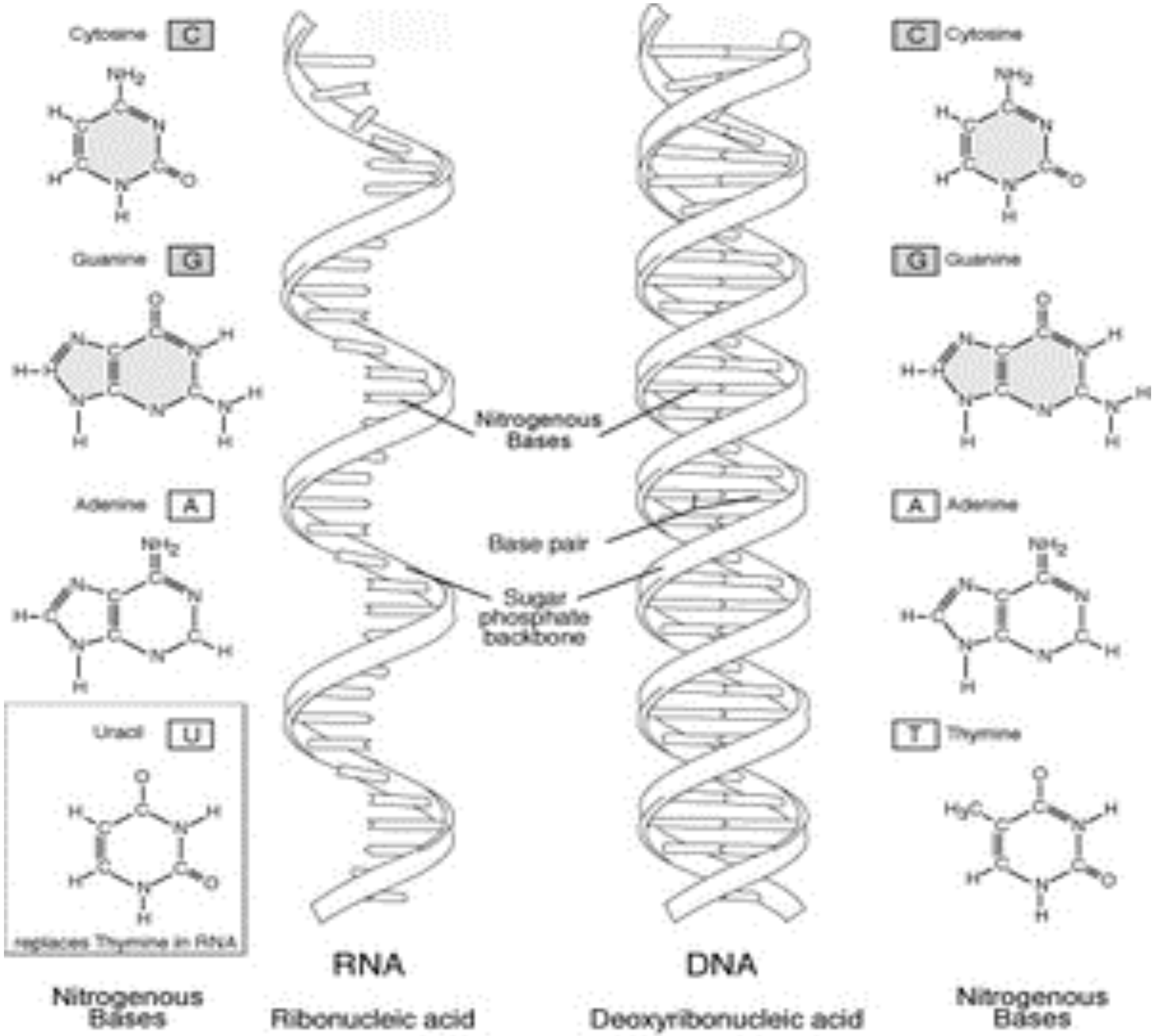



DNA
Transcription
mRNA
Amino acid
tRNA
Growing peptide chain
Transport to the cytoplasm for protein synthesis (Translation)
mRNA


DNA
transcription
RNA
translation
Protein

Central Dogma




Cytosine
C
Guanine
G
Adenine
A
Uracil
U
replaces Thymine in RNA
Nitrogenous Bases
RNA
Ribonucleic acid
Nitrogenous Bases
Base pair
Sugar phosphate backbone
DNA
Deoxyribonucleic acid
C Cytosine
G Guanine
A Adenine
T Thymine
Nitrogenous Bases

Differences between RNA and DNA

- DNA uses GA**T**C
- RNA uses GA**U**C
- RNA is temporary copy of DNA
- RNA is made by RNA polymerase
- RNA is single stranded, DNA is double stranded

Genes

- A gene is region of DNA that encodes all the information to make a protein.
- Genes are often considered the basic units of heredity.
- Often named for the function of the protein for which it encodes
- One gene…one protein
- Show incredible diversity in size, organization, and have no typical structure, but some conserved features

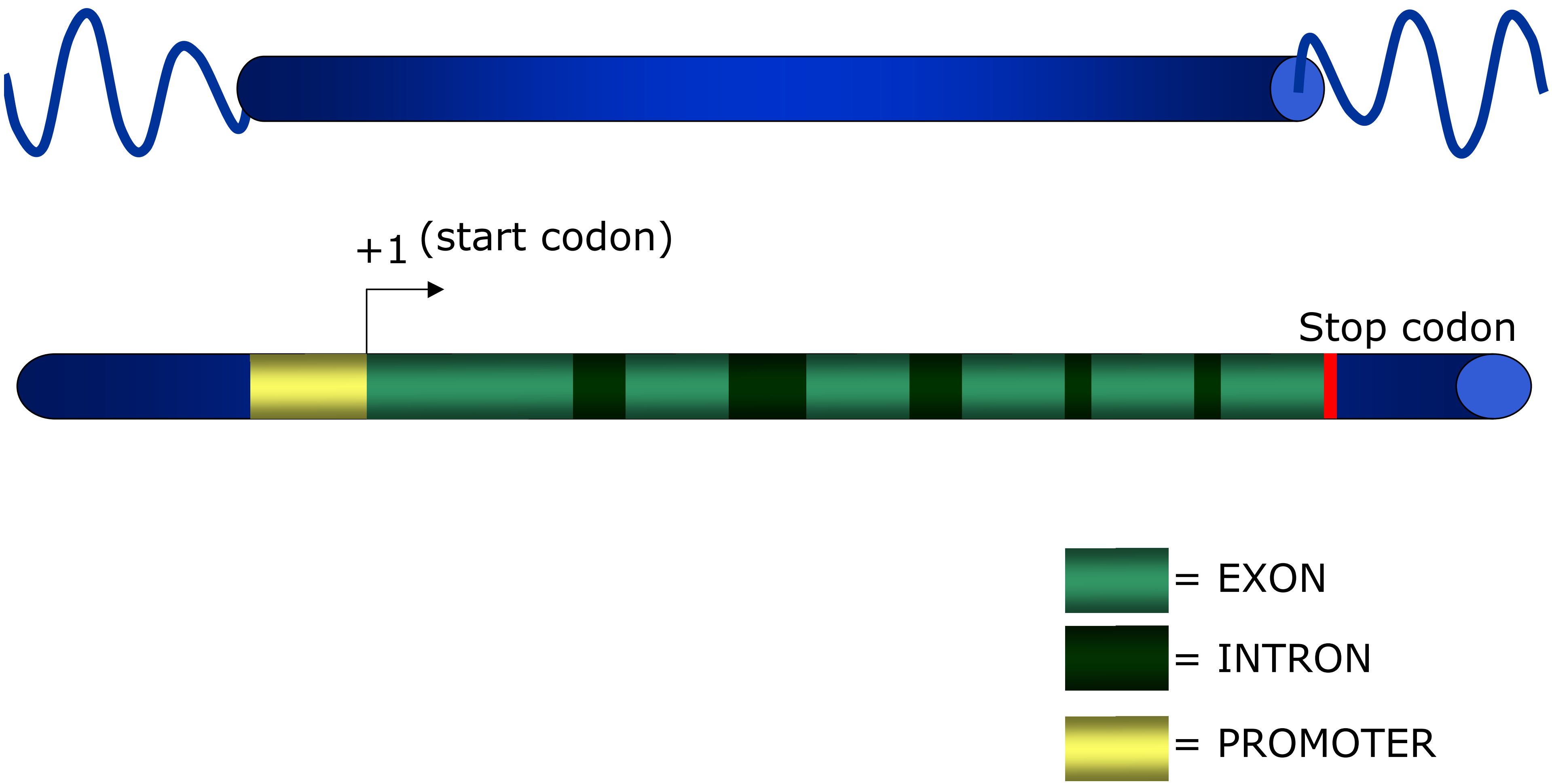
Protein Encoding Genes

- Boundaries of genes are defined by the start and end of transcription
- The core of the gene is called the coding region
- The gene sequence inscribed in the coding region of DNA, and in RNA, is composed of tri-nucleotide units called **codons**, each coding for a single amino acid

Basic Gene components

- Start Codon - ATG
- Stop Codons – TAA, TAG, TGA
- Promoter – non-coding sequence, directs RNA polymerase to the start of gene
- Exon – coding sequence (sequences that encode the protein)
- Intron – non-coding sequence

Schematic of gene structure



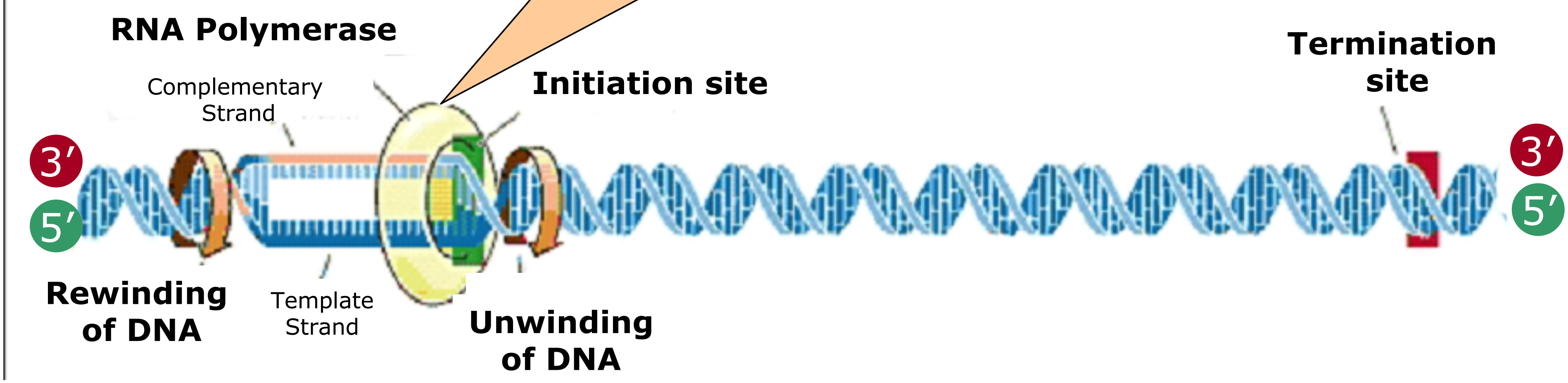
TRANSCRIPTION

- Copying of one strand of DNA into a complementary RNA sequence by the enzyme RNA polymerase
- Necessary components for Transcription
  - DNA template
  - RNA polymerase
  - Nucleoside triphosphates
- Three phases of transcription
  - Initiation
  - Elongation
  - Termination

Basic steps of transcription

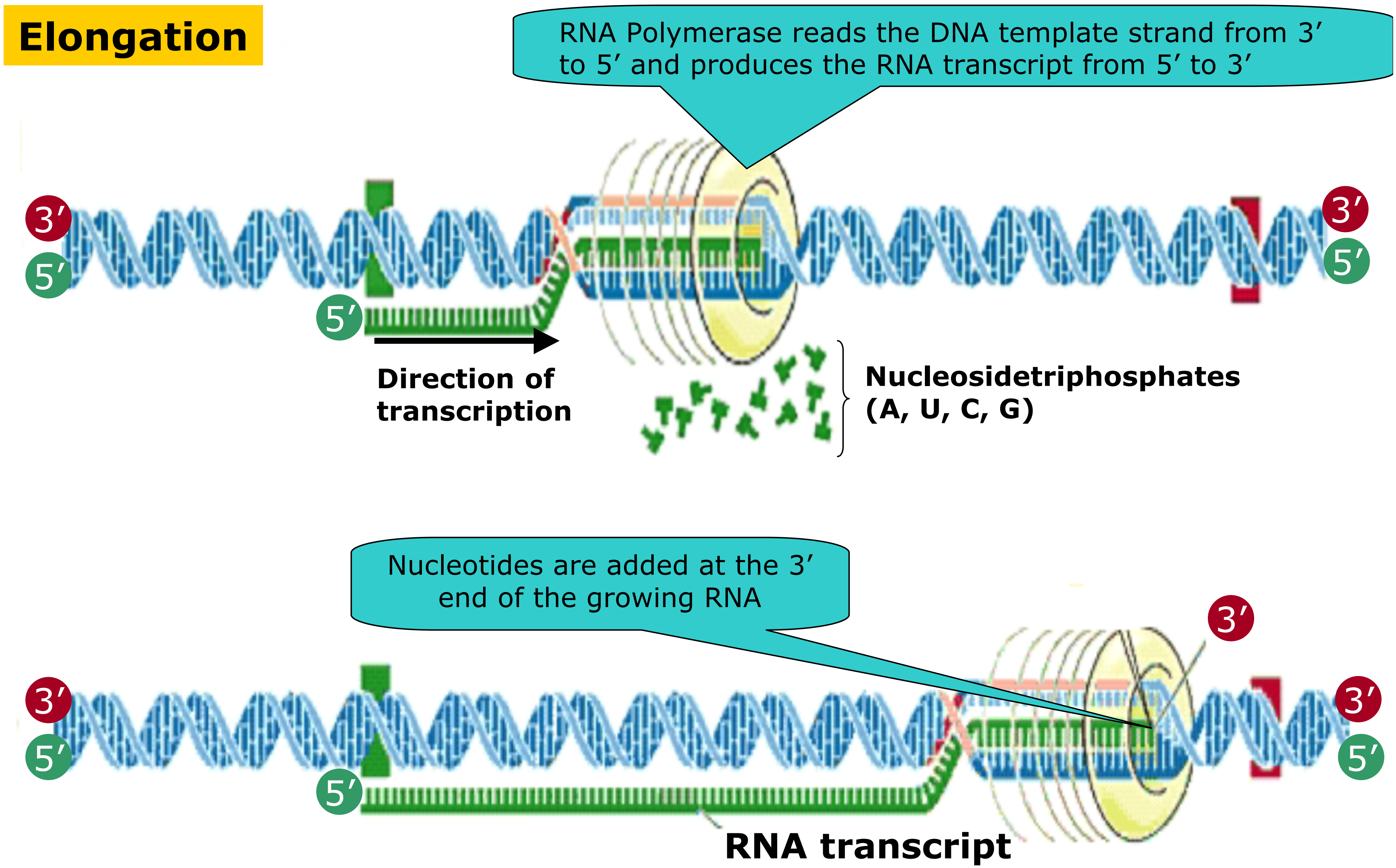
Initiation

RNA polymerase binds to the promoter and starts to unwind the DNA strands



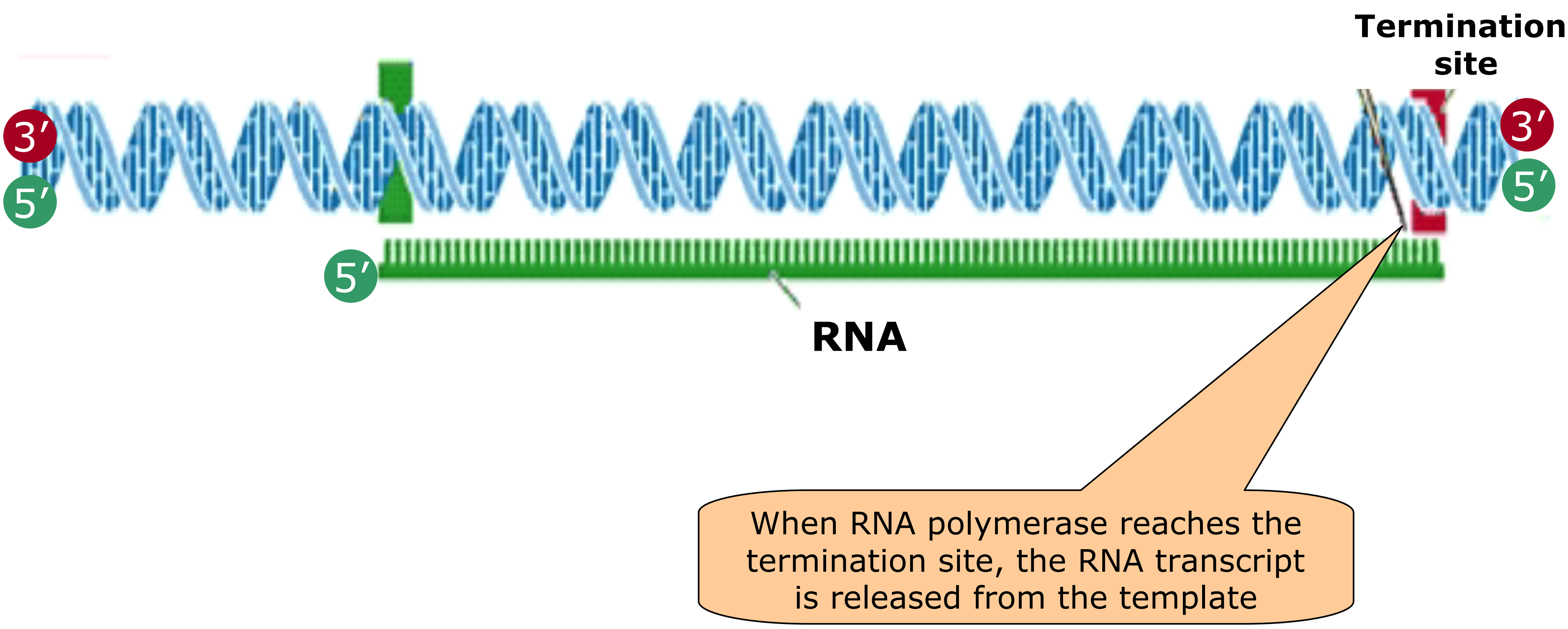
= promoter

Basic steps of transcription



Basic steps of transcription

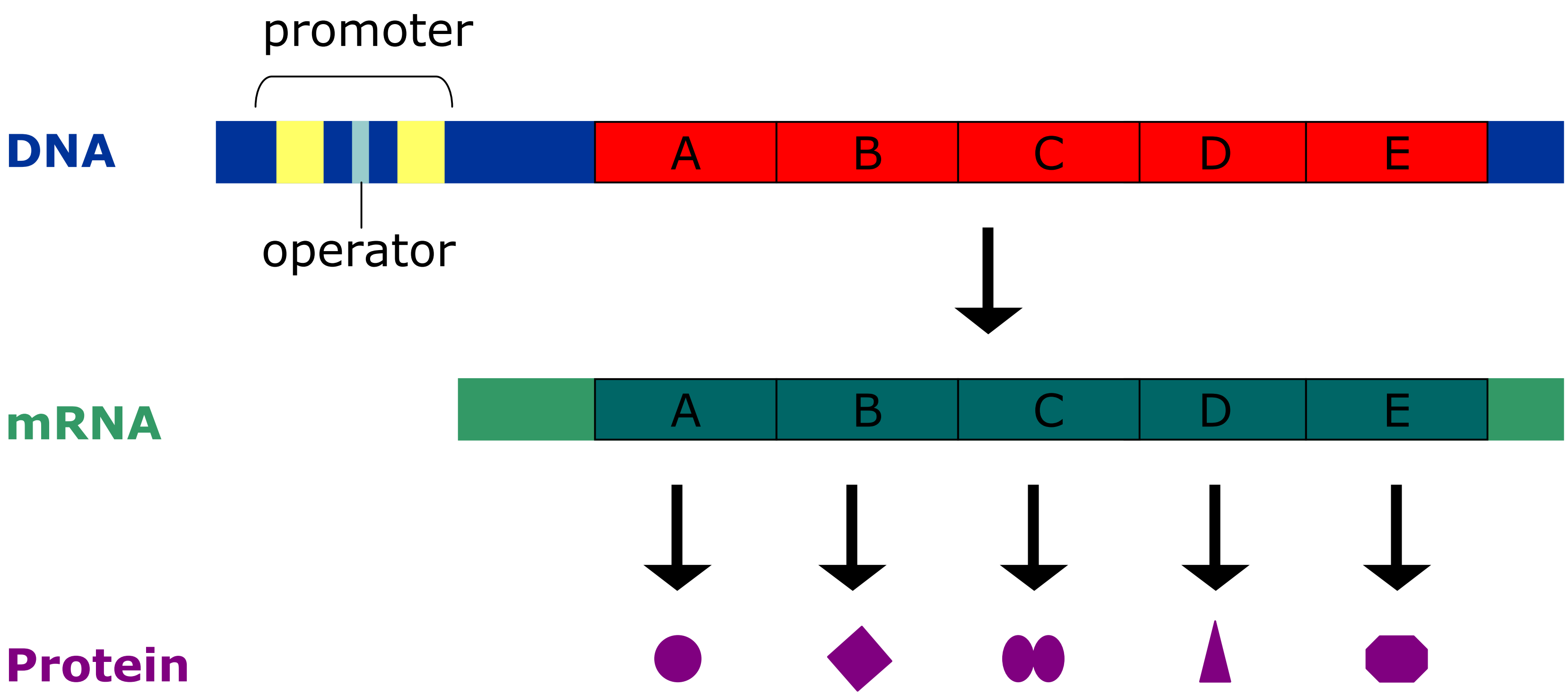
Termination



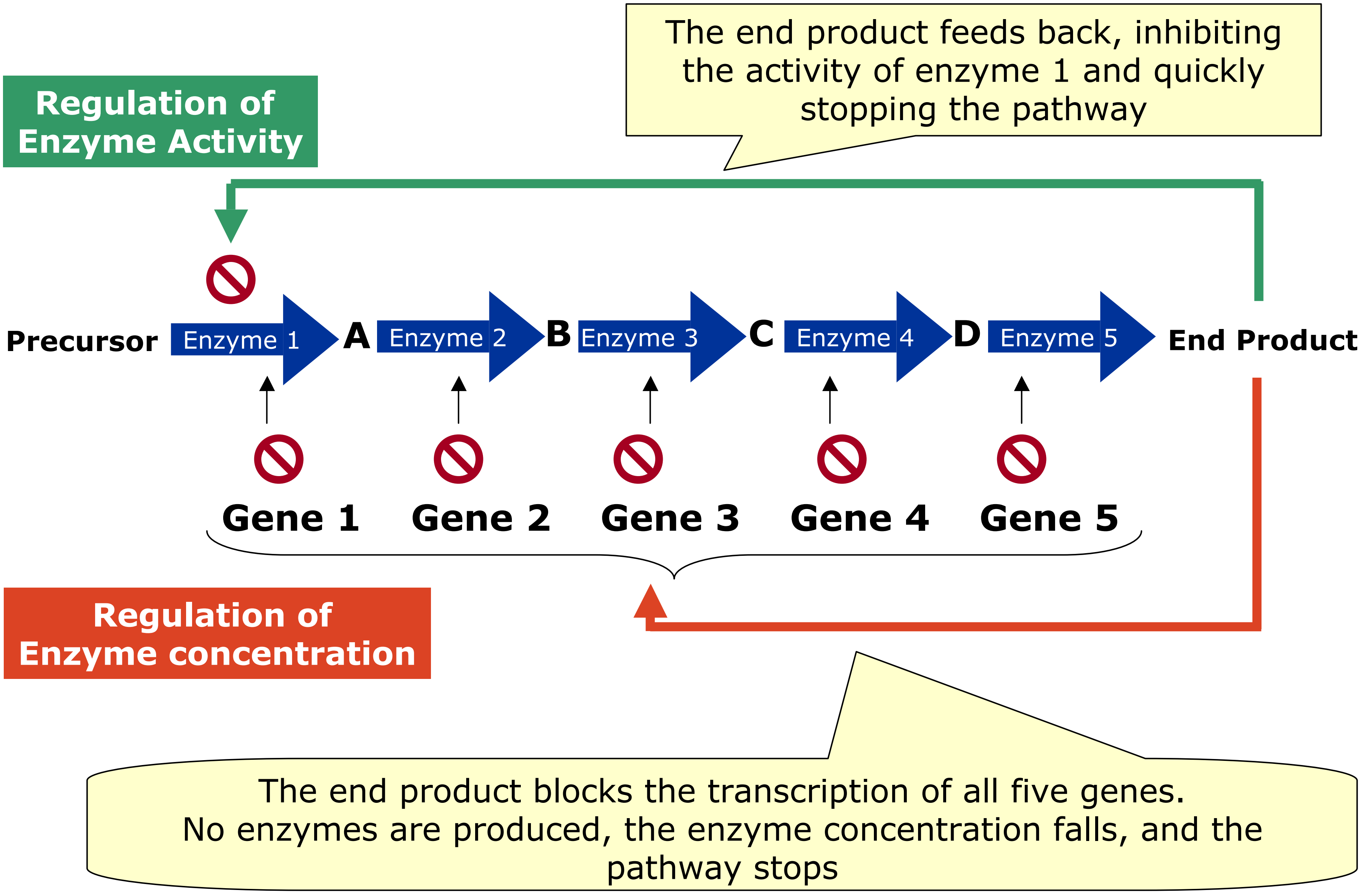
Operons

- In bacteria, half of the genes in are grouped into operons
- An operon is a group of contiguous genes that are transcribed into a single mRNA molecule
- The proteins that these genes encode are often involved in a similar pathway or function
- Operons have a promoter, operator and structural genes
- We will look at two – trp and lac

Operon schematic



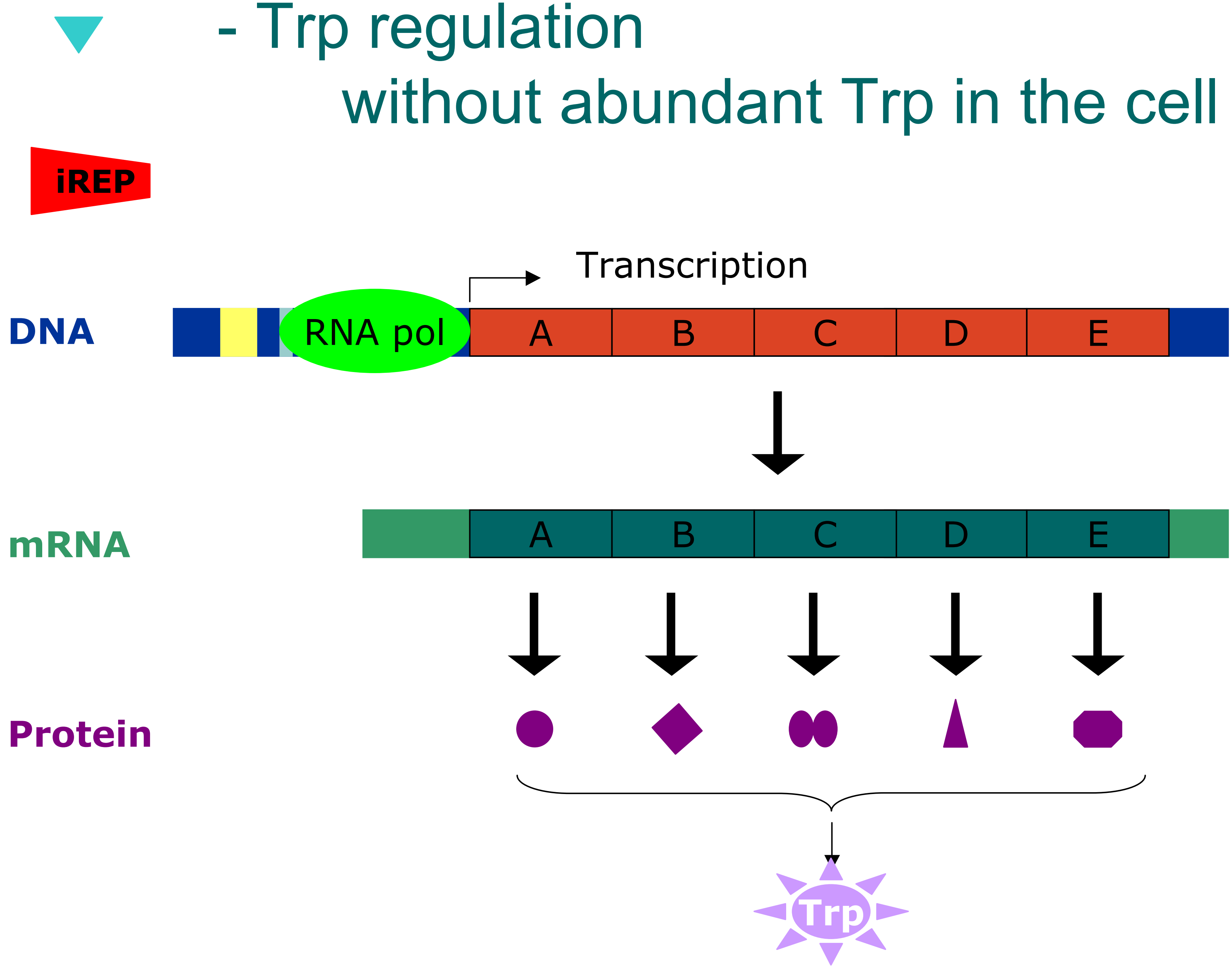
Operon – feedback loops



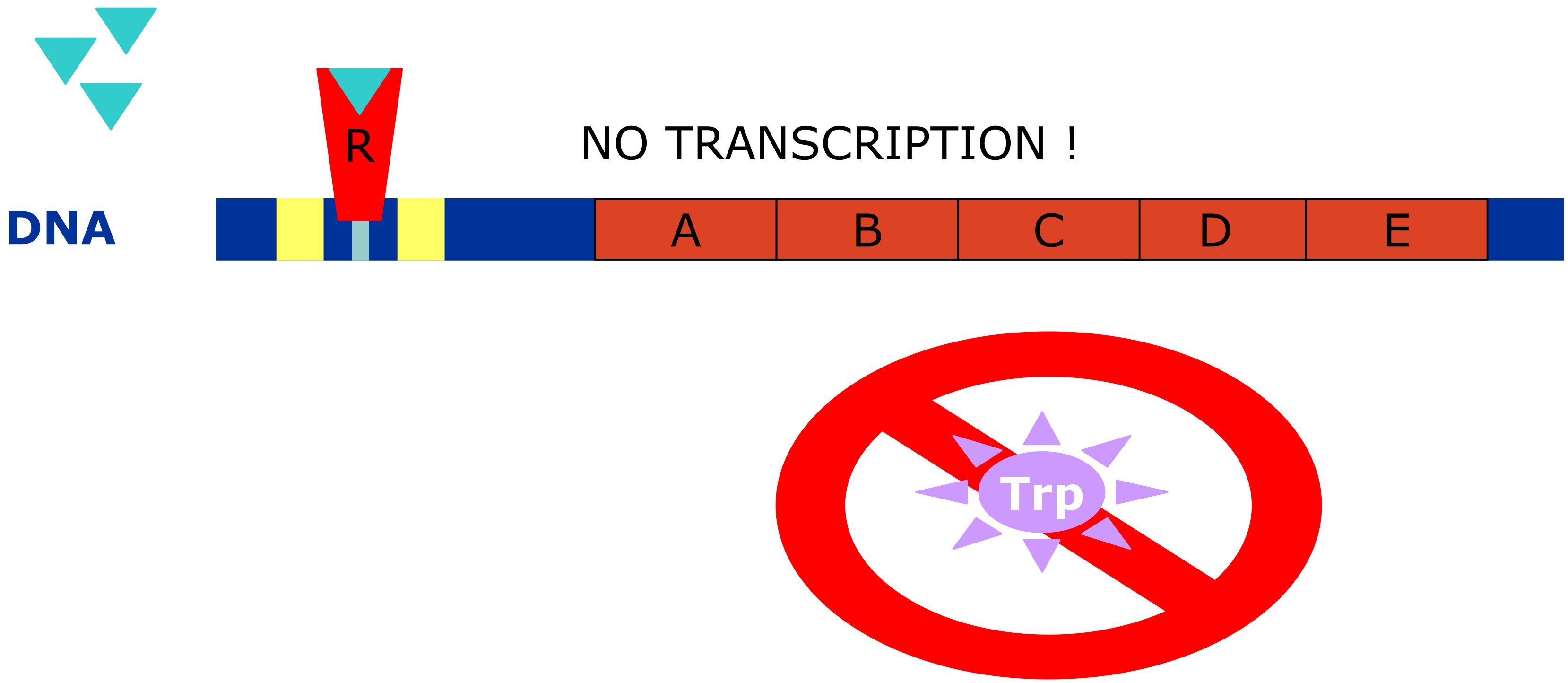
Trp operon

- Trp operon has five genes that code for the enzymes responsible for the manufacture the amino acid tryptophan
- Trp is necessary for transcription of operon, i.e. when Trp is abundant in a cell, the cell no longer needs to make Trp and shuts off their transcription
- When Trp is at lower concentrations in the cell, the cell needs to boost its Trp production
- How does this work?

- Trp regulation
without abundant Trp in the cell



Trp regulation
– with abundant Trp in the cell



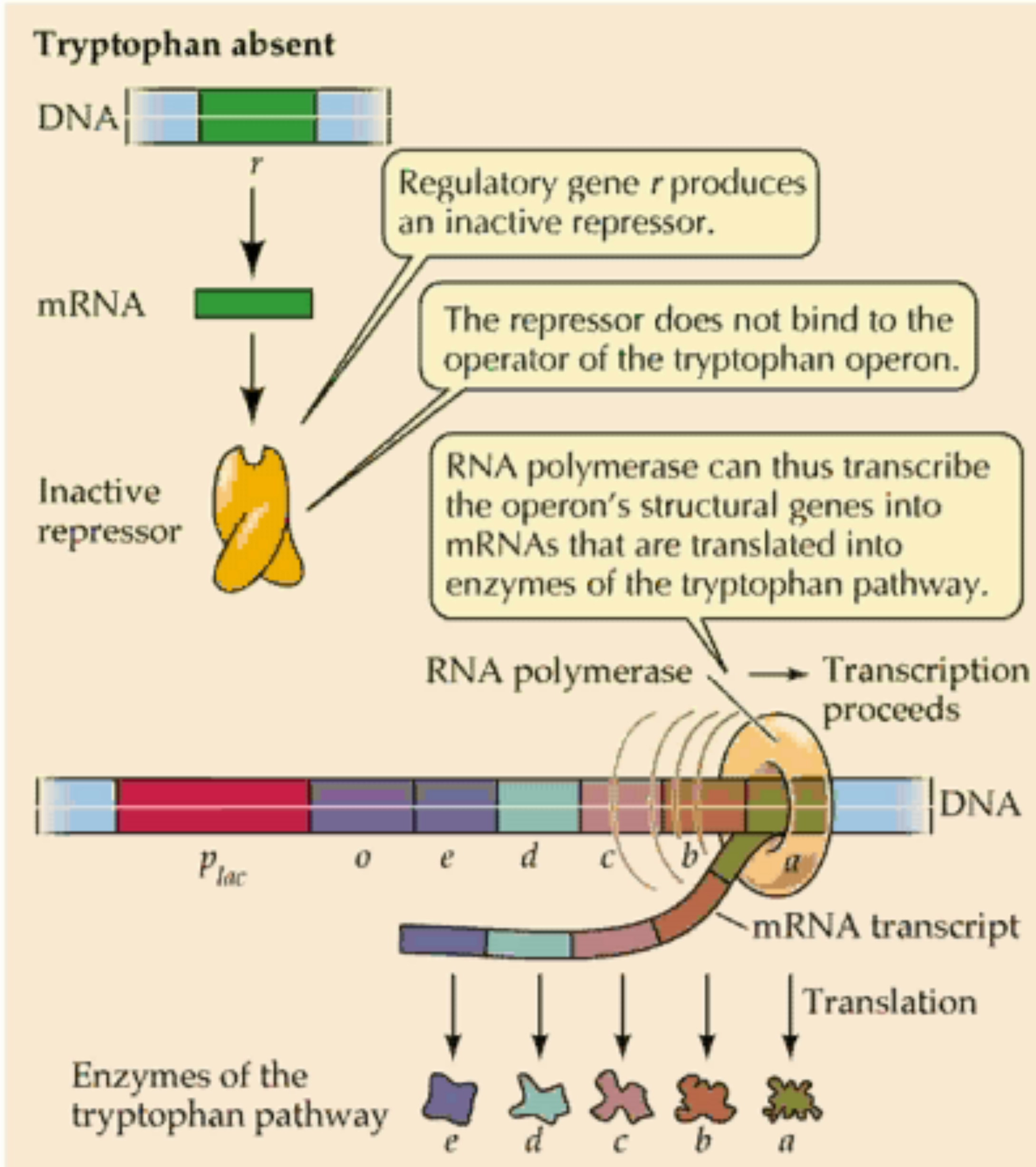
No Trp is made

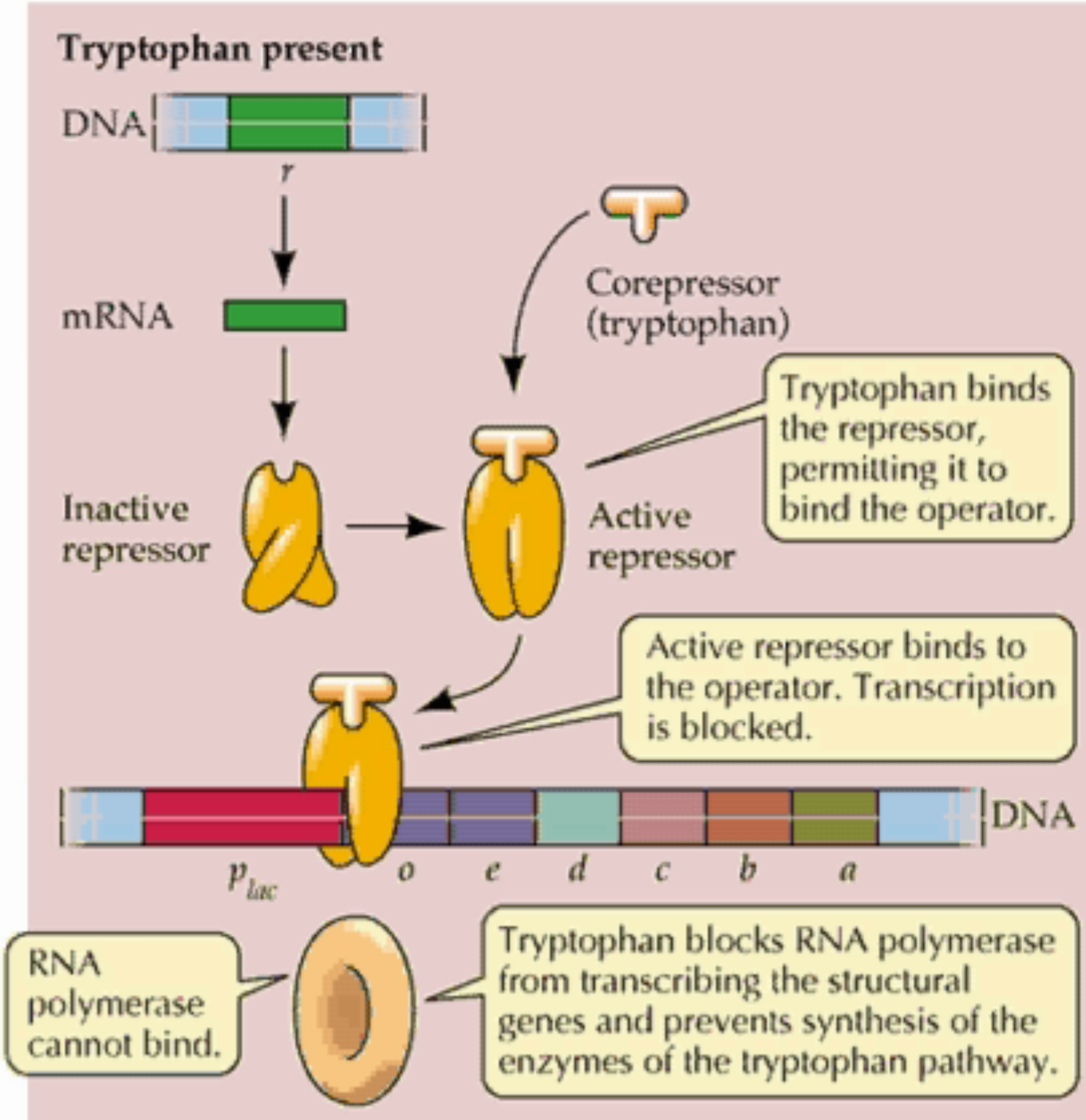
Trp binds to the inactive repressor, activating it

They bind the promoter, blocking RNA Polymerase.

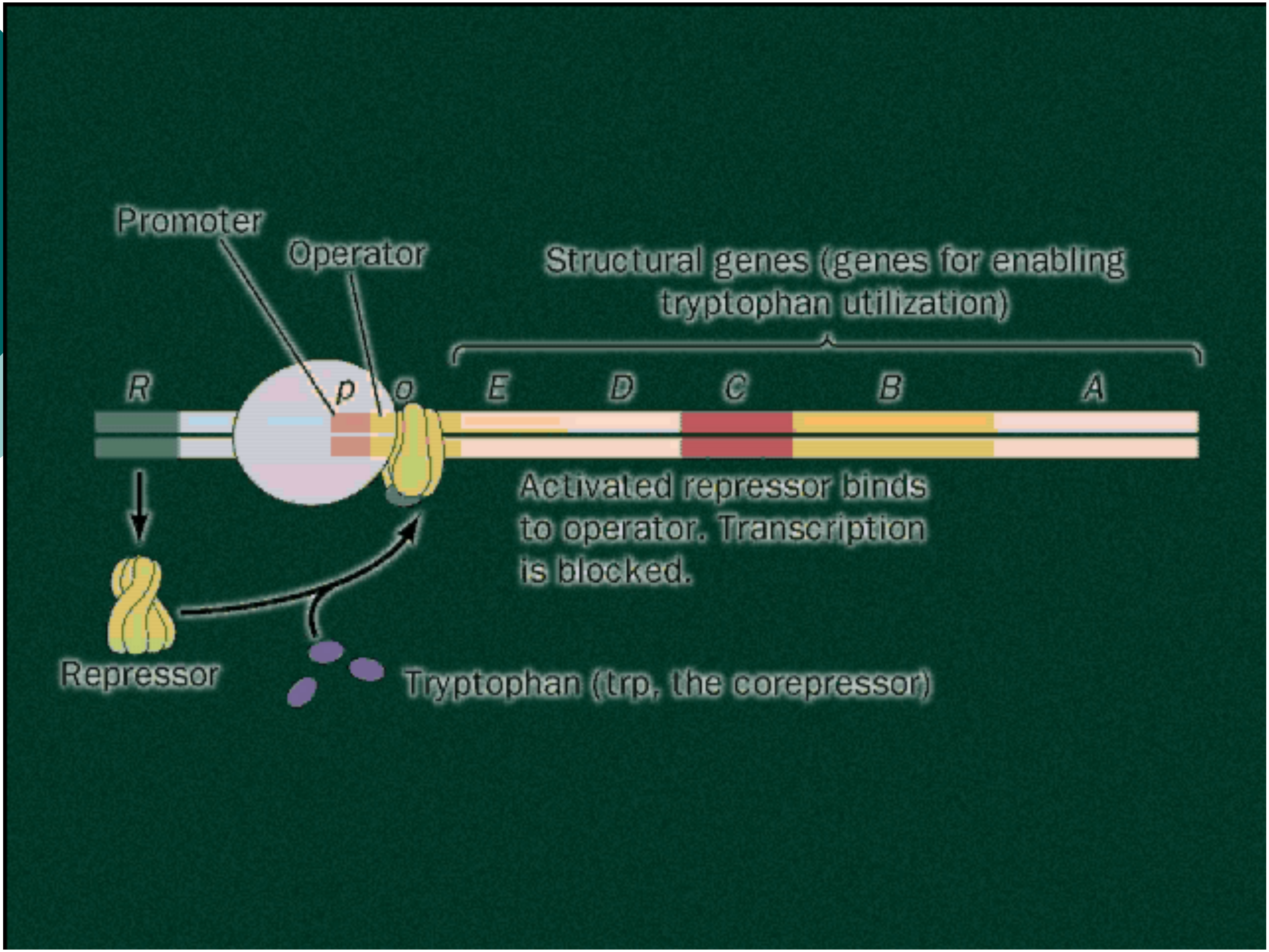
Resulting in no transcription of trp

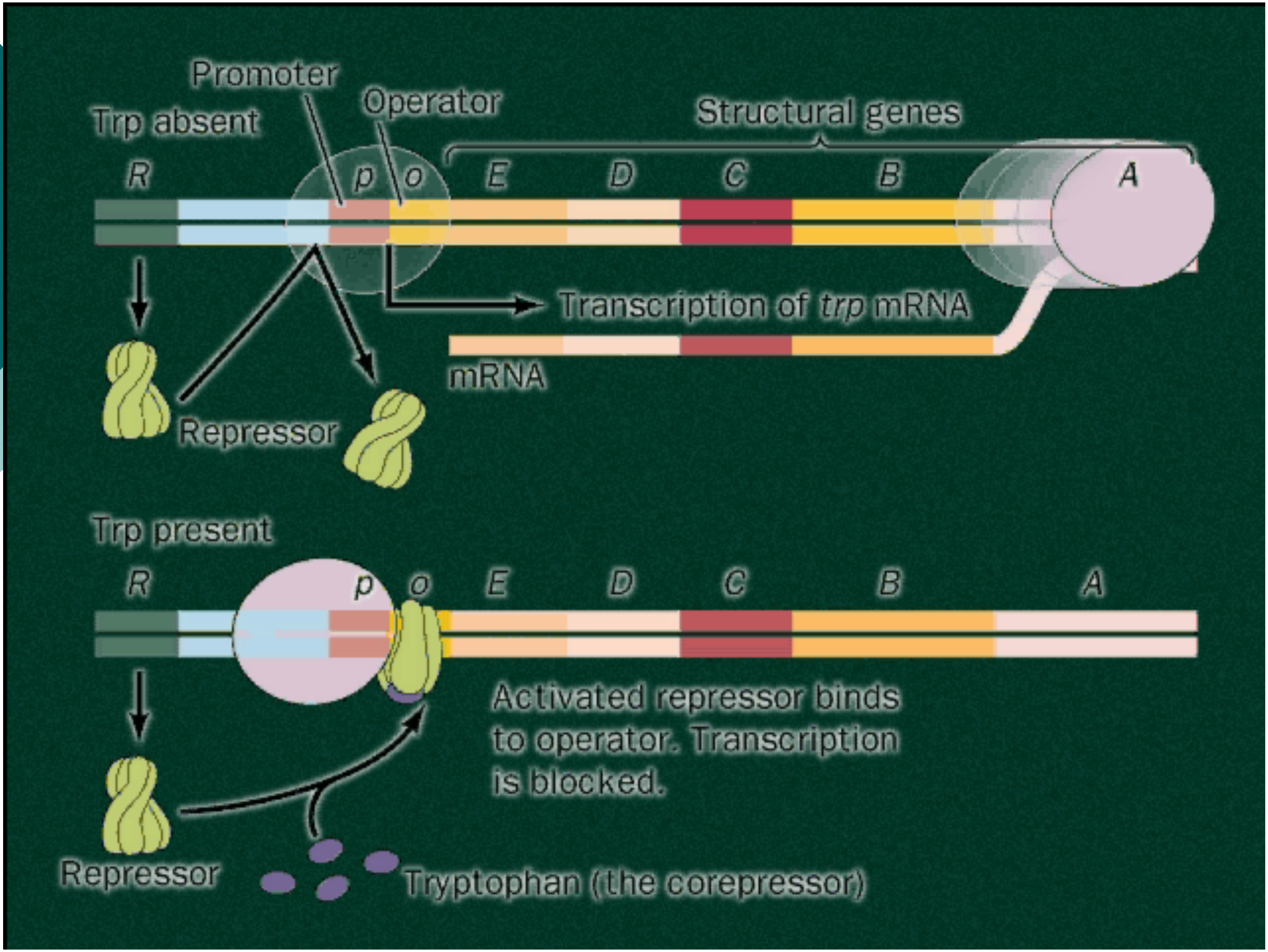
Operator- Synthesis of Tryptophan





Tryptophan present
DNA
r
mRNA
Corepressor (tryptophan)
Tryptophan binds the repressor, permitting it to bind the operator.
Inactive repressor
Active repressor
Active repressor binds to the operator. Transcription is blocked.
DNA
p_{lac}
o
e
d
c
b
a
RNA polymerase cannot bind.
Tryptophan blocks RNA polymerase from transcribing the structural genes and prevents synthesis of the enzymes of the tryptophan pathway.


Promoter
Operator
Structural genes (genes for enabling tryptophan utilization)
R
p
o
E
D
C
B
A
Activated repressor binds to operator. Transcription is blocked.
Repressor
Tryptophan (trp, the corepressor)



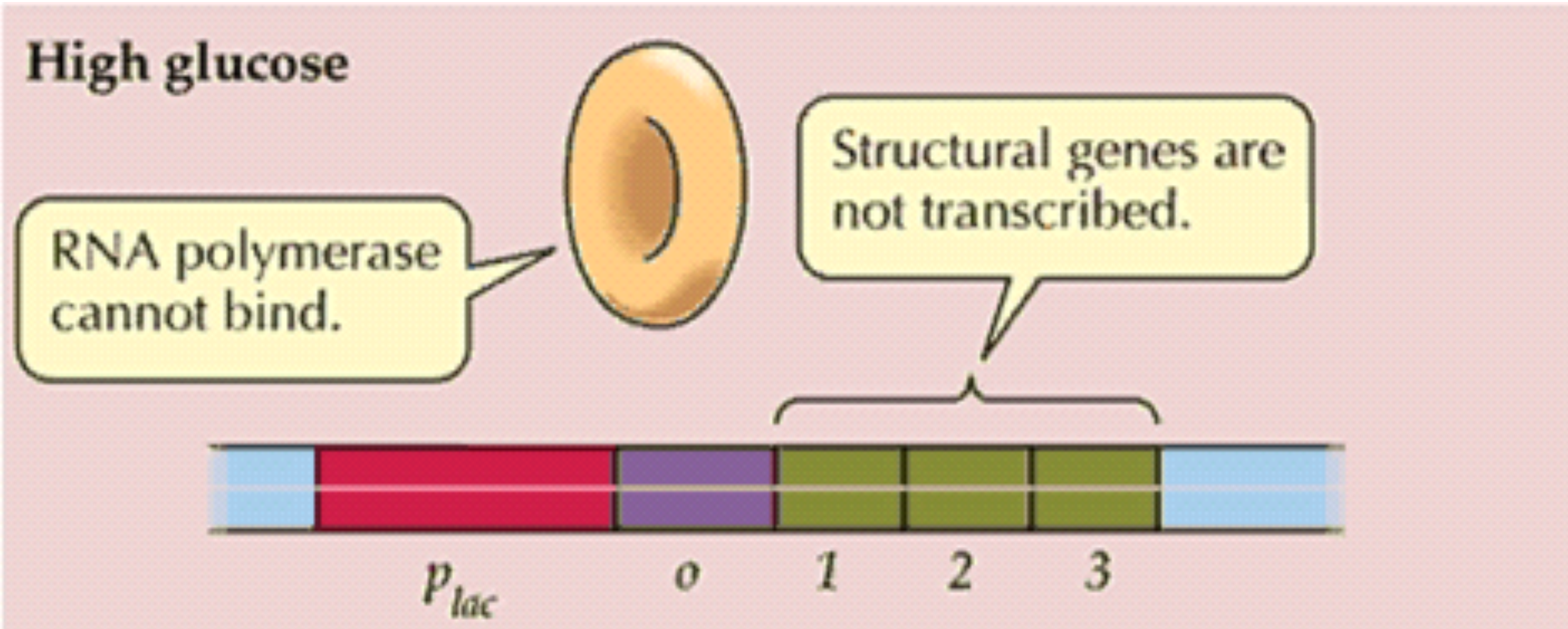
Promoter
Operator
Trp absent
Structural genes
R
p
o
E
D
C
B
A
Transcription of trp mRNA
mRNA
Repressor
Trp present
R
p
o
E
D
C
B
A
Activated repressor binds to operator. Transcription is blocked.
Repressor
Tryptophan (the corepressor)

Lac operon

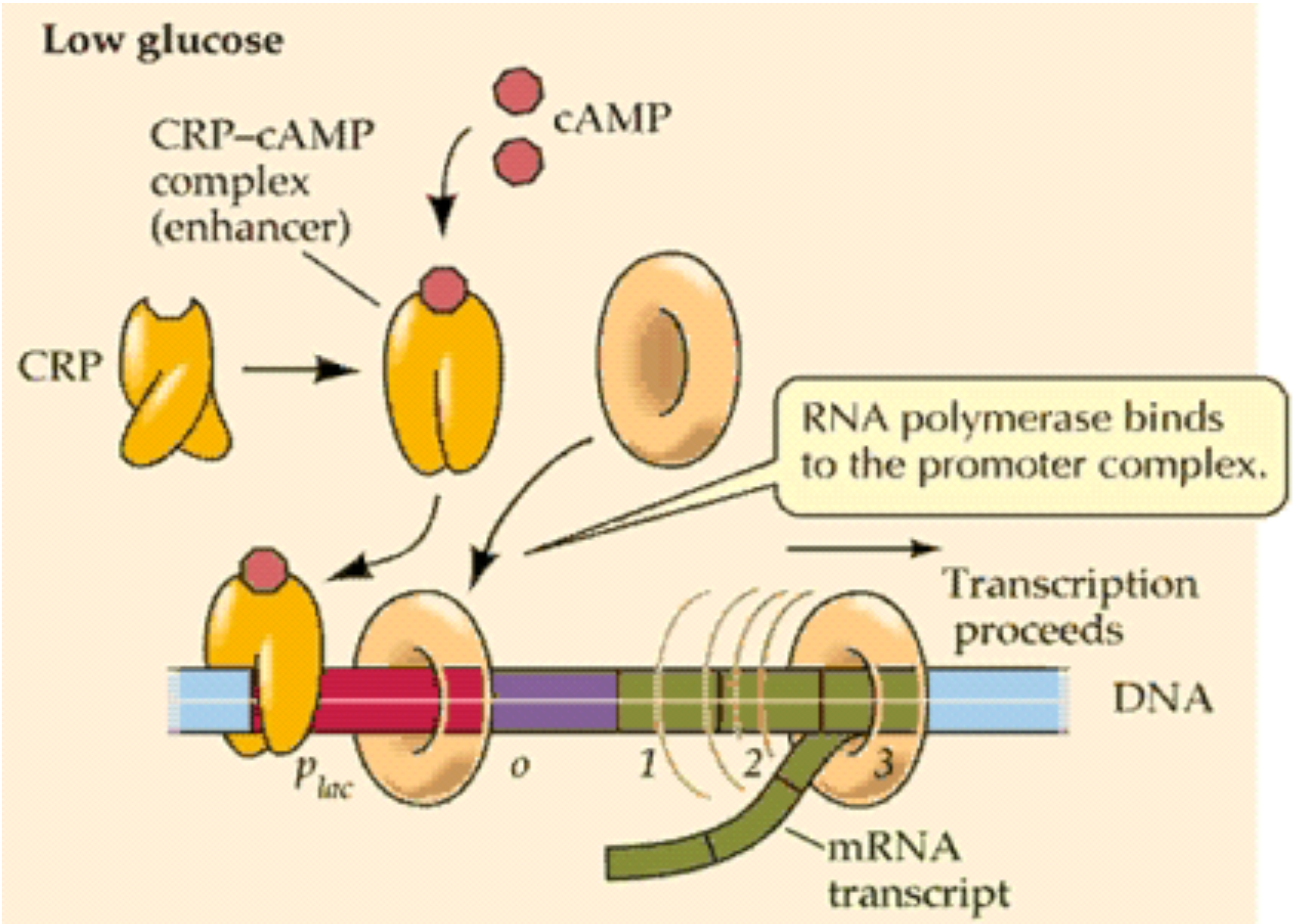
- Lac operon encodes the proteins required to transport the disaccharide lactose into the cell and break it down
- Three genes controlled by this operon
- This operon is under both positive (CAP) and negative (Lac Repressor) transcriptional control
- Lactose and glucose control the initiation of transcription of this operon

Role of cAMP in Bacteria

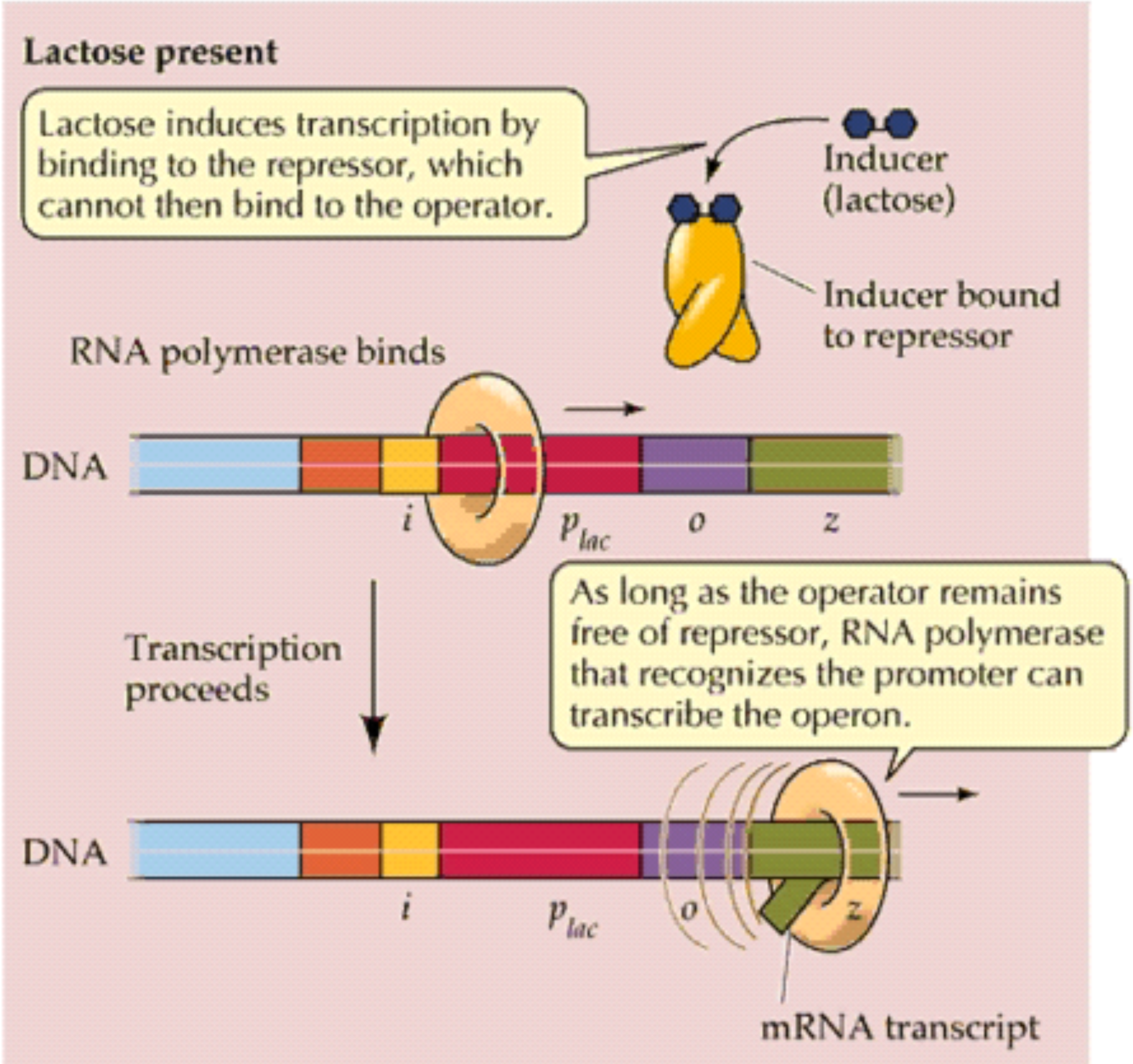
- In bacteria, the level of cAMP **varies** depending on the medium used for growth. Glucose inhibits the formation of cAMP, so cAMP is low when glucose is present.
- The transcription factor “cAMP receptor protein” (**CRP**) or **CAP** (catabolite gene activator protein) forms a complex with cAMP and thereby is activated to bind to DNA.
- CRP-cAMP increases **expression** of a large number of genes, including some encoding enzymes that can supply energy independent of glucose.



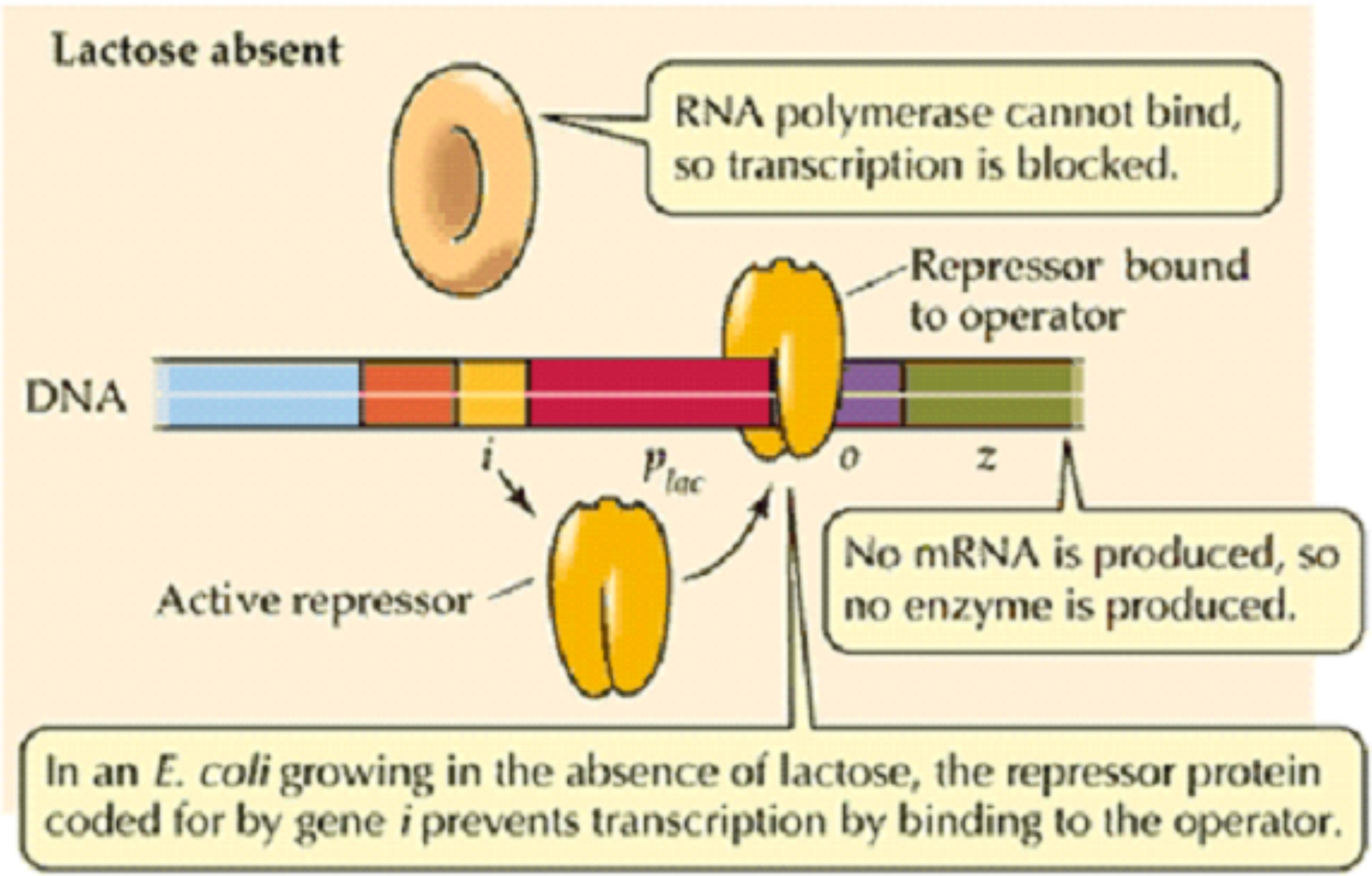
A cell that contains ample glucose and does not require energy from other sources contains little cAMP and little CRP–cAMP; in such a cell, the structural genes are not transcribed and the catabolic enzymes are not formed.

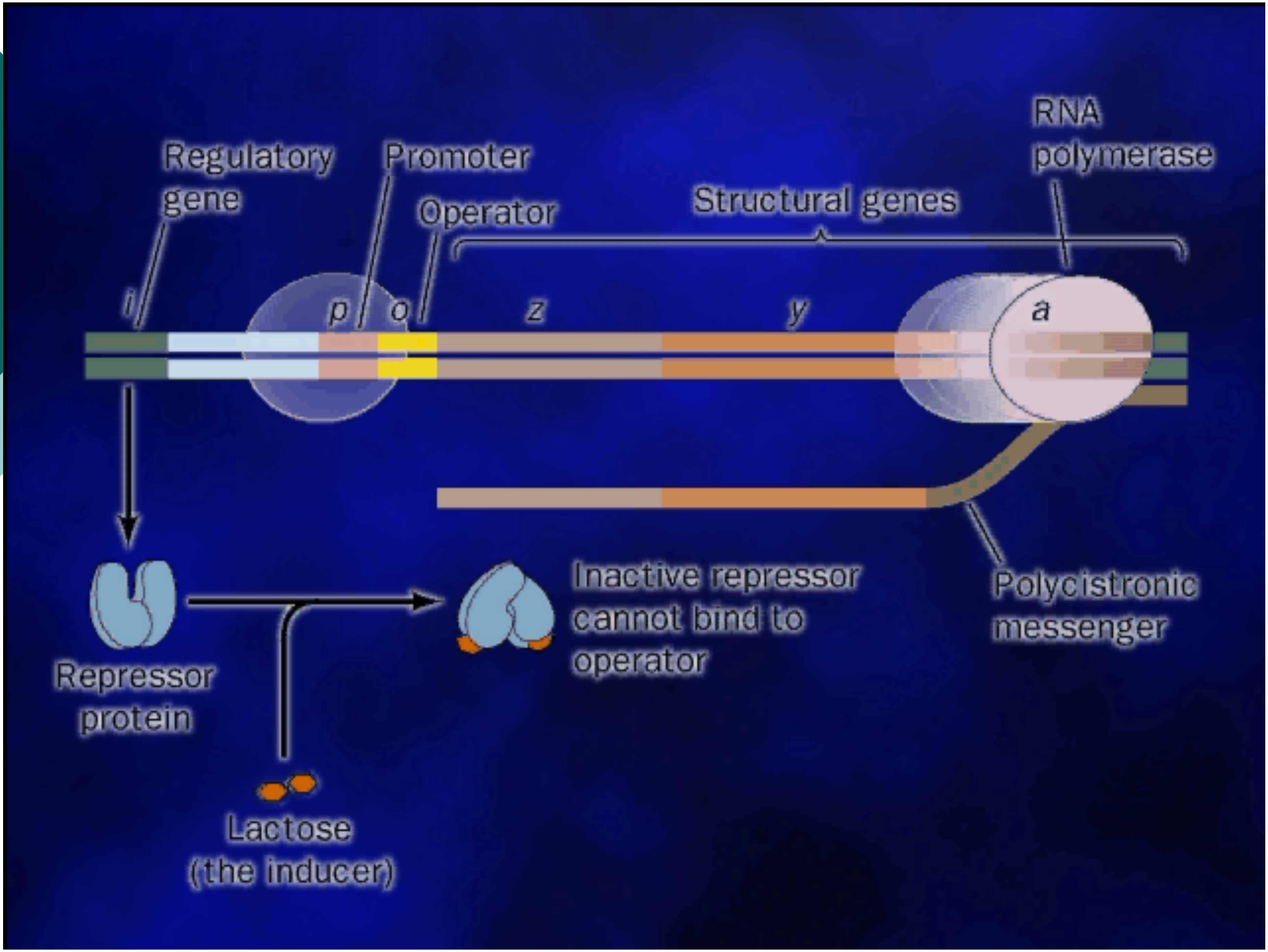


When supplies of glucose are low, a receptor protein (CRP) and cAMP form a complex that binds to the promoter and activates it, allowing transcription of structural genes that encode enzymes for catabolizing the alternative energy source.

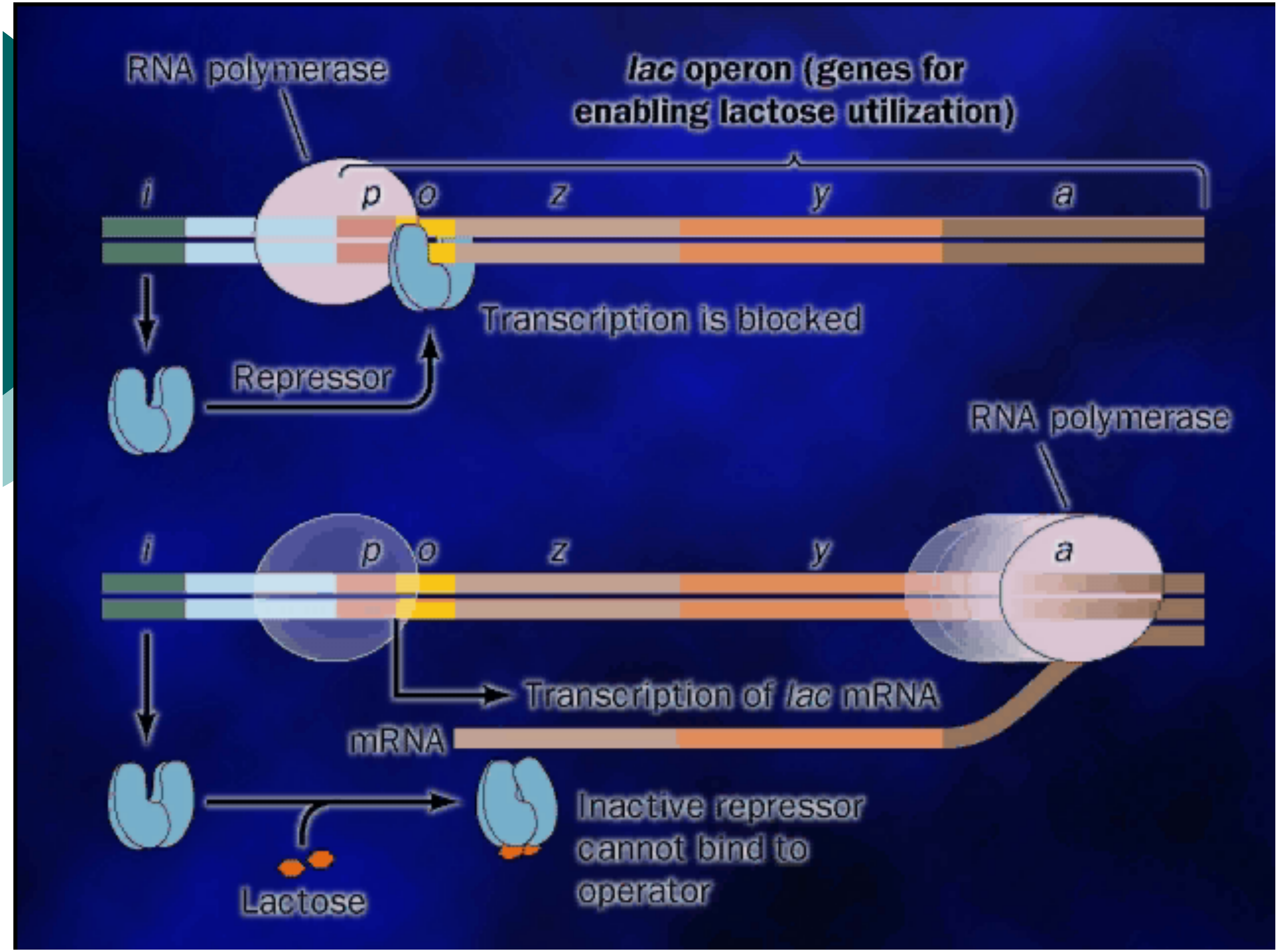

Lactose present
Lactose induces transcription by binding to the repressor, which cannot then bind to the operator.
Inducer (lactose)
Inducer bound to repressor
RNA polymerase binds
DNA
i
p_{lac}
o
z
Transcription proceeds
As long as the operator remains free of repressor, RNA polymerase that recognizes the promoter can transcribe the operon.
DNA
i
p_{lac}
o
z
mRNA transcript

Catabolism of Lactose

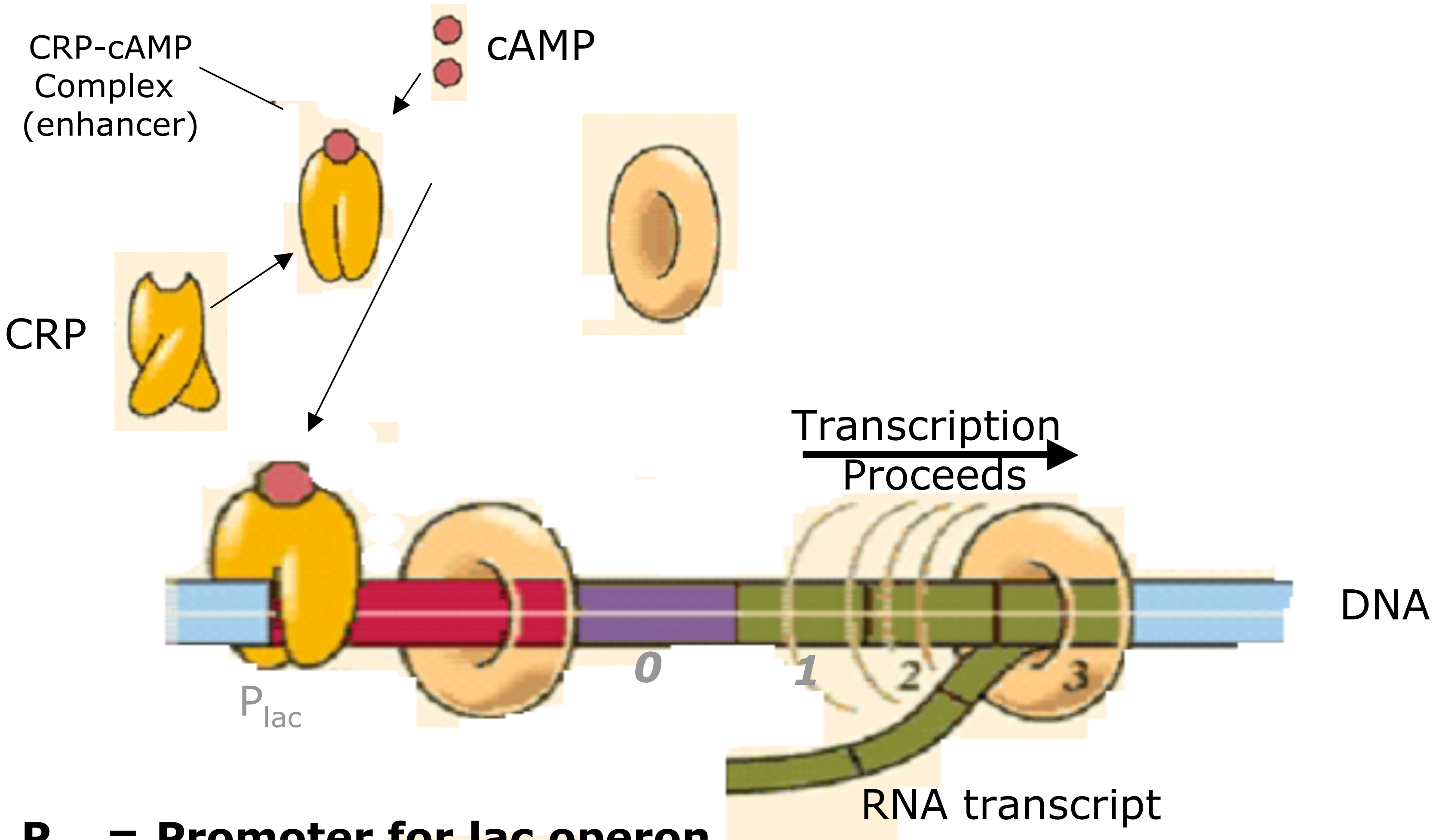




Regulatory gene
Promoter
Operator
Structural genes
RNA polymerase
i
p
o
z
y
a
Repressor protein
Inactive repressor cannot bind to operator
Polycistronic messenger
Lactose (the inducer)


RNA polymerase
lac operon (genes for enabling lactose utilization)
i
p
o
z
y
a
Transcription is blocked
Repressor
RNA polymerase
i
p
o
z
y
a
Transcription of lac mRNA
mRNA
Inactive repressor cannot bind to operator
Lactose

Low Glucose



P_{lac} = Promoter for lac operon

Lac regulation