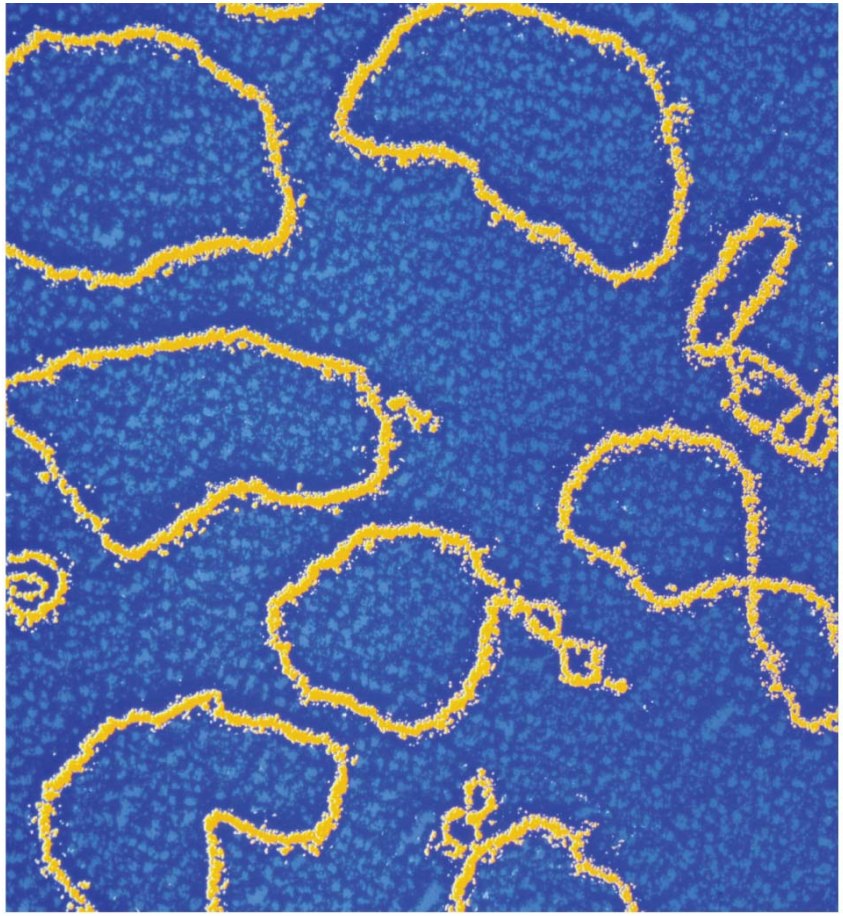
Chapter 8

Microbial Genetics



Terminology

- **Heredity =** all the traits of a living organisms // traits are encoded in the genetic material of the cell
  - Traits (coded information) also include antibiotic resistance of microbes
  - emerging diseases: microbes may gain pathogenic traits from other organisms **// e.g. plasmids – carry genetic code for drug resistance**
- **Chromosome**: the structures that contain coded information // chemical polymer called DNA // bacteria have “one” circular chromosome
  - carries hereditary information // individual traits are arranged as linear nucleotides called genes

Terminology

- **Genetics:** science of heredity
- **Gene**: a segment of DNA that encodes a functional product, usually a protein (structural vs functional or enzyme)
- **Genome**: all the genetic information in a cell (chromosome plus plasmids)
- **Genomics**: the molecular study of genomes
- **Genotype**: the genes of an organism
- **Phenotype**: expression of the genes / determined by those genes that are turned on

Terminology

- **Genetics**: the study of genes
  - how genes carry information
  - how information is expressed
  - how genes (chromosomes) are replicated
  - how genes maybe transferred

Determine Relatedness

Minor changes in the sequence of the "code" forming the genetic "alphabet" results in different "microbial stains" // each with a different genotype

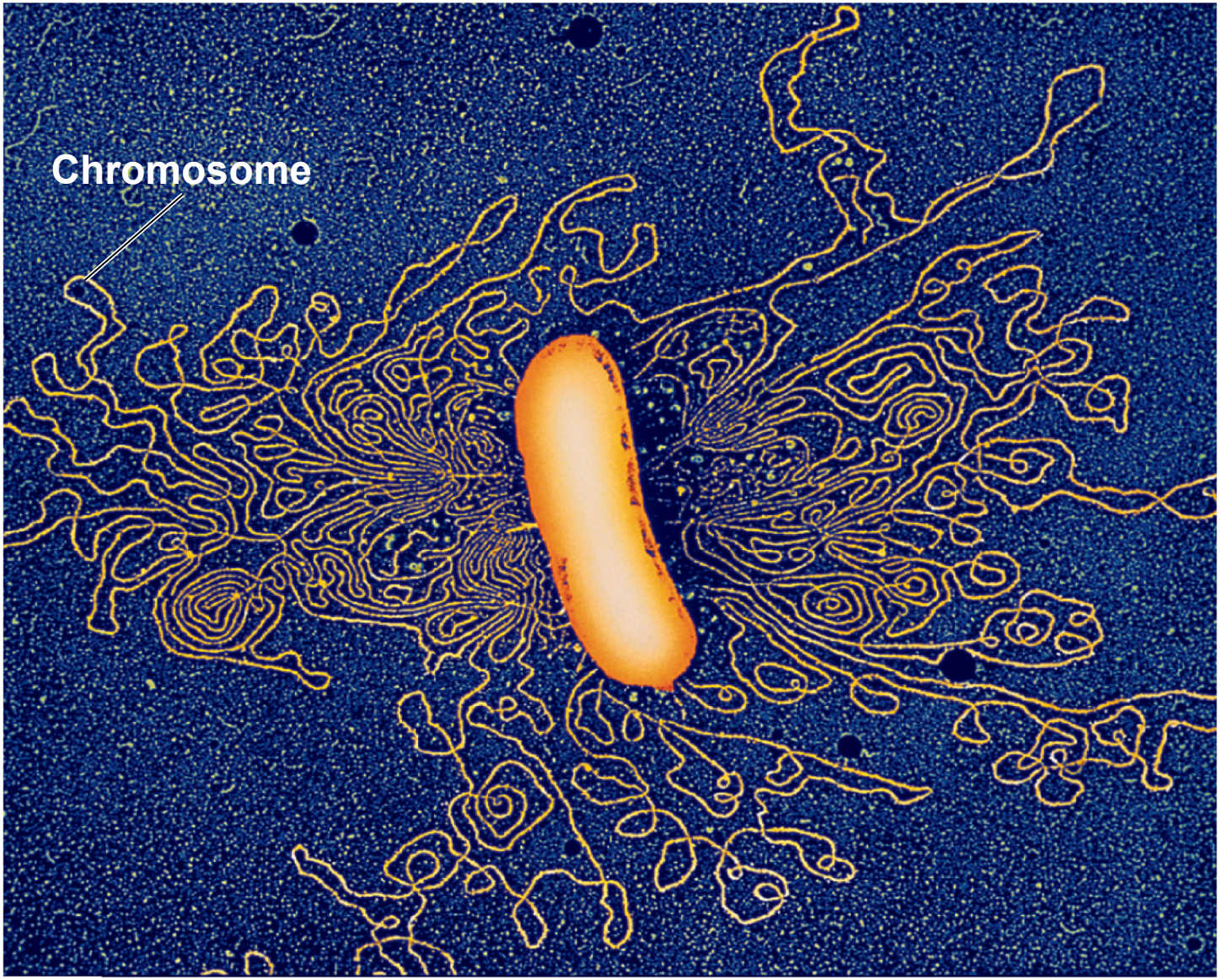
Australia	A	C	C	C	C	G	T	C	C	A	C	C	C	T	T	T	C	A	A	T	T
Egypt	A	A	T	C	G	A	T	C	A	T	C	T	T	C	G	T	C	G	A	T	C
France	A	A	T	C	G	A	T	C	A	T	C	G	T	C	G	T	C	G	A	T	C
Israel	A	T	C	C	A	T	T	C	A	T	C	C	T	C	A	T	C	G	A	T	T
Italy	A	T	C	C	A	C	T	C	A	T	C	C	T	C	G	T	C	G	A	T	T
Kenya	A	T	C	C	A	C	T	C	A	T	C	C	T	C	G	T	C	G	A	T	T
Mexico	A	A	C	C	C	T	T	C	C	T	C	C	C	C	T	T	C	G	A	T	T
United States	A	A	C	C	C	C	T	C	C	T	C	C	C	C	T	T	C	G	A	T	T
Uganda	A	T	A	C	G	A	T	C	A	T	G	C	T	C	G	T	C	C	A	T	C

Determine Relatedness

- Which strain is more closely related to the Uganda strain?

Strain	% Similar to Uganda
Kenya	71%
U.S.	51%

A prokaryotic chromosome.

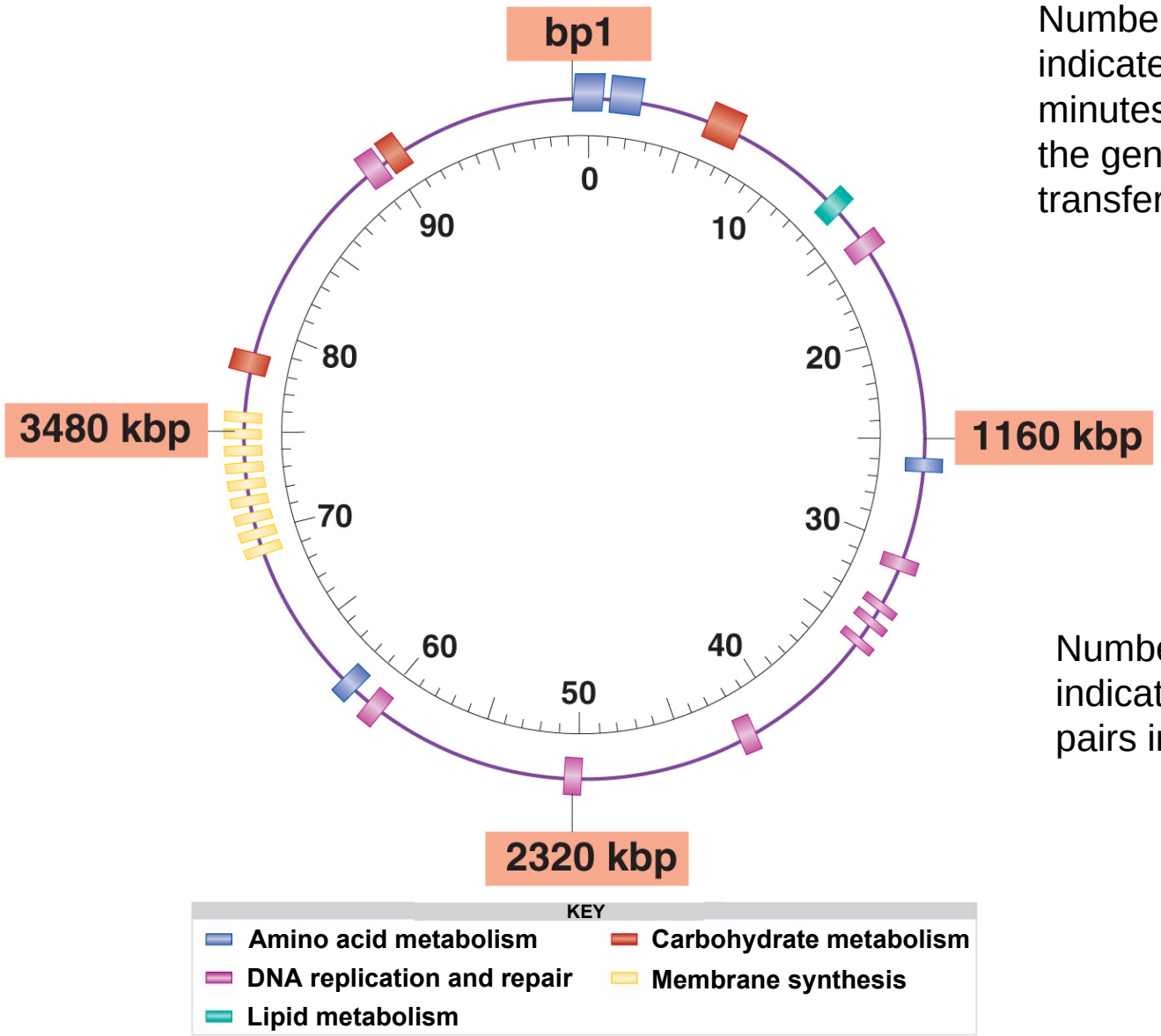


Disrupted *E. coli* cell

TEM

1 μm

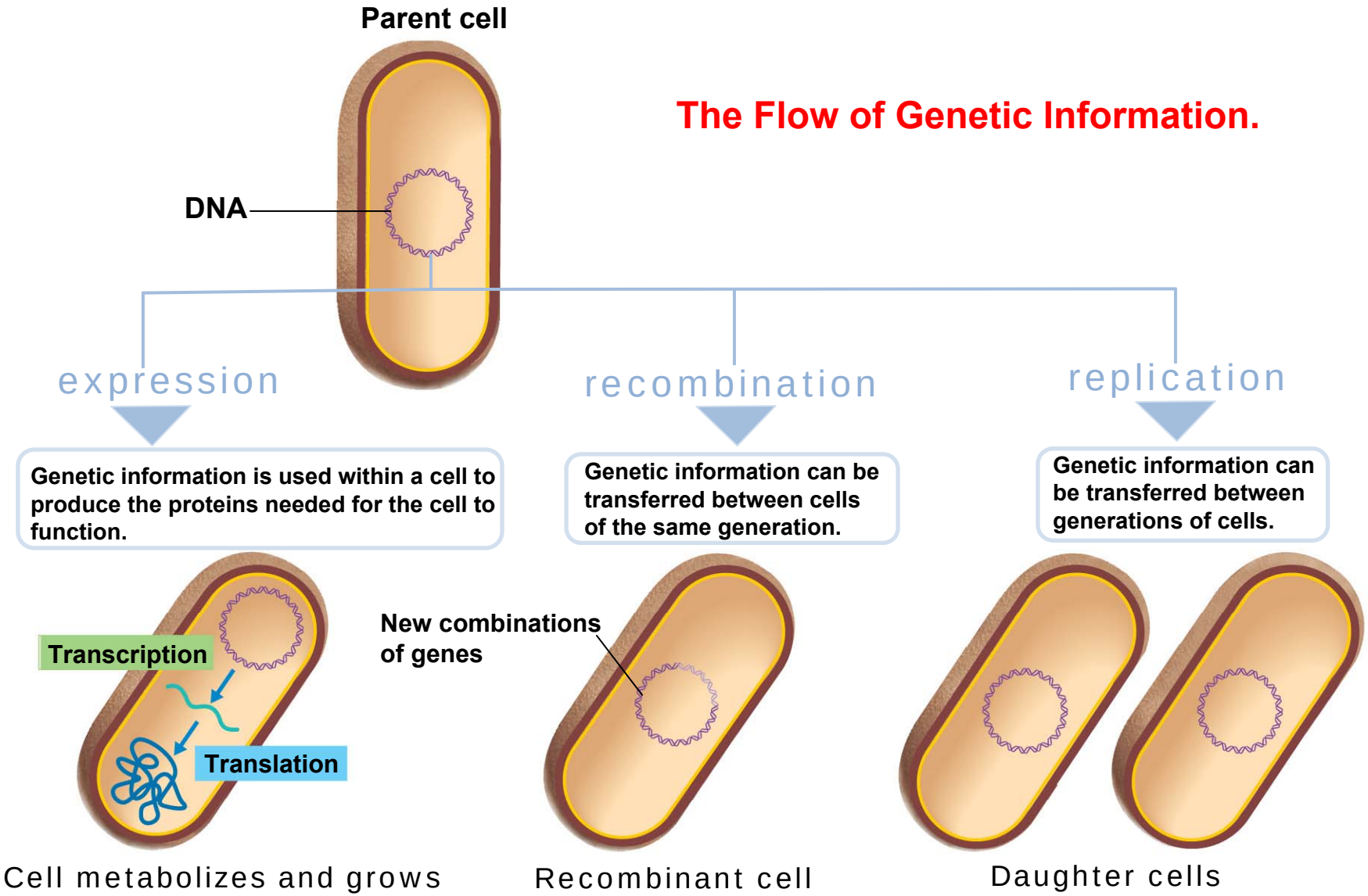
A prokaryotic chromosome. // Genetic map of the chromosome of *E. coli*



Number inside the circle indicate the number of minutes it takes to transfer the genes during gene transfer

Number inside color boxes indicate number of base pairs in gene

The Flow of Genetic Information.

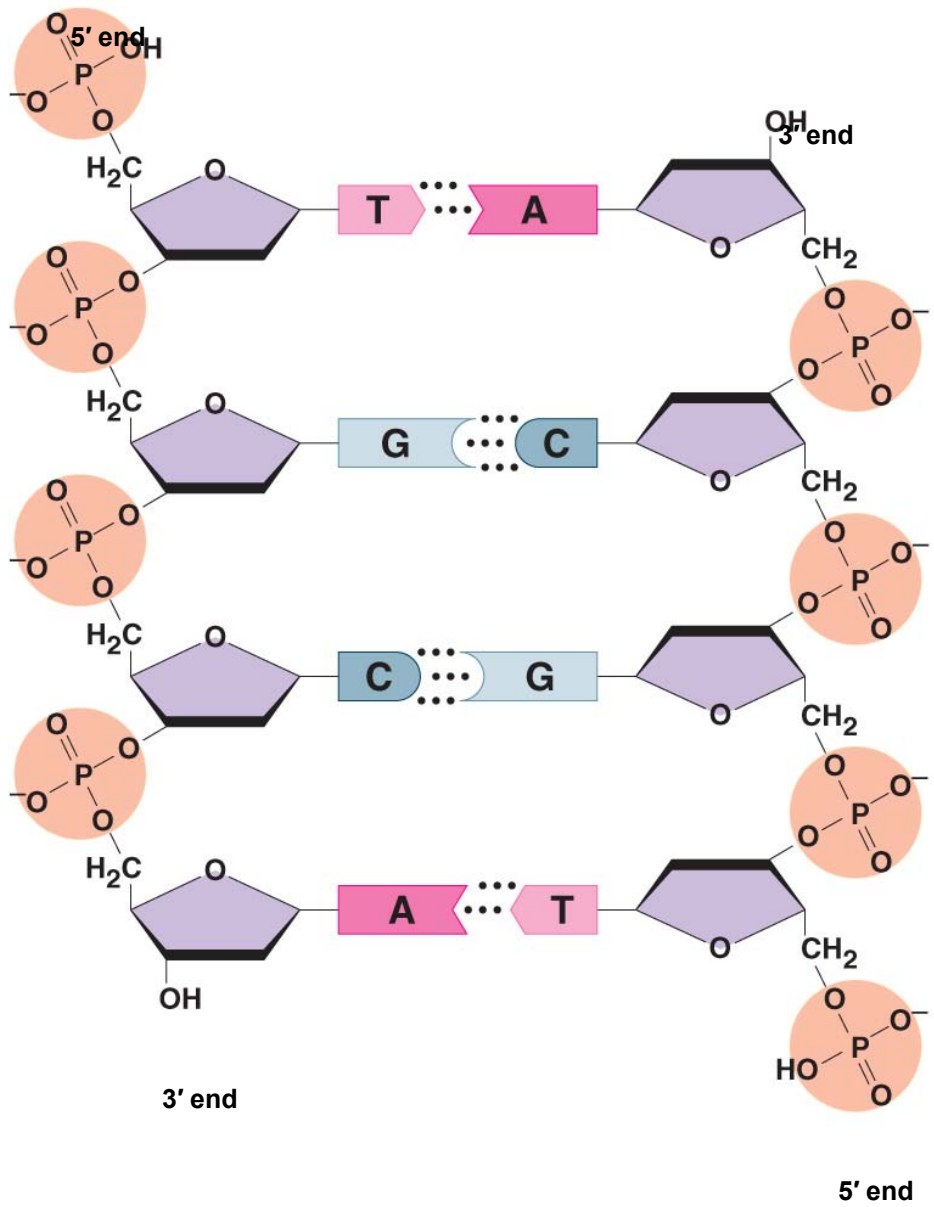


DNA

- Polymer of nucleotides: adenine, thymine, cytosine, and guanine
- Double helix associated with proteins
- “Backbone” is deoxyribose-phosphate
- Strands are held together by hydrogen bonds between AT and CG
- Strands are anti-parallel (5-3 vs 3-5 direction)

DNA replication.

This occurs during binary fission

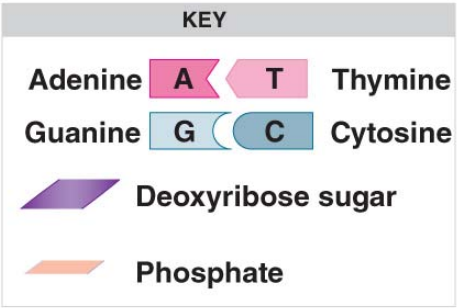


The two strands of DNA are anti-parallel. The sugar-phosphate backbone of one strand is upside down relative to the backbone of the other strand. Turn the book upside down to demonstrate this.

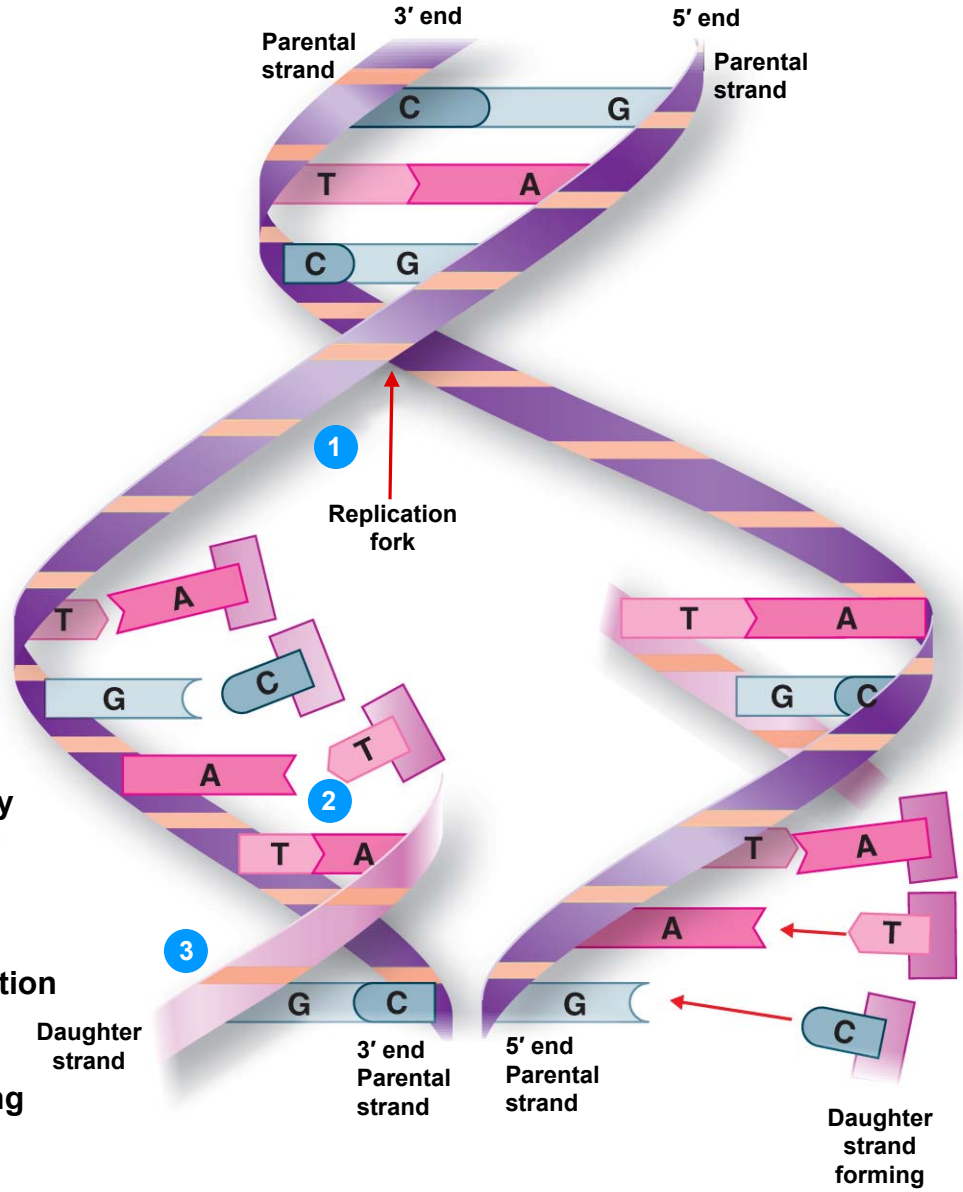
DNA

- A gene is “coded information” (a recipe) for how to string amino acids together in order to form a new protein (structural vs functional)
- The Law of Complementary Base Pairing
- The Law of Semi-Conservative Replication

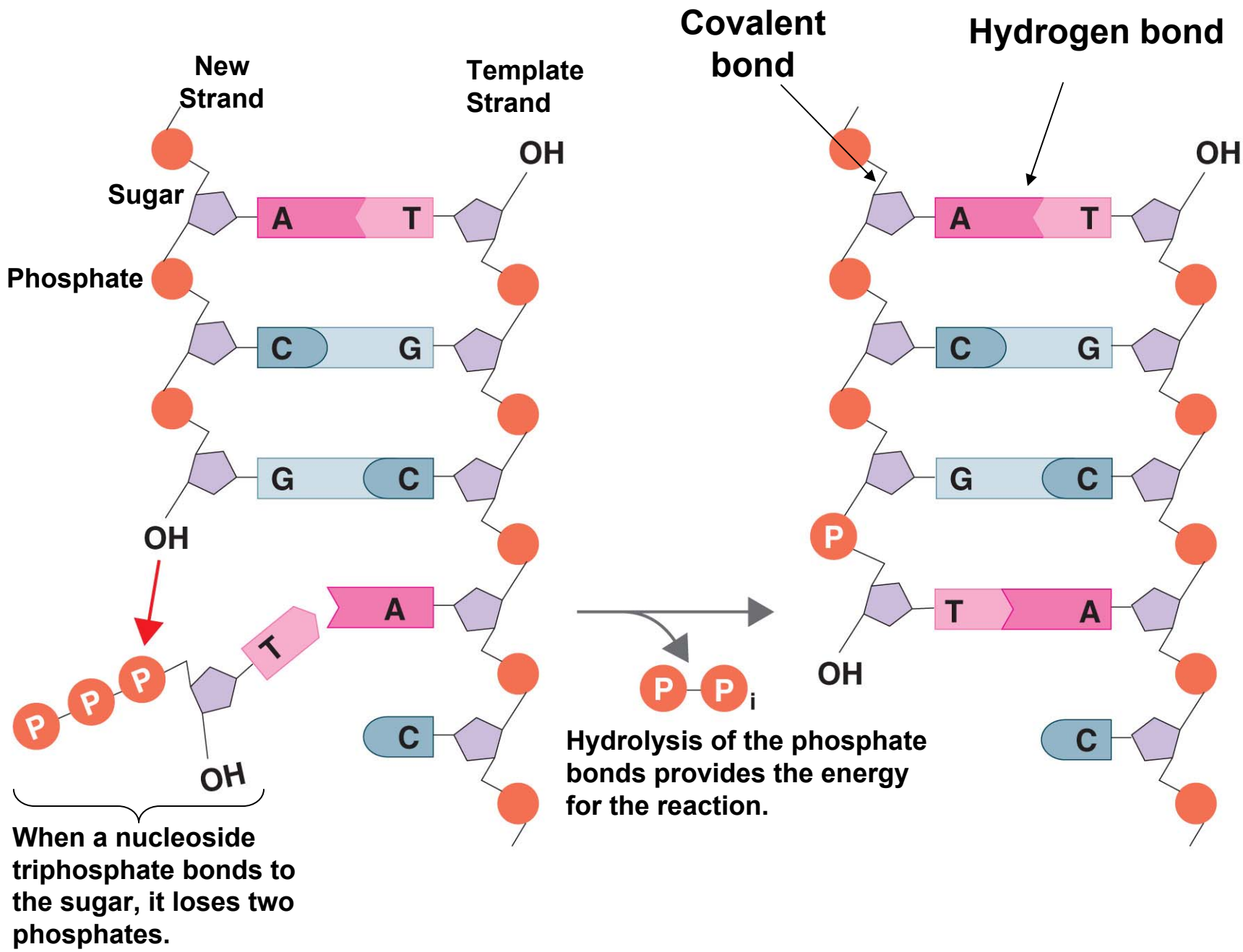
DNA replication.



1. The double helix of the parental DNA separates as weak hydrogen bonds between the nucleotides on opposite strands break in response to the action of replication enzymes.
2. Hydrogen bonds form between new complementary nucleotides and each strand of the parental template to form new base pairs.
3. Enzymes catalyze the formation of sugar-phosphate bonds between sequential nucleotides on each resulting daughter strand.



The replication fork


New Strand
Template Strand
Covalent bond
Hydrogen bond
Sugar
Phosphate
OH
A
T
C
G
G
C
A
T
C
P
P
P
OH
OH
P
P
i
OH
OH
A
T
C
G
G
C
T
A
C
When a nucleoside triphosphate bonds to the sugar, it loses two phosphates.
Hydrolysis of the phosphate bonds provides the energy for the reaction.

DNA Synthesis

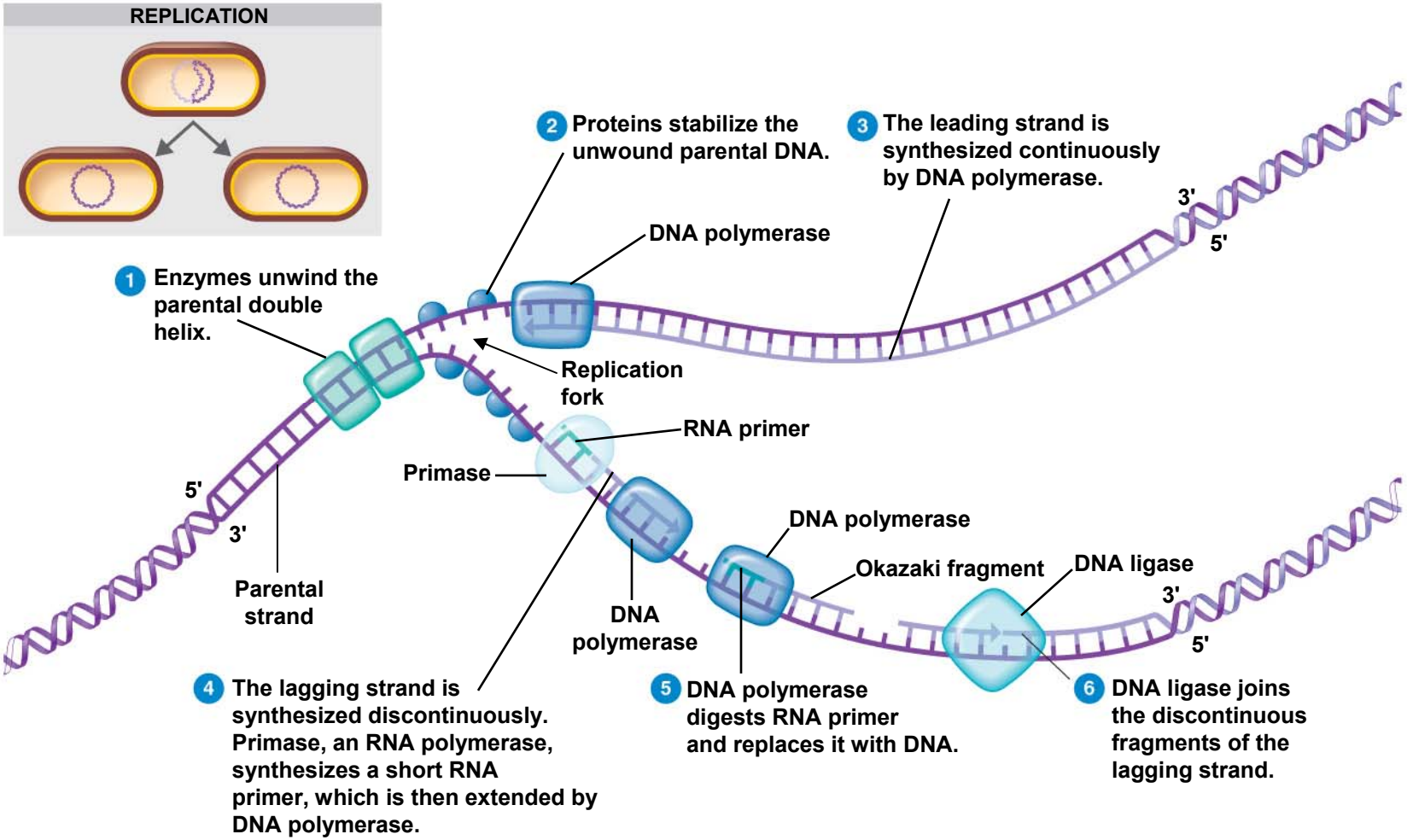
- DNA is copied by DNA polymerase
  - In the 5' $\rightarrow$ 3' direction (polymerase adds nucleotides only to the 3’ end)
  - Initiated by an RNA primer
  - Leading strand is synthesized continuously
  - Lagging strand is synthesized discontinuously // creates Okazaki fragments
  - RNA primers are removed and Okazaki fragments joined by a DNA polymerase and DNA ligase

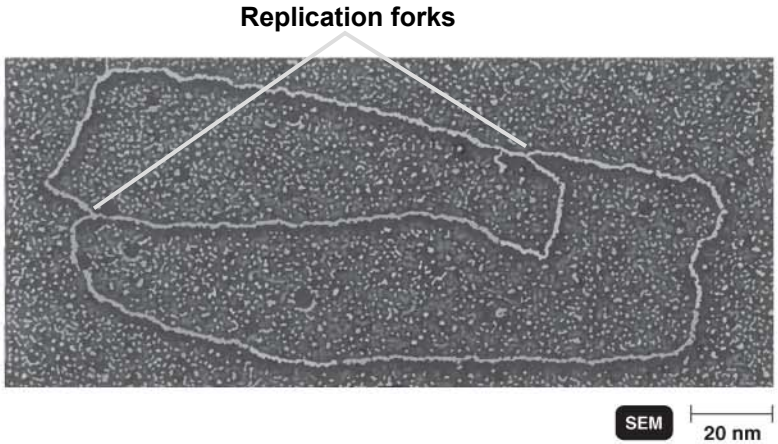
Important Enzymes in DNA Replication, Expression, and Repair

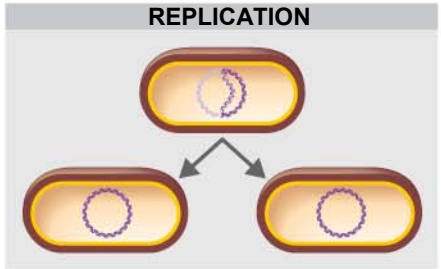
TABLE 8.1 Important Enzymes in DNA Replication, Expression, and Repair

*	DNA Gyrase	Relaxes supercoiling ahead of the replication fork
*	DNA Ligase	Makes covalent bonds to join DNA strands; joins Okazaki fragments and new segments in excision repair
*	DNA Polymerase	Synthesizes DNA; proofreads and repairs DNA
*	Endonucleases	Cut DNA backbone in a strand of DNA; facilitate repair and insertions
	Exonucleases	Cut DNA from an exposed end of DNA; facilitate repair
*	Helicase	Unwinds double-stranded DNA
	Methylase	Adds methyl group to selected bases in newly made DNA
	Photolyase	Uses visible light energy to separate UV-induced pyrimidine dimers
	Ribozyme	RNA enzyme that removes introns and splices exons together
*	RNA Polymerase	Copies RNA from a DNA template
	RNA Primase	An RNA polymerase that makes RNA primers from a DNA template
	snRNP	RNA-protein complex that removes introns and splices exons together
*	Topoisomerase	Relaxes supercoiling ahead of the replication fork; separates DNA circles at the end of DNA replication
	Transposase	Cuts DNA backbone, leaving single-stranded “sticky ends”

A summary of events at the DNA replication fork.

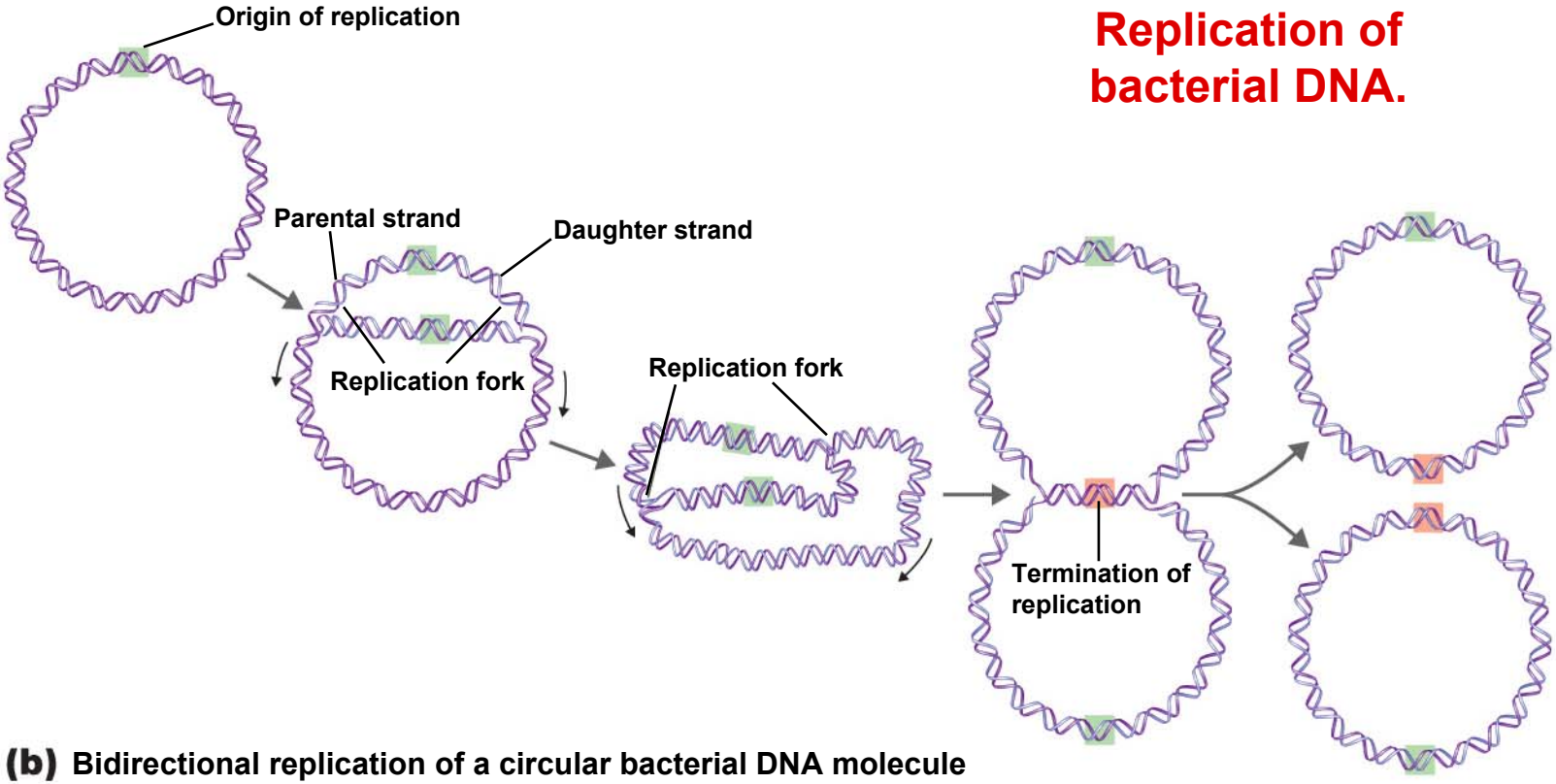






(a) An *E. coli* chromosome in the process of replicating

Replication of bacterial DNA.

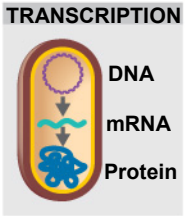


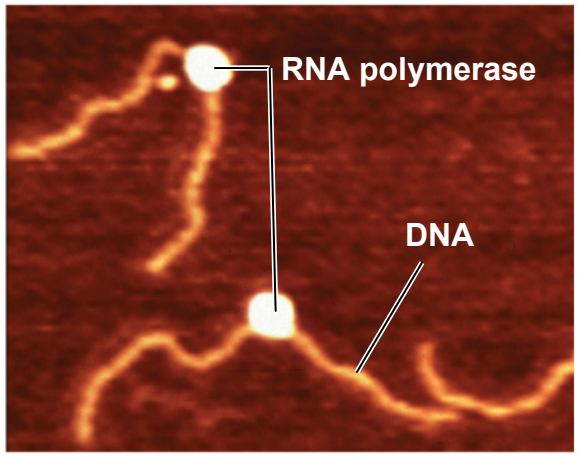
(b) Bidirectional replication of a circular bacterial DNA molecule

Transcription

- DNA is transcribed to make messenger RNA (mRNA)
  - Other important RNAs involved in protein synthesis // transfer RNA, and ribosomal RNA
- Transcription begins when RNA polymerase binds to the **promoter** sequence
- Transcription proceeds in the 5' $\rightarrow$ 3' direction
- Transcription stops when it reaches the **terminator** sequence

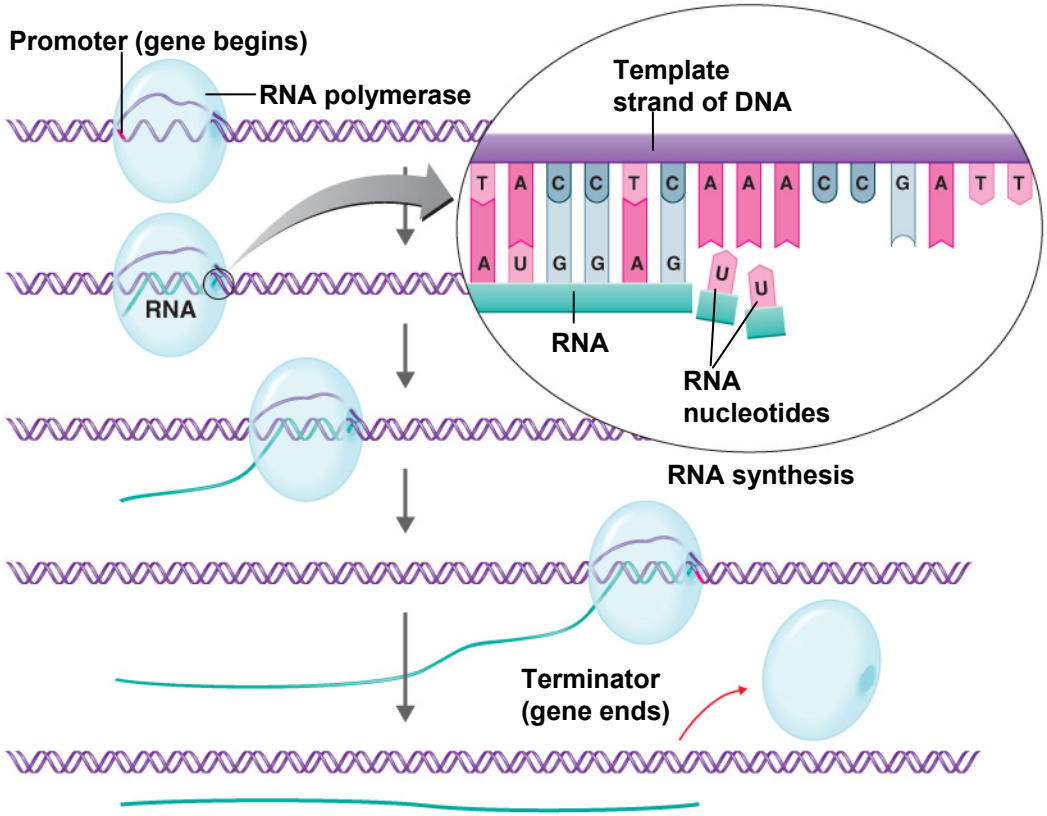
The process of transcription.





RNA polymerase bound to DNA AFM 6 nm

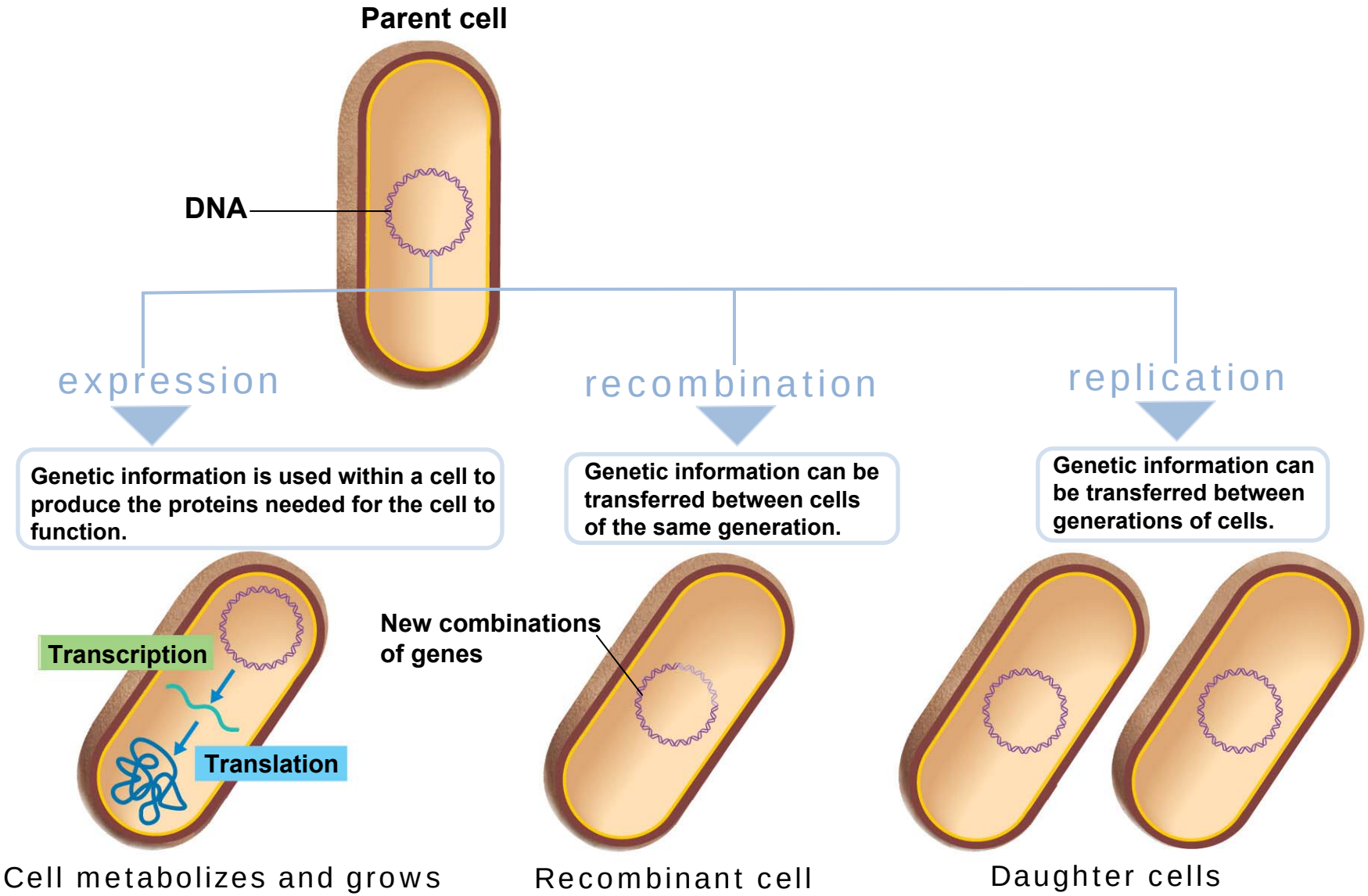
1. RNA polymerase binds to the promoter, and DNA unwinds at the beginning of a gene.
2. RNA is synthesized by complementary base pairing of free nucleotides with the nucleotide bases on the template strand of DNA.
3. The site of synthesis moves along DNA; DNA that has been transcribed rewinds.
4. Transcription reaches the terminator.
5. RNA and RNA polymerase are released, and the DNA helix re-forms.



Translation

- Three nucleotide on DNA called the **base triplet**
- mRNA is translated by “codons” (three nucleotides on mRNA)
- Translation of mRNA begins at the **start codon**: AUG
- Translation ends at **nonsense codons**: UAA, UAG, UGA (stop codons of mRNA)

The Flow of Genetic Information.



The Genetic Code

- Base triplet → codon → anticodon
- Base triplet three nucleotides on chromosome // condon three nucleotides on messenger RNA // anticodon three nucleotides on tRNA
- 64 **sense codons** on mRNA encode the 20 amino acids
- The genetic code is **degenerate**
  - several different codons mapping the same a.a. // also – the third nucleotide “wobbles” and often has little influence on determining the a.a.

The genetic code.

First position	Second position: U	C	A	G	Third position
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met/start	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

64 codons (only 20 a.a.)

61 = sense codons / code for a.a.

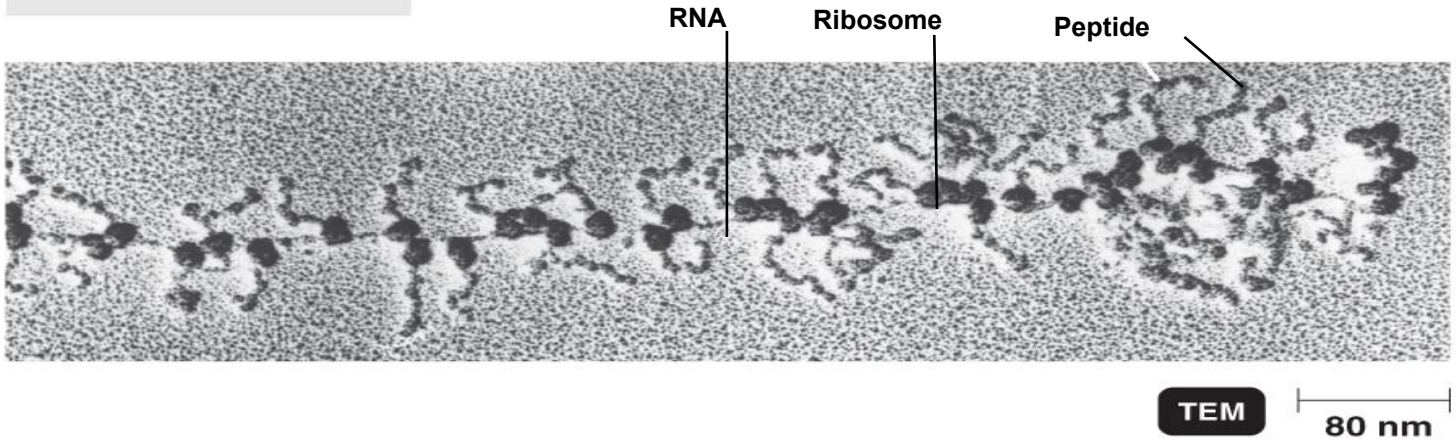
3 = non-sense / these are stop codons

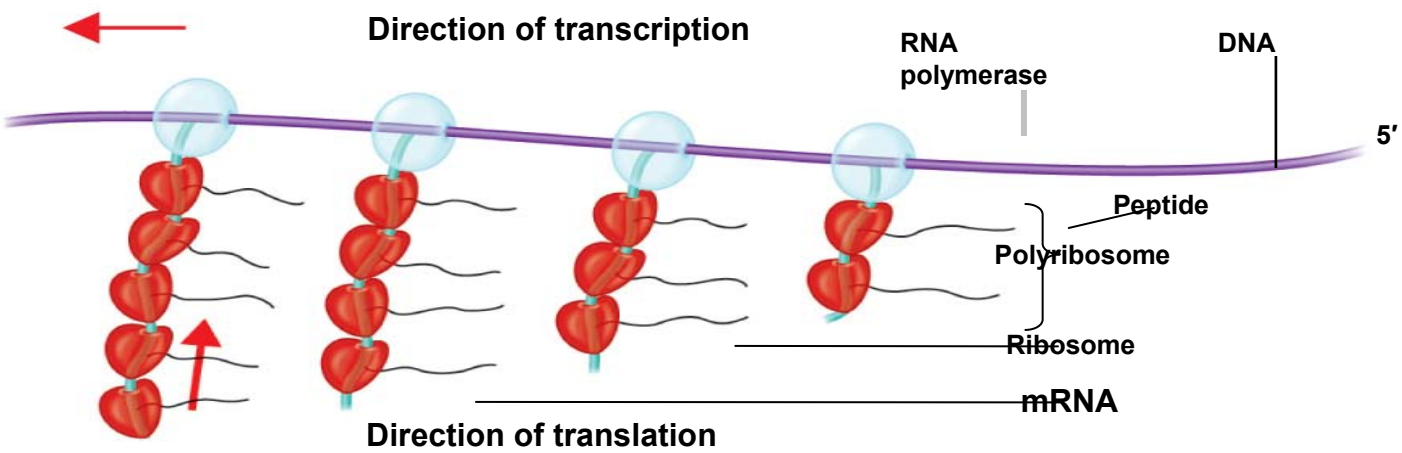
1 = start codon / AUG for methionine

Note: degeneracy – most a.a. are coded for by several different codons

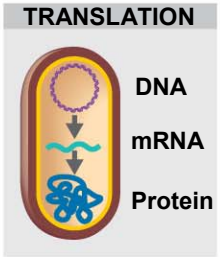
therefore a point mutation may not actually change the a.a. and not effect the protein

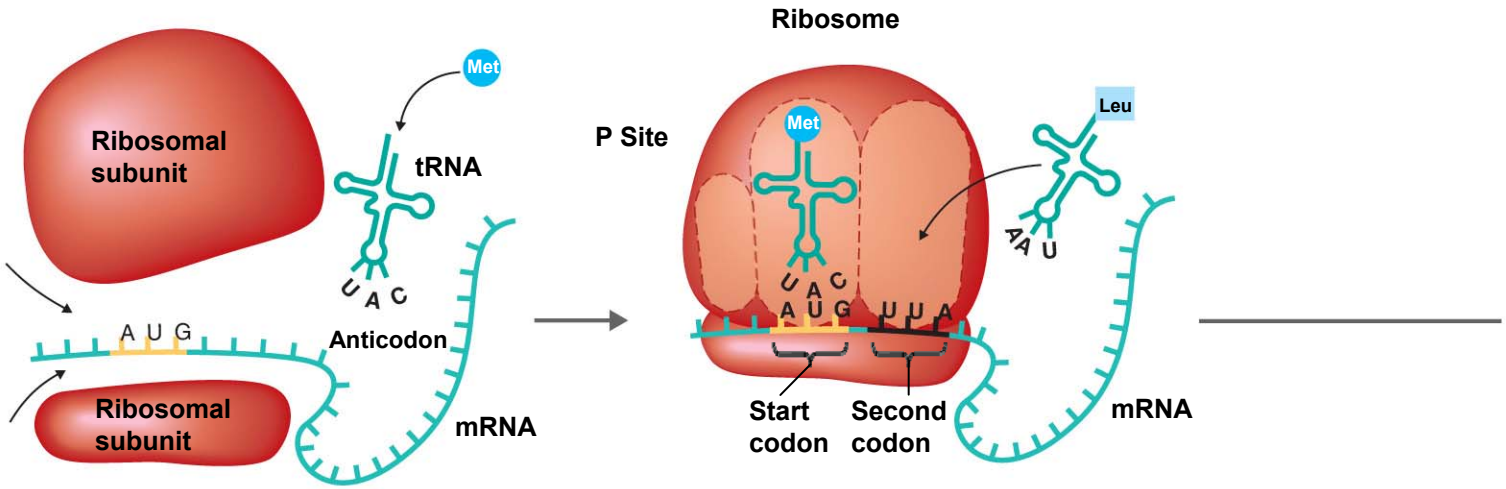
Simultaneous transcription and translation in bacteria.





The process of translation.

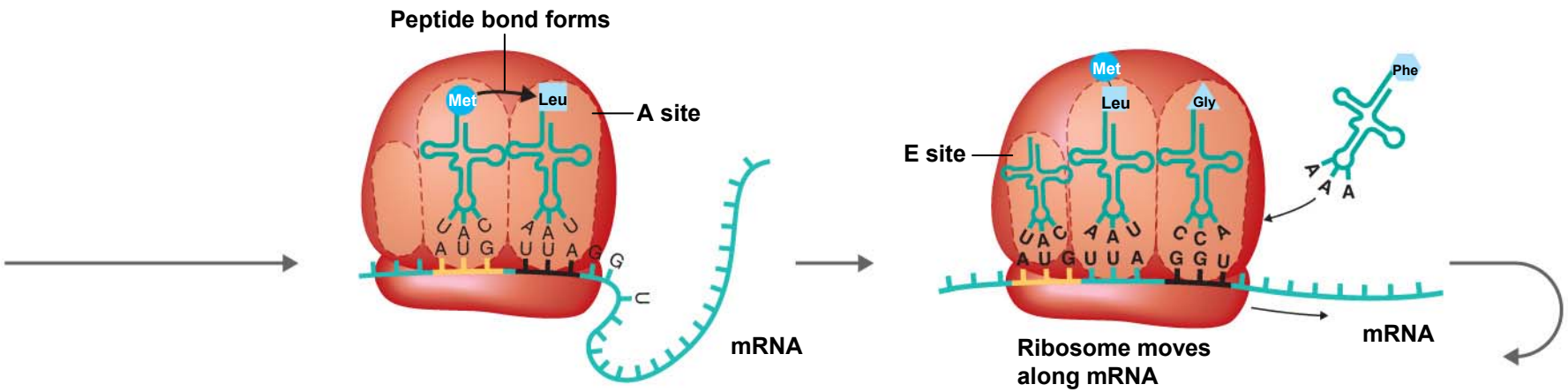




1 Components needed to begin translation come together.

2 On the assembled ribosome, a tRNA carrying the first amino acid is paired with the start codon on the mRNA. The place where this first tRNA sits is called the P site. A tRNA carrying the second amino acid approaches.

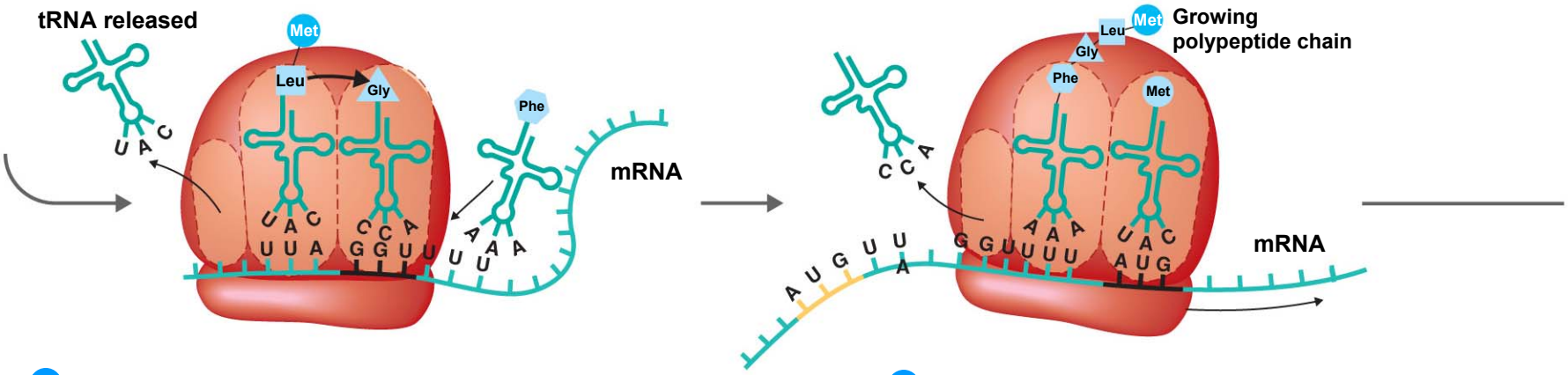
The process of translation.



3 The second codon of the mRNA pairs with a tRNA carrying the second amino acid at the A site. The first amino acid joins to the second by a peptide bond. This attaches the polypeptide to the tRNA in the P site.

4 The ribosome moves along the mRNA until the second tRNA is in the P site. The next codon to be translated is brought into the A site. The first tRNA now occupies the E site.

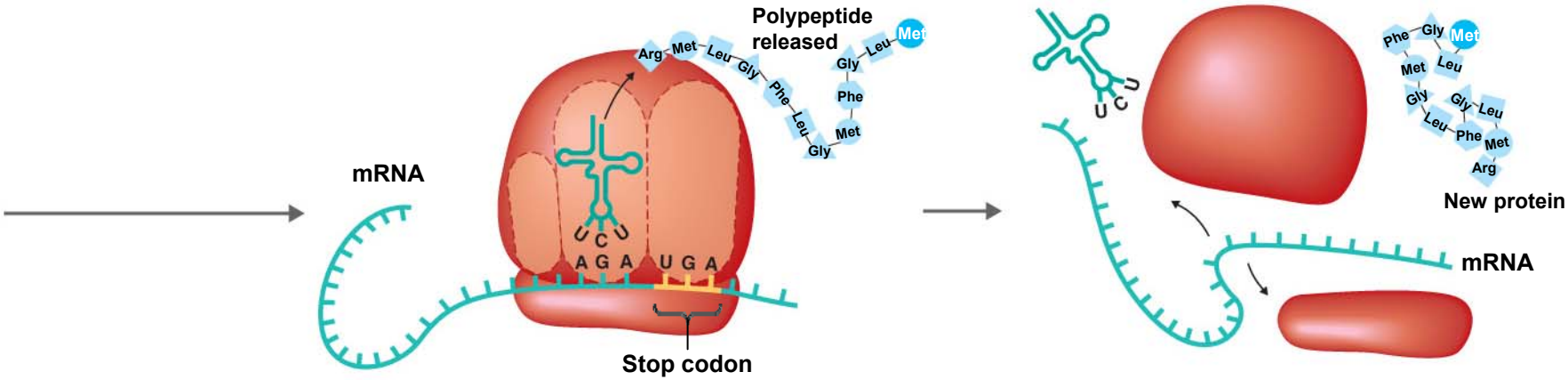
The process of translation.



5 The second amino acid joins to the third by another peptide bond, and the first tRNA is released from the E site.

6 The ribosome continues to move along the mRNA, and new amino acids are added to the polypeptide.

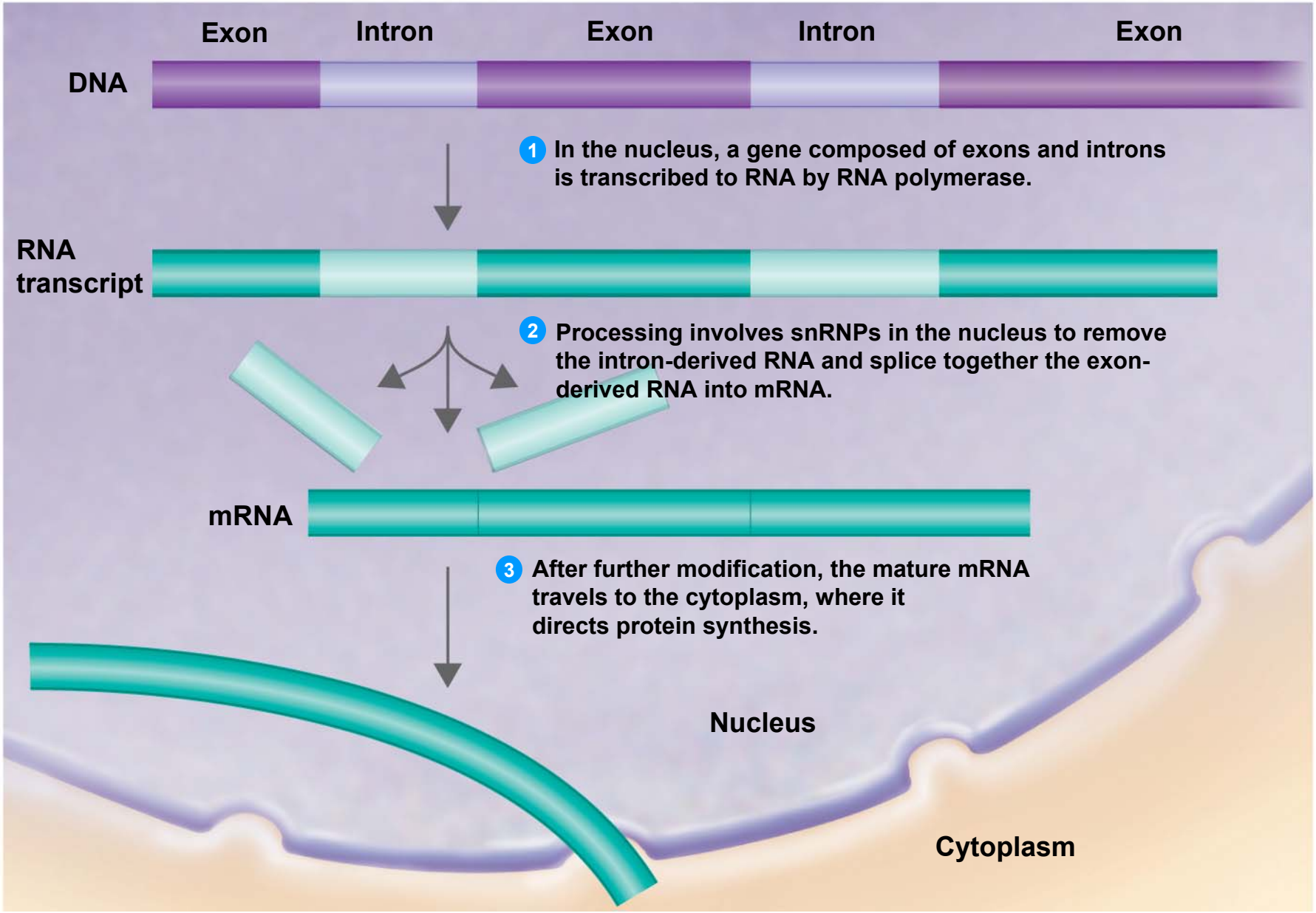
The process of translation.



7 When the ribosome reaches a stop codon, the polypeptide is released.

8 Finally, the last tRNA is released, and the ribosome comes apart. The released polypeptide forms a new protein.

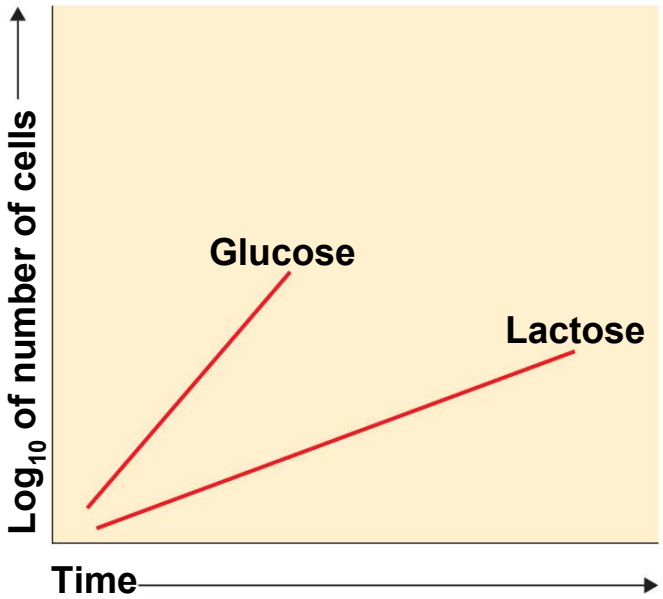
RNA processing in eukaryotic cells. // Different Than Prokaryotes



Regulation

- **Constitutive genes** are expressed at a fixed rate (e.g. glycolysis enzymes)
- Other genes are expressed only as needed
  - **Inducible** genes / default position is <u>turned off</u> **//** **the** enzyme is produced in response to the presence of a substrate
  - **Repressible** genes / default position is <u>turned on</u> // the enzyme production is stopped by build up off end product

The growth rate of *E. coli* on glucose and lactose.

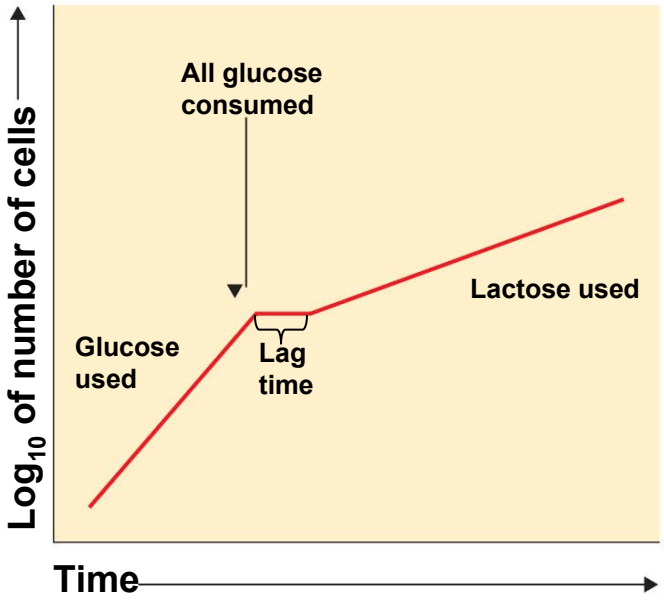


(a) Bacteria growing on glucose as the sole carbon source

grows faster than on glucose

However, if no glucose is available microbe can use lactose as carbon source

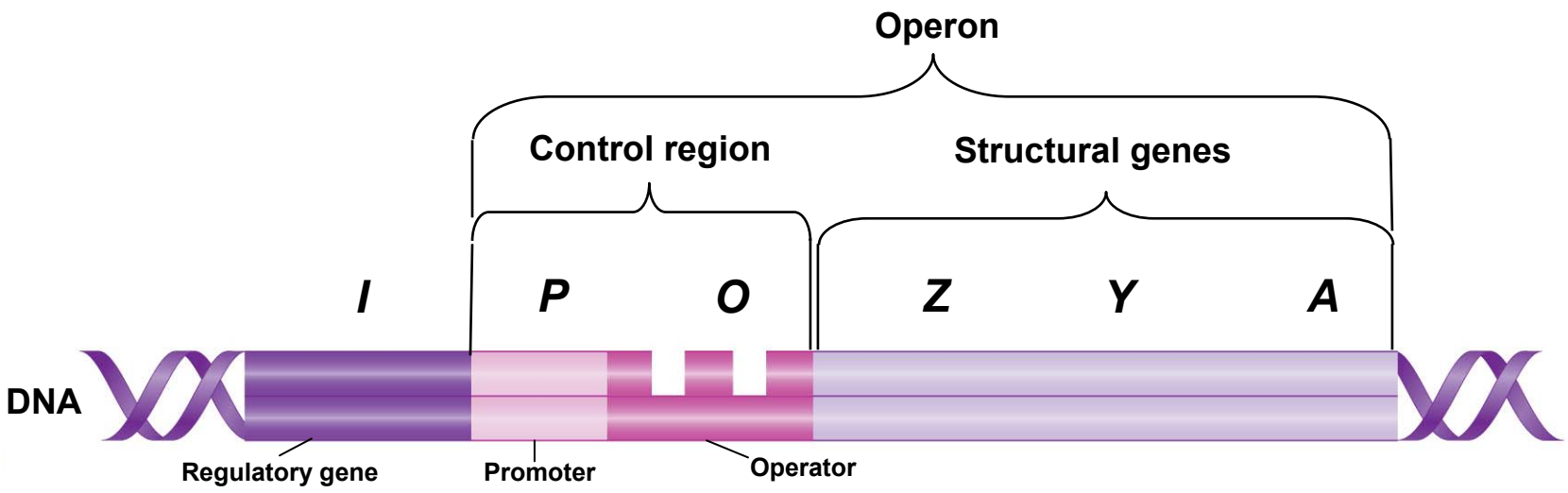
Microbe will need to "make new enzymes to metabolize lactose



(b) Bacteria growing in a medium containing glucose and lactose first consume the glucose and then, after a short lag time, the lactose.

During the lag time, intracellular cAMP increases, the *lac* operon is transcribed, more lactose is transported into the cell, and β-galactosidase is synthesized to break down lactose.

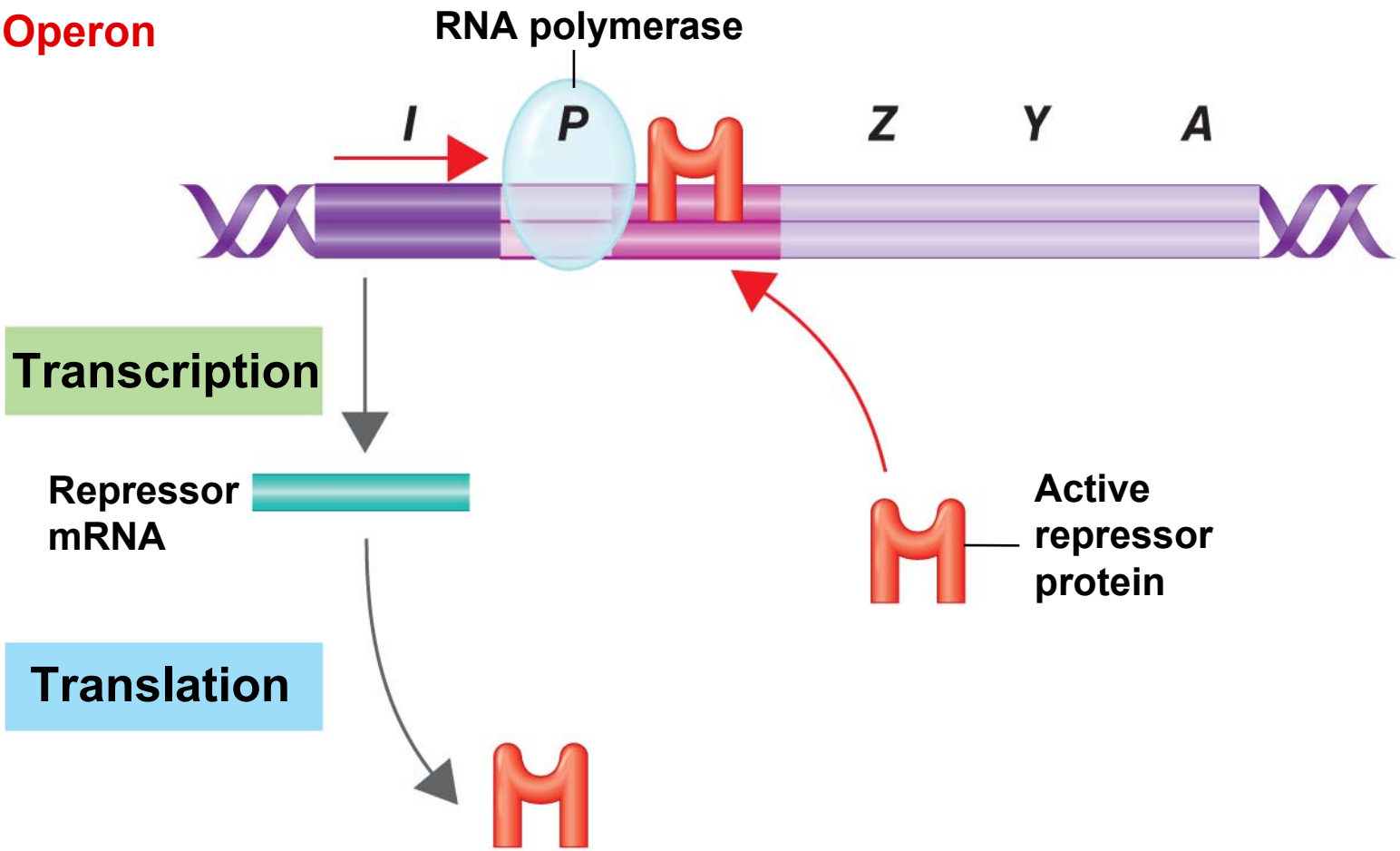
An inducible operon (e.g. lactose operon)



1 Structure of the operon. The operon consists of the promoter (*P*) and operator (*O*) sites and structural genes that code for the protein.

The operon is regulated by the product of the regulatory gene (*I*).

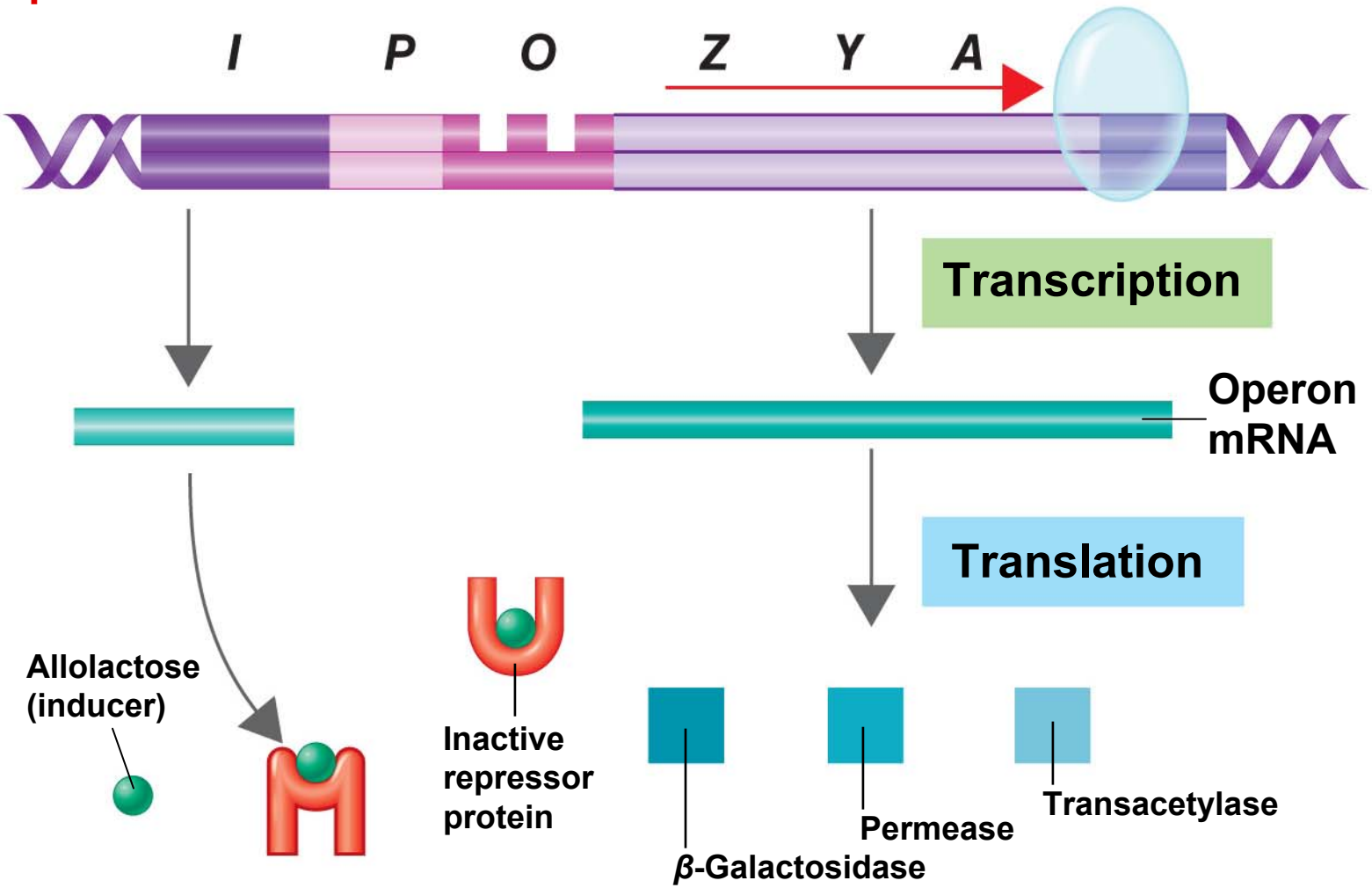
Inducible Operon



2 Repressor active, operon off. The repressor protein binds with the operator, preventing transcription from the operon.

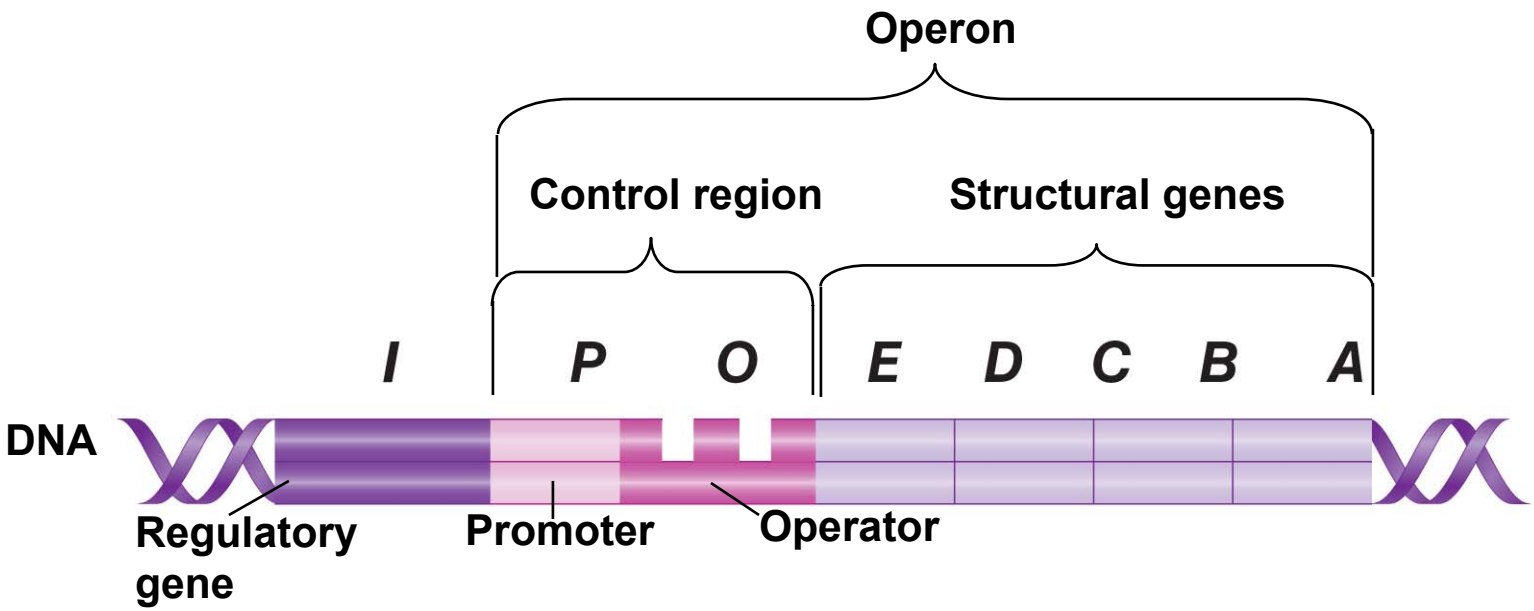
Note: inducible operon default setting is “off” (active repressor protein is being made by “I” – the regulatory gene)

Inducible Operon



3 Repressor inactive, operon on. When the inducer allolactose binds to the repressor protein, the inactivated repressor can no longer block transcription. The structural genes are transcribed, ultimately resulting in the production of the enzymes needed for lactose catabolism.

Repressible Operon.

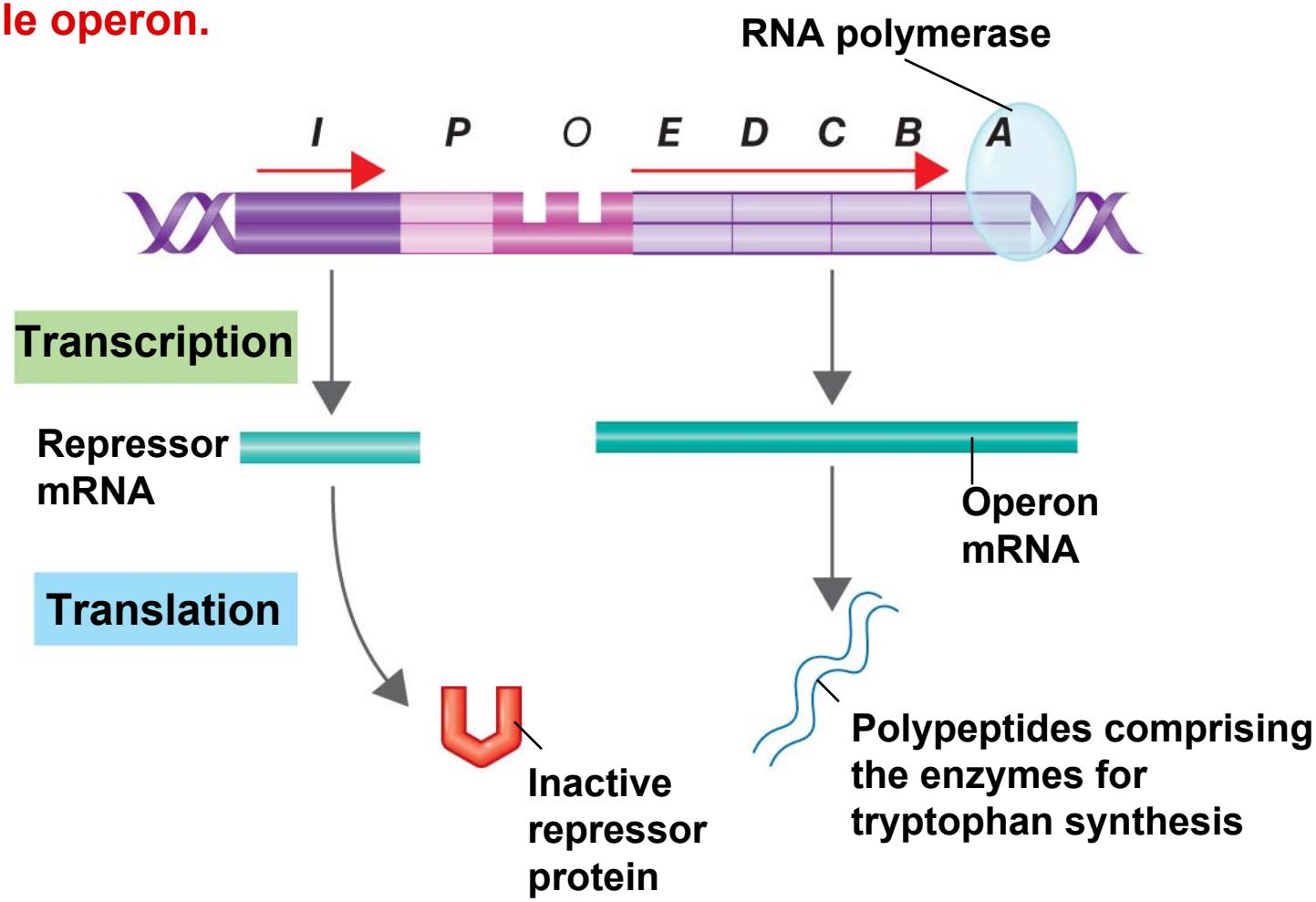


1 Structure of the operon. **The operon consists of the promoter (*P*) and operator (*O*) sites and structural genes that code for the protein.**

The operon is regulated by the product of the regulatory gene (*I*).

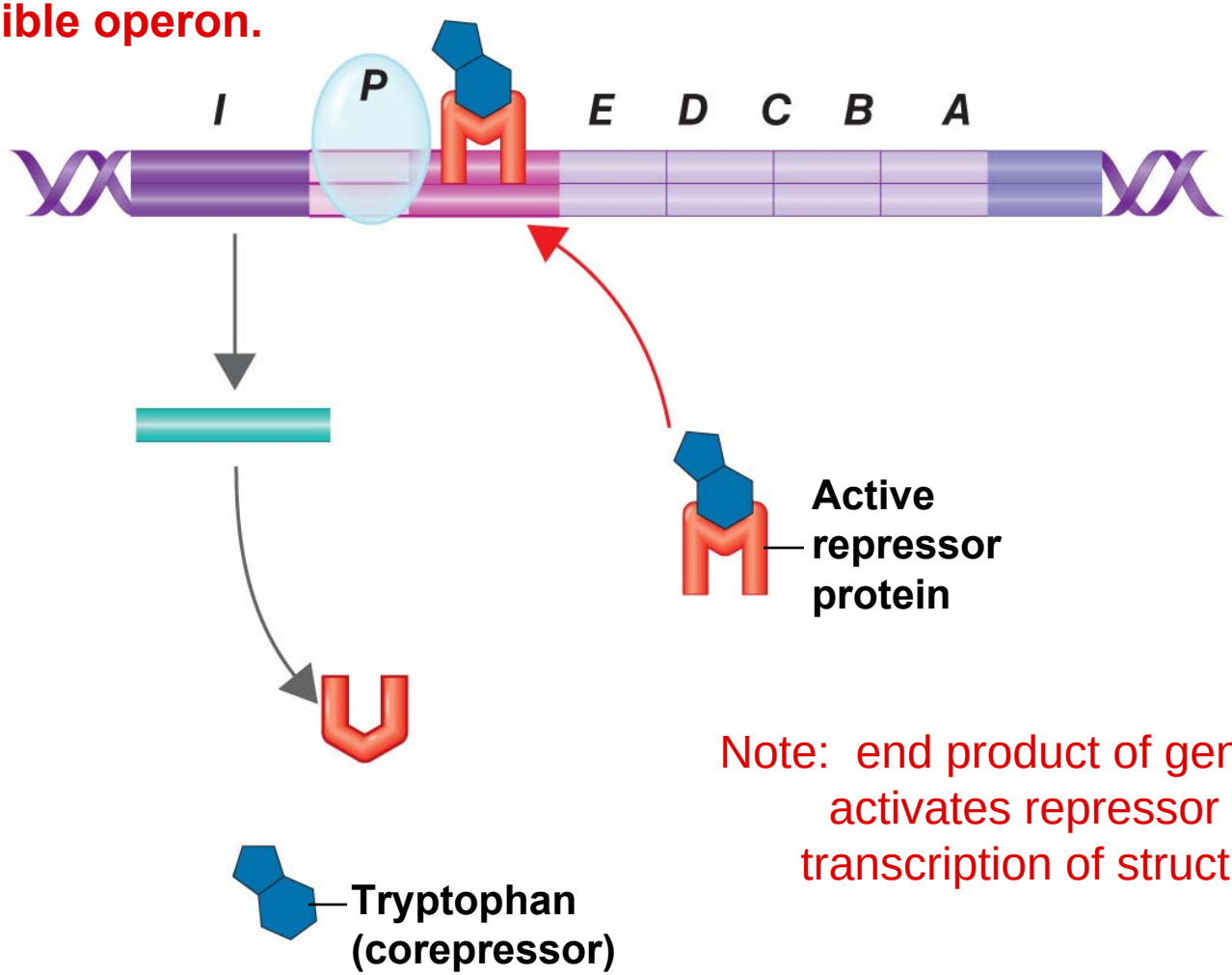
Note: Repressible operon default setting is “on” // structural genes are made

A repressible operon.



2 Repressor inactive, operon on. The repressor is inactive, and transcription and translation proceed, leading to the synthesis of tryptophan.

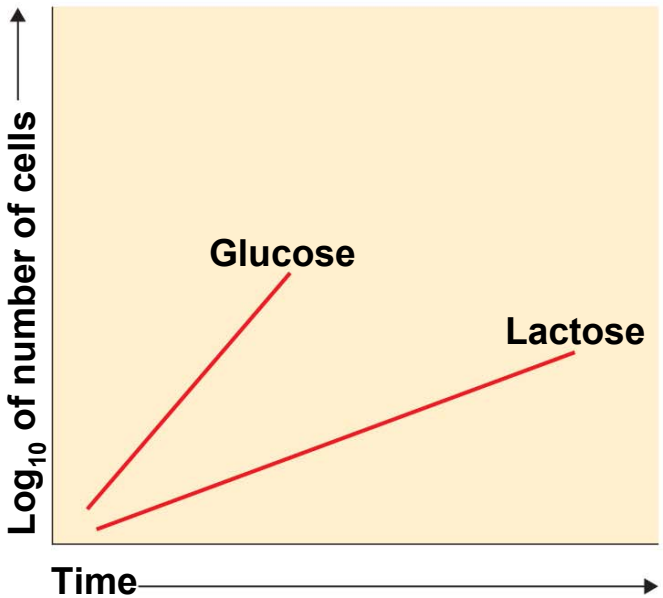
A repressible operon.



Note: end product of gene (trytophan) activates repressor // stops transcription of structural gene

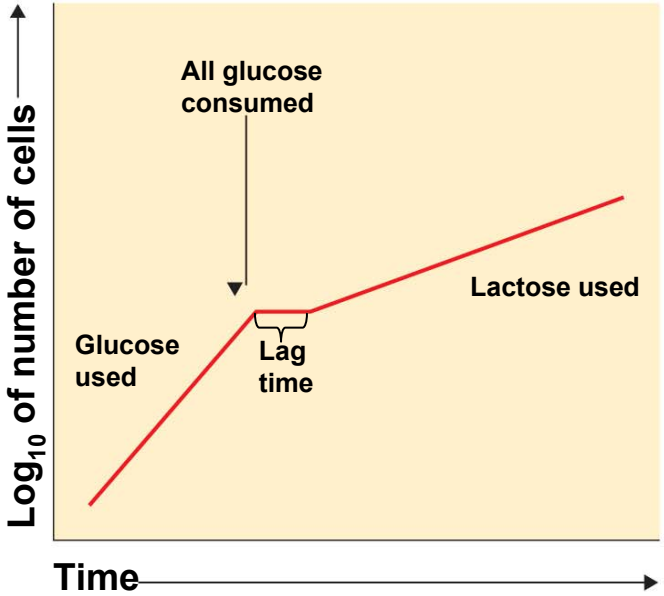
3 Repressor active, operon off. **When the corepressor tryptophan binds to the repressor protein, the activated repressor binds with the operator, preventing transcription from the operon.**

The growth rate of *E. coli* on glucose and lactose.



(a) Bacteria growing on glucose as the sole carbon source

grow faster than on lactose.



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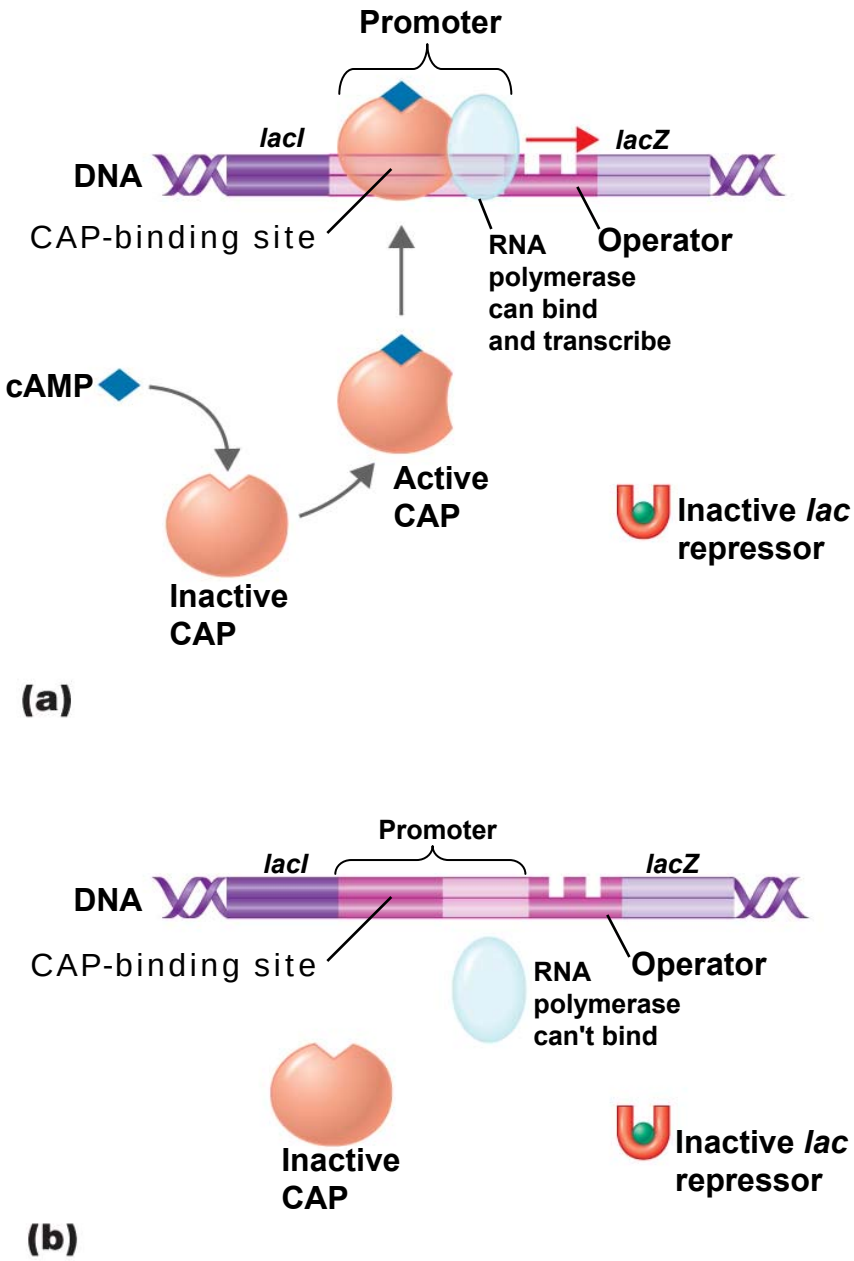
Positive regulation of the *lac* operon.



When lactose present, but glucose scarce (cAMP level is high)

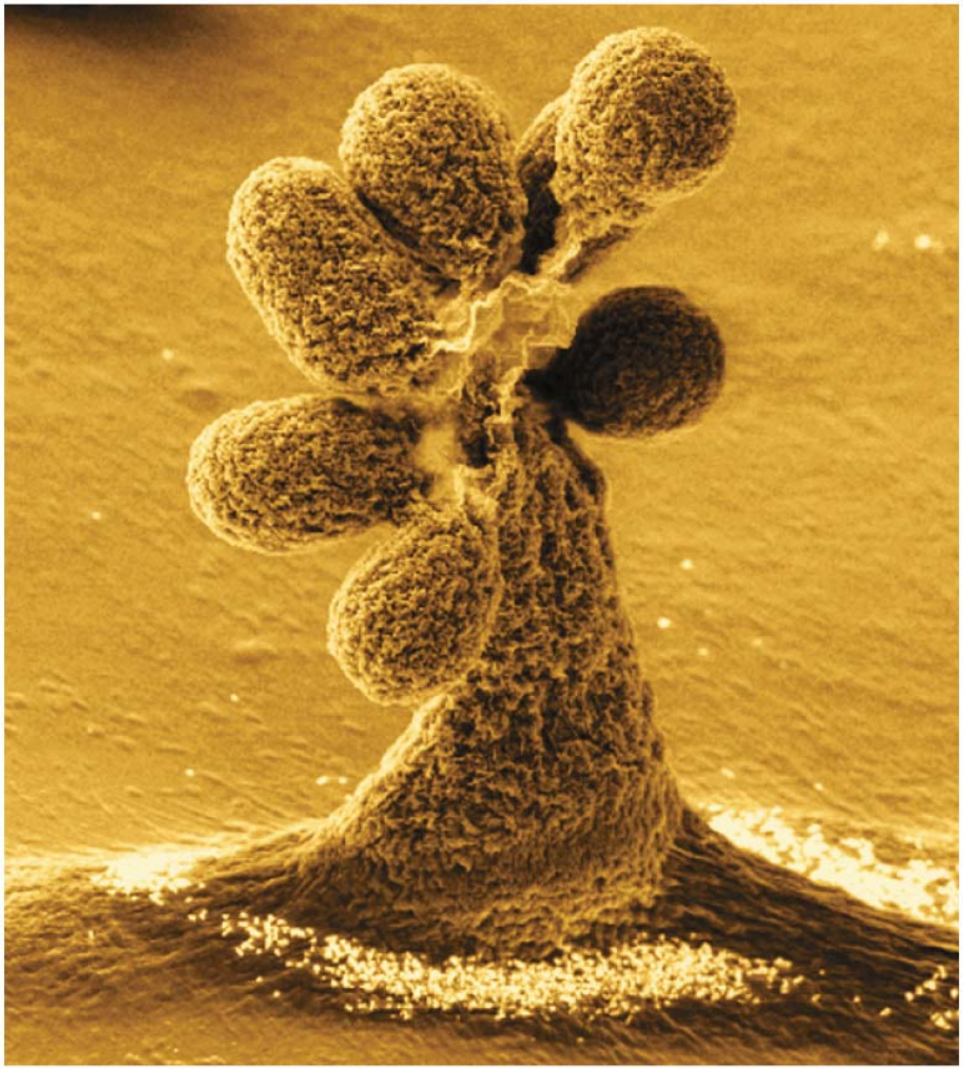
If glucose is scarce, the high level of cAMP activates CAP (catabolic active protein), and the *lac* operon produces large amounts of mRNA for lactose digestion.

Lactose present, glucose present (cAMP level low).
When glucose is present, cAMP is scarce, and CAP is unable to stimulate transcription.

Epigenetic Control

- Methylating nucleotides affects transcription of DNA
- Methylated genes are able to be passed to offspring cells (vertical transfer)
- However not permanent / unlike a mutation in genetic code
- Believed to influence biofilm behavior

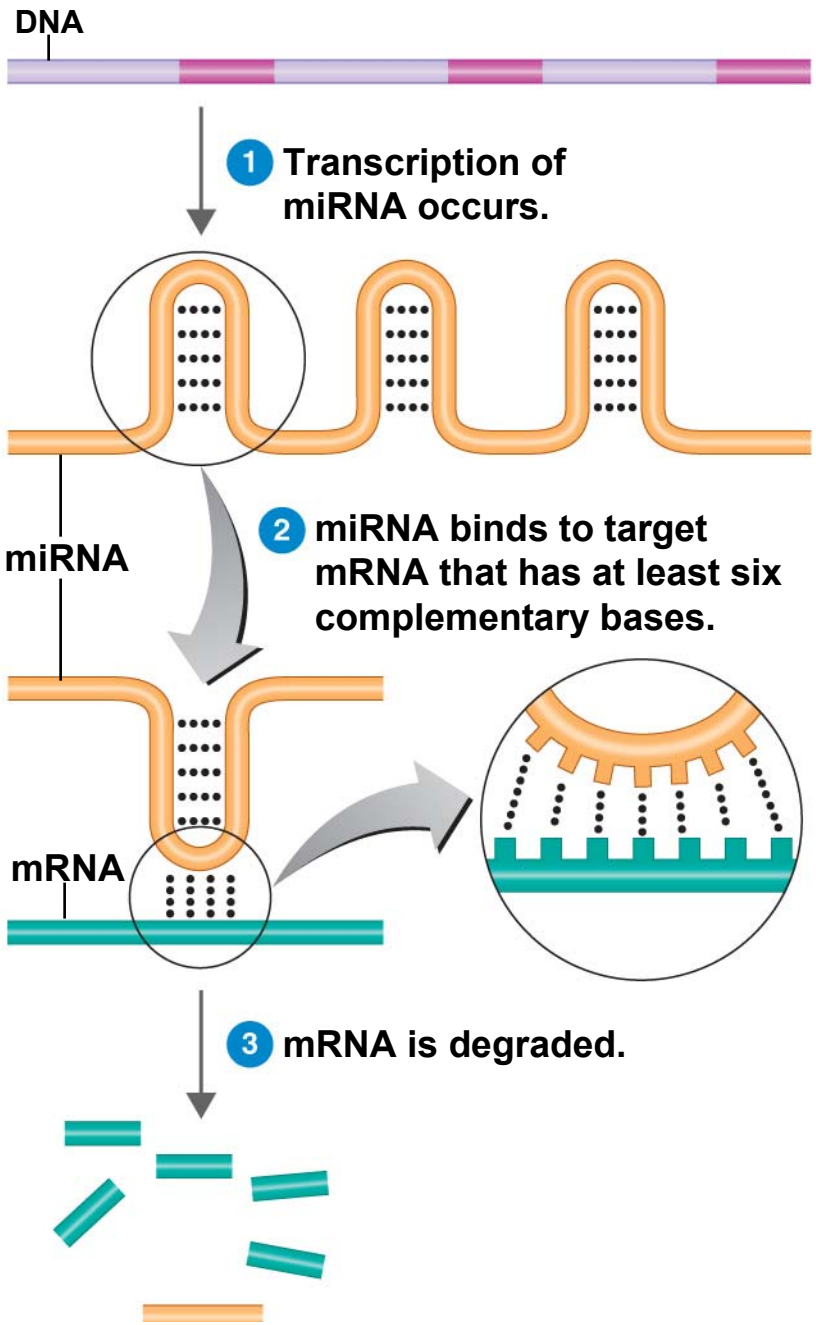
Paenibacillus.



10μm

SEM

A fruiting body of a myxobacterium.



MicroRNAs control a wide range of activities in cells.

Stop protein synthesis after transcription

Post-transcriptional control

Inhibit protein synthesis in eukaryotic cells

Allows different cell types in multicellular organisms to produce different type of proteins from similar genetic code. (explains how heart tissue and skin tissue make different proteins)

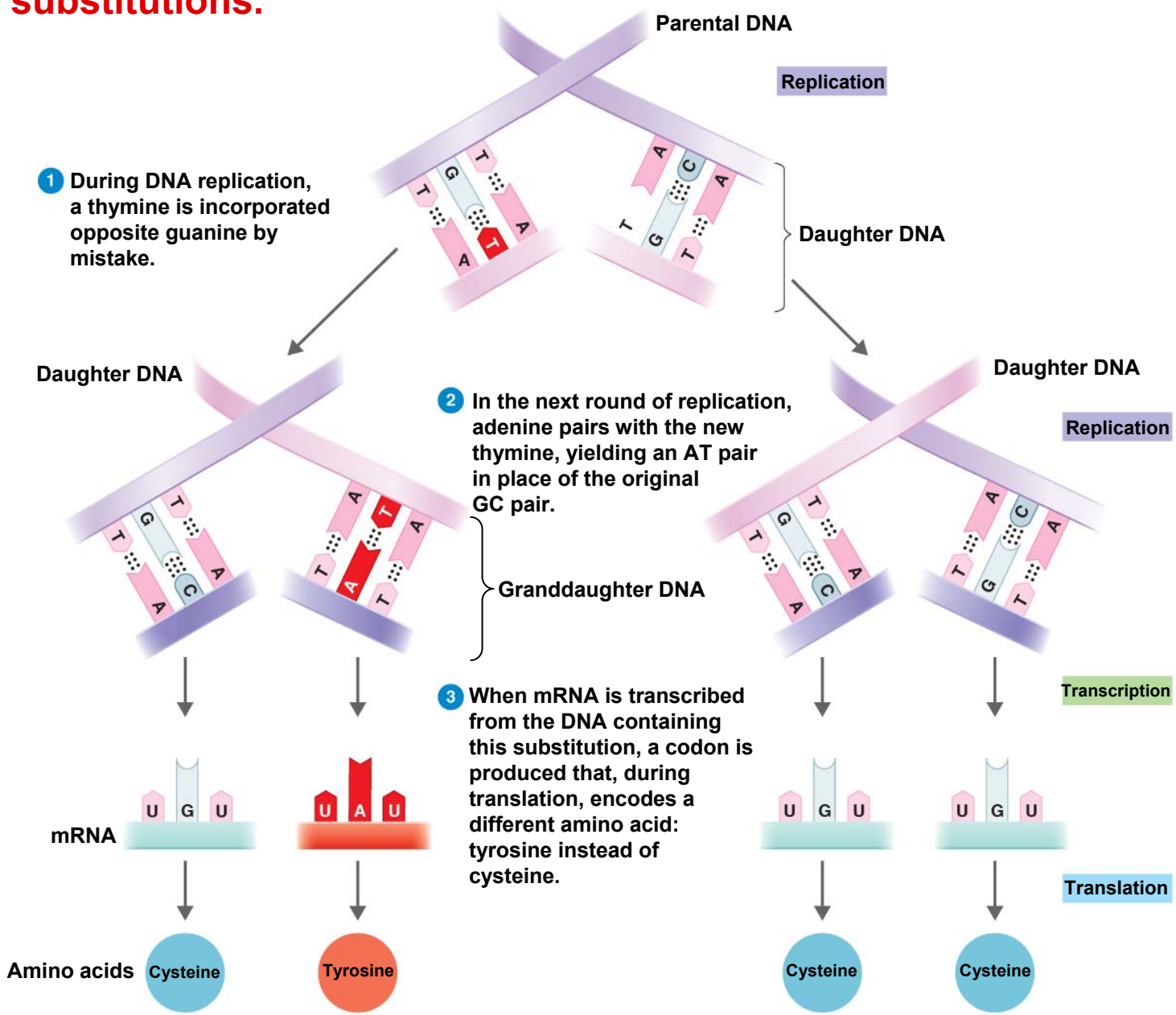
Mutation / Several Different Types

- A change in the genetic material
- Mutations may be *neutral, beneficial, or harmful*
- **Mutagen:** agent that causes mutations
- **Spontaneous mutations:** occur in the absence of a mutagen

Mutation #1

- **Base substitution** (point mutation)
  // Change in one base

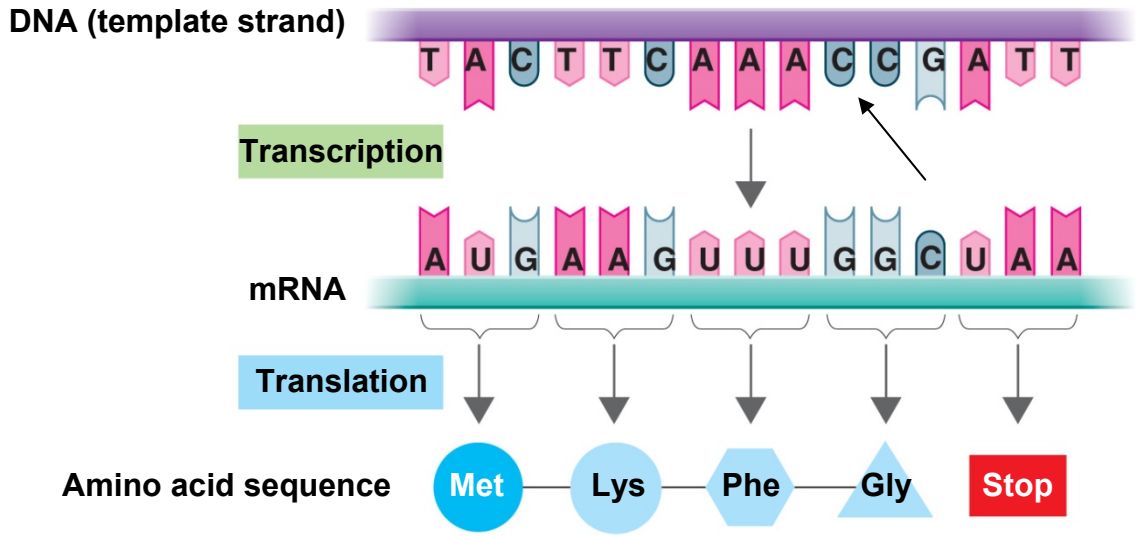
Base substitutions.



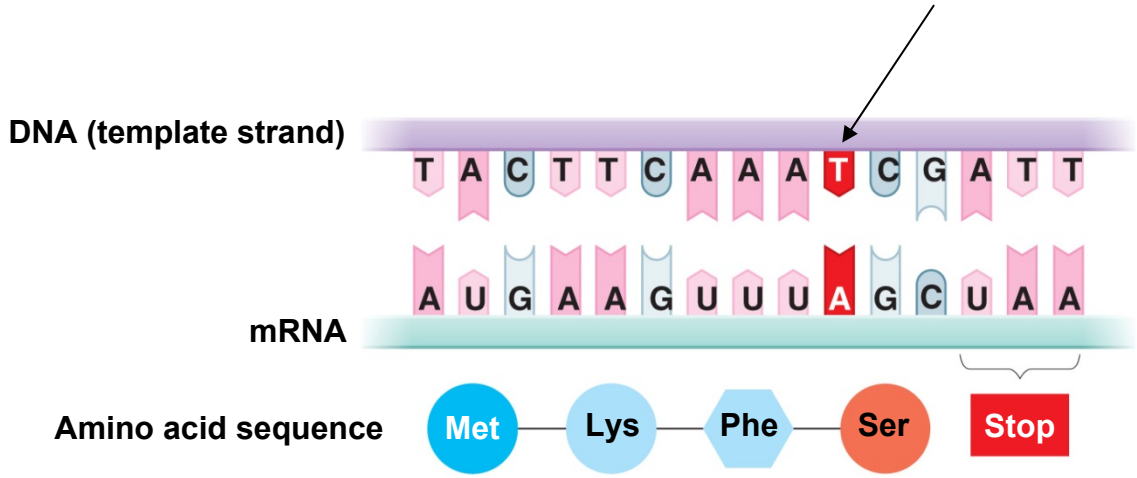
Mutation #2

- **Missense mutation** // Base substitution results in change in amino acid

Types of mutations and their effects on the amino acid sequences of proteins.



(a) Normal DNA molecule



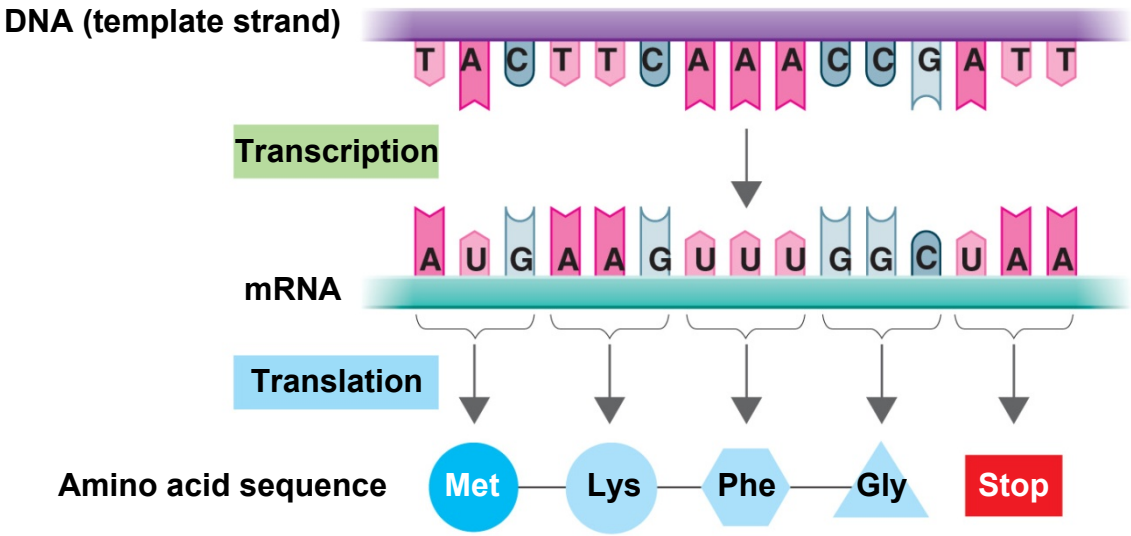
(b) Missense mutation

Mutation #3

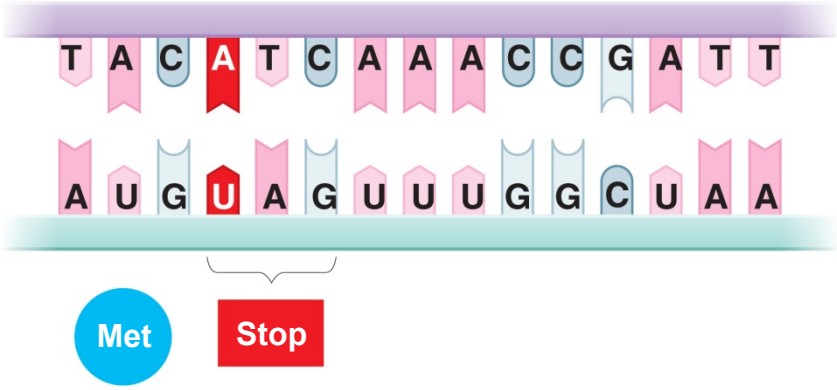
- **Nonsense mutation //** Base substitution results in a nonsense codon

- E.g. // Tells the transcription process to “stop” in the middle of the gene // results in a non-functional protein

Types of mutations and their effects on the amino acid sequences of proteins.



(a) Normal DNA molecule

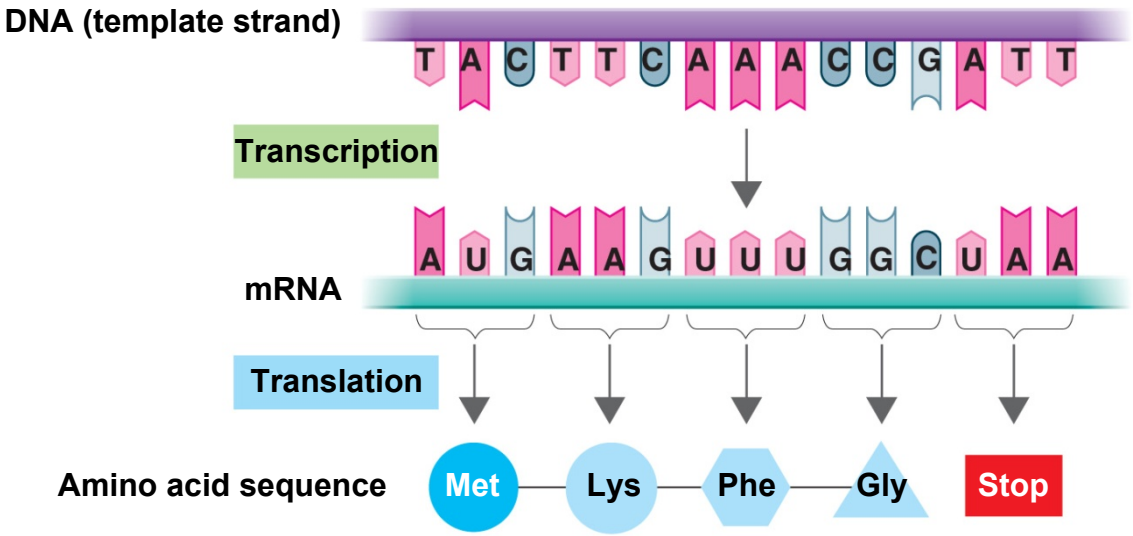


(c) Nonsense mutation

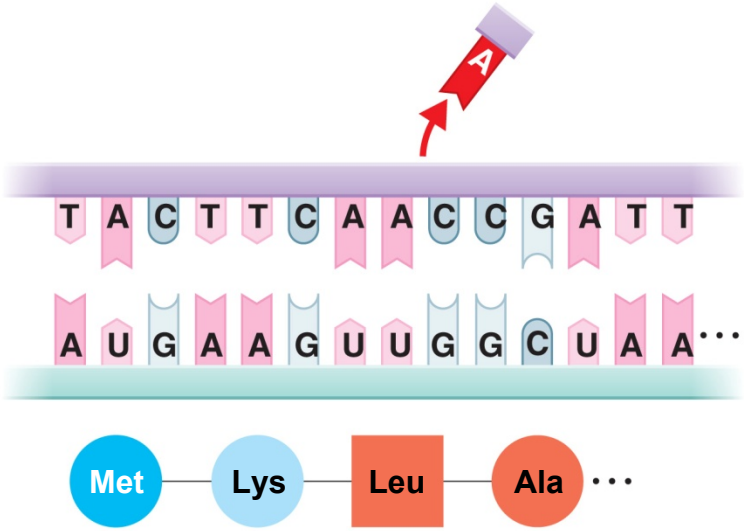
Mutation #4

- **Frameshift mutation //** Insertion or deletion of one or more nucleotide pairs
  - This type of mutation will cause the most damage to the genetic code.
  - Note: If “shift” is three or multiple of three nucleotides then resulting “damage” less

Types of mutations and their effects on the amino acid sequences of proteins.



(a) Normal DNA molecule



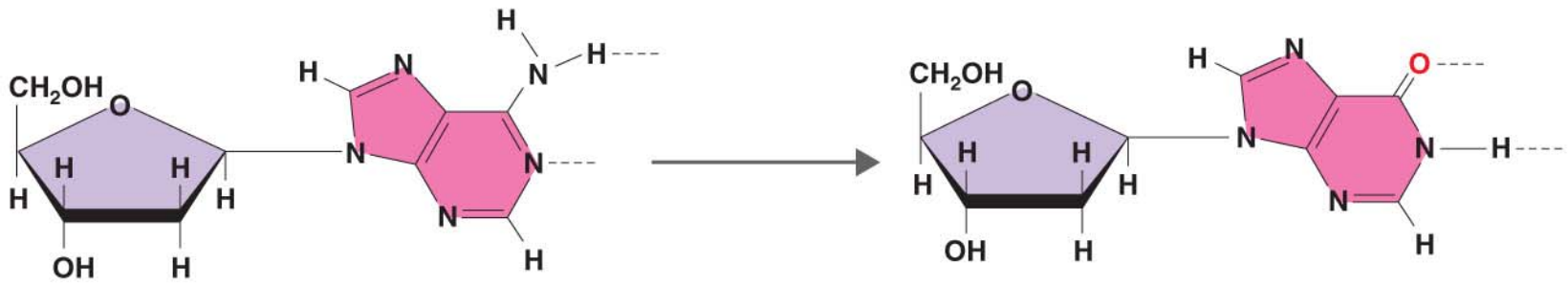
(d) Frameshift mutation

The Frequency of Mutation

- **Spontaneous mutation** rate = 1 in 10^9 replicated base pairs or 1 in 10^6 replicated genes

- **Mutagens** // something that increase the incidence of errors // increase to 10^{-5} or 10^{-3} per replicated gene

Oxidation of nucleotides makes a mutagen.



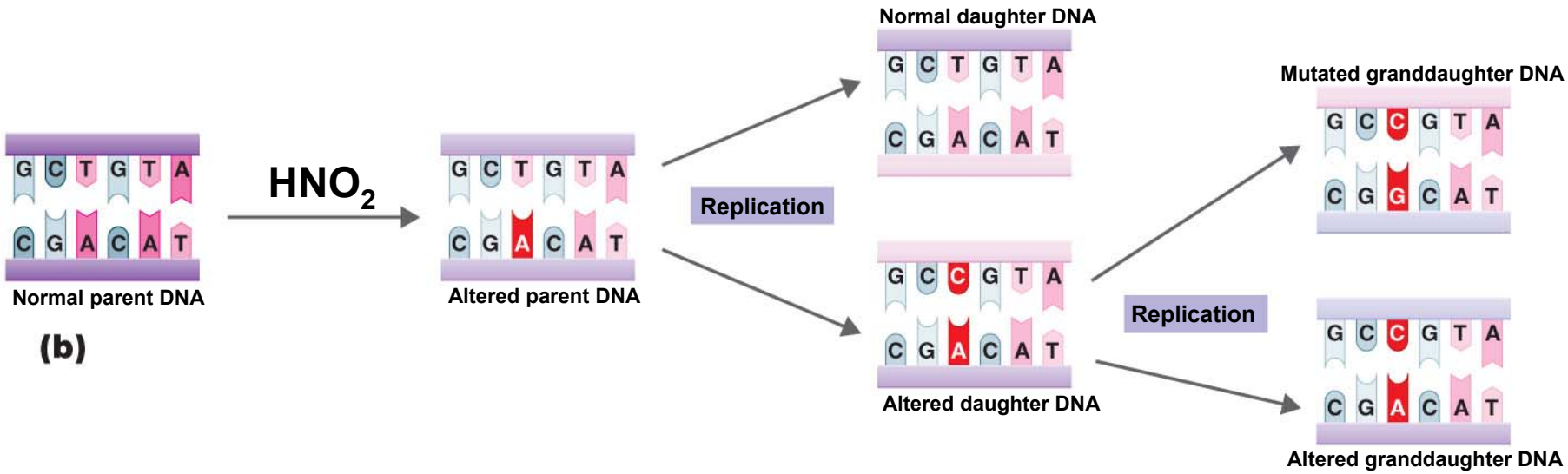
(a)

Adenosine nucleoside normally base-pairs by hydrogen bonds with an oxygen and a hydrogen of a thymine or uracil nucleotide.

Altered adenine will hydrogen bond with a hydrogen and a nitrogen of a cytosine nucleotide.

Oxidation of nucleotides makes a mutagen.

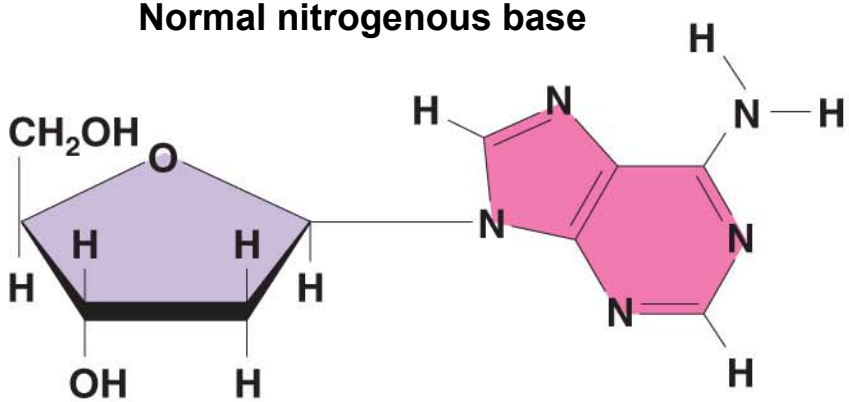
Nitrous acid / convert base adenine to form that no longer pairs with thymine



The altered adenine pairs with cytosine instead of thymine.

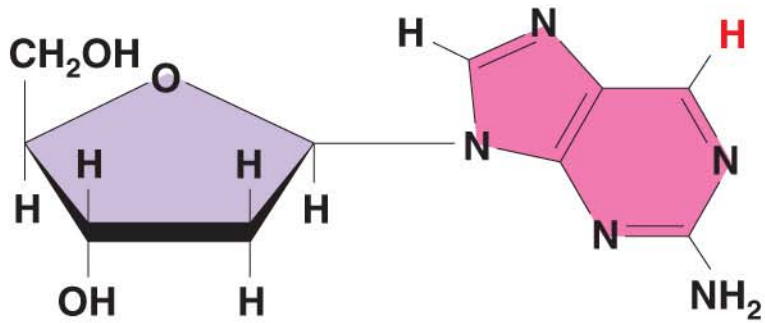
Nucleoside analogs and the nitrogenous bases they replace.

Normal nitrogenous base



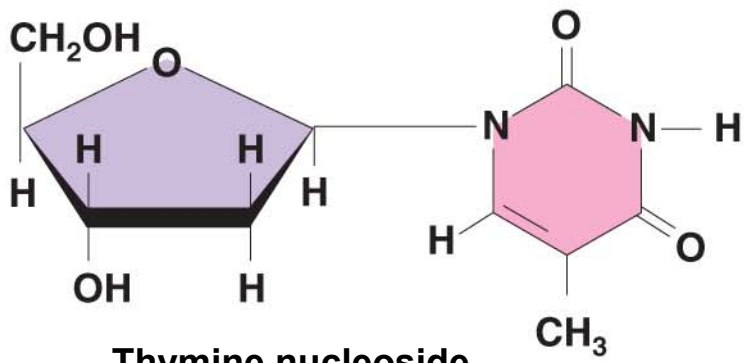
Adenine nucleoside

Analog

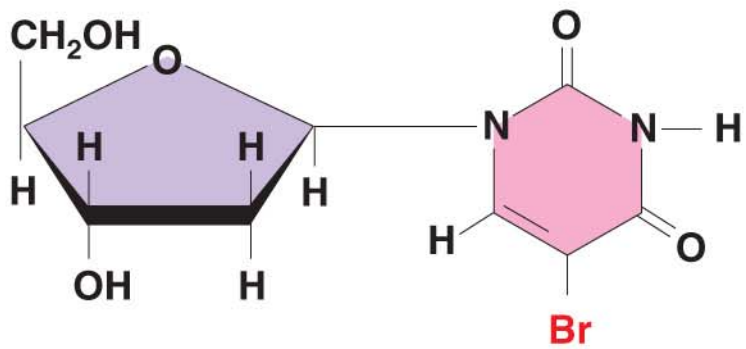


2-Aminopurine nucleoside

(a) The 2-aminopurine is incorporated into DNA in place of adenine but can pair with cytosine, so an AT pair becomes a CG pair.



Thymine nucleoside



5-Bromouracil nucleoside

(b) The 5-bromouracil is used as an anticancer drug because it is mistaken for thymine by cellular enzymes but pairs with cytosine. In the next DNA replication, an AT pair becomes a GC pair.

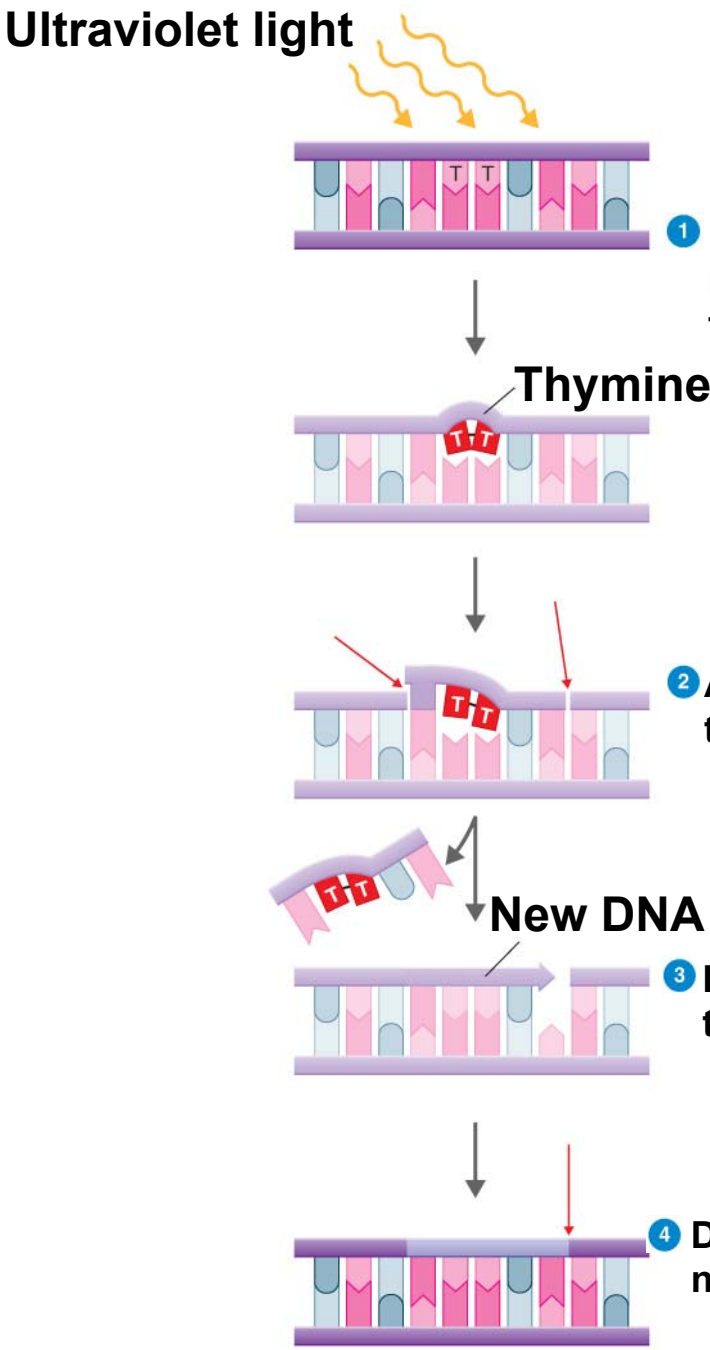
Radiation

- **Ionizing radiation**
  - (X rays and gamma rays) causes the formation of ions that can react with nucleotides and the deoxyribose-phosphate backbone // cause mutations
  - More serious outcome
    - breakage of covalent bonds in sugar phosphate “backbone” – breaks chromosome!

Radiation

- UV radiation causes thymine dimers

The creation and repair of a thymine dimer caused by ultraviolet light.



1. Exposure to ultraviolet light causes adjacent thymines to become cross-linked, forming a thymine dimer and disrupting their normal base pairing.

2. An endonuclease cuts the DNA, and an exonuclease removes the damaged DNA.

3. DNA polymerase fills the gap by synthesizing new DNA, using the intact strand as a template.

4. DNA ligase seals the remaining gap by joining the old and new DNA.

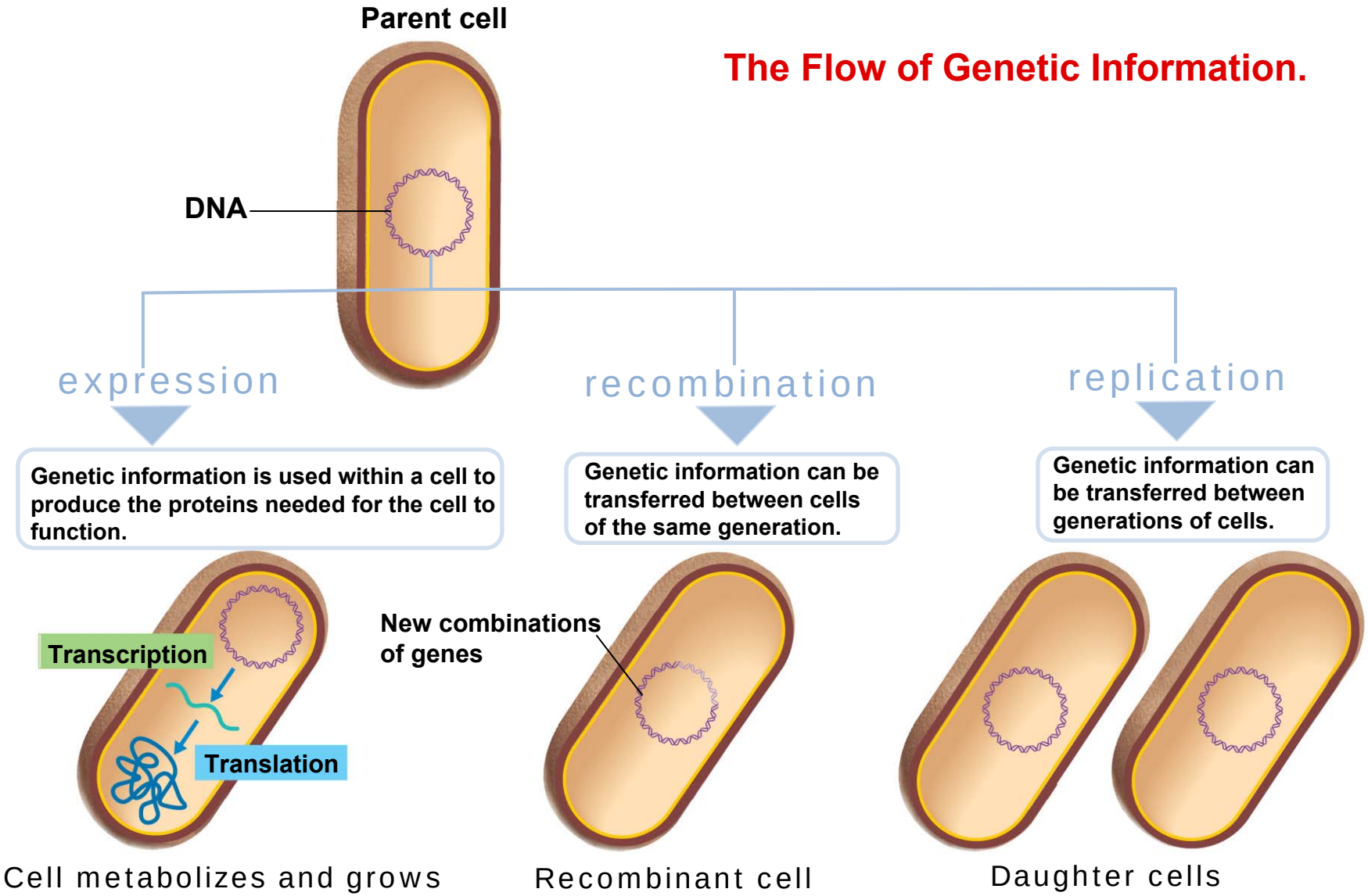
Repair

- **Photolyases** separate thymine dimers
- **Nucleotide excision repair** // repair mutations from UV and other forms of mutagens

Genetic Recombination

- **Horizontal gene transfer**: the transfer of genes between cells of the same generation
- **Vertical gene transfer**: occurs during reproduction between generations of cells

The Flow of Genetic Information.



Genetic Recombination

- Exchange of genes between two DNA molecules
- Crossing over occurs when two chromosomes break and rejoin
- In meiosis this is a normal process which adds genetic variability // associated with organisms which replicate sexually – gamete formation
- May also occurs in prokaryotes

Genetic Recombination in Prokaryotes

- Crossing Over
- Transformation
- Conjugation
- Transduction
- Transposons (jumping genes)

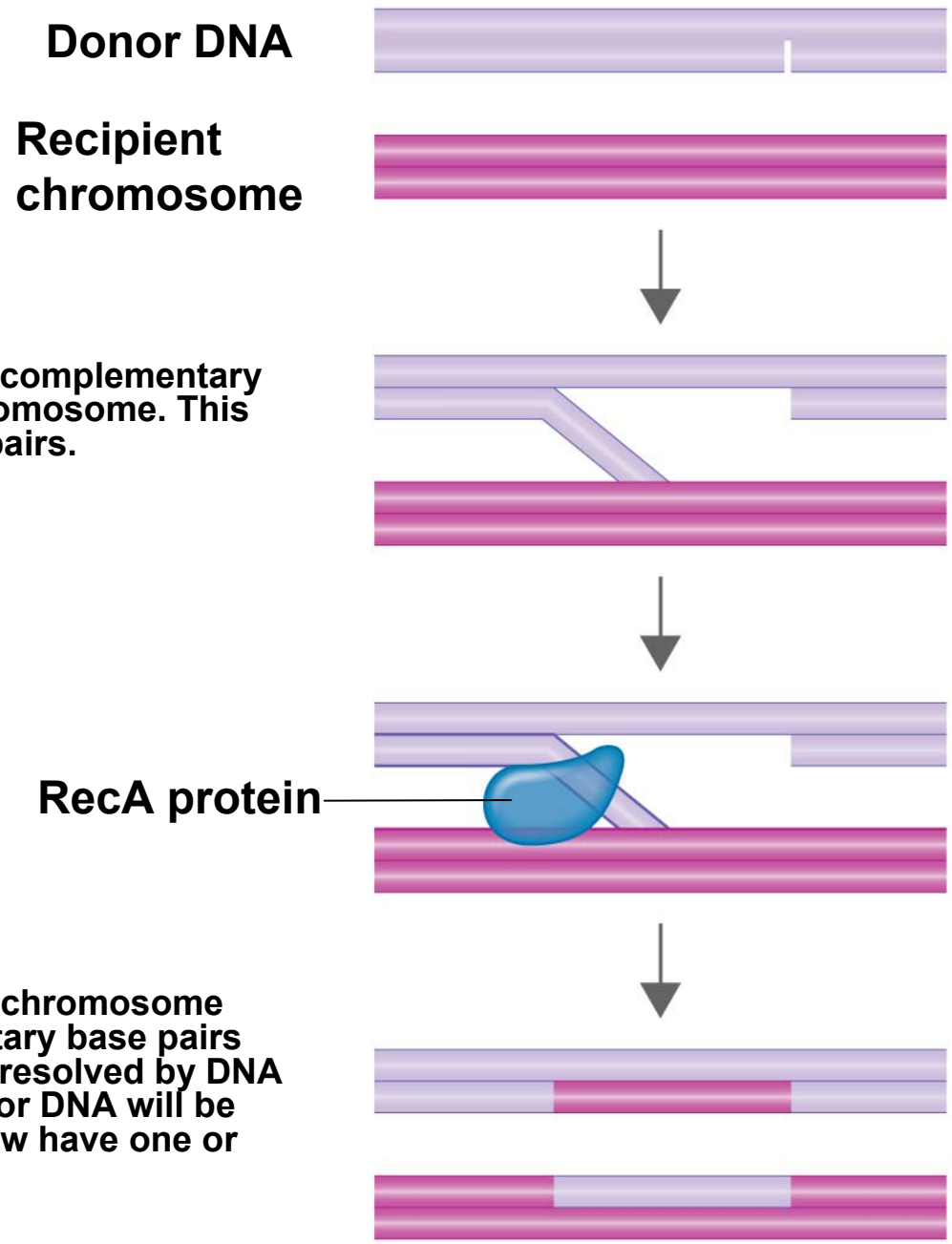
Genetic recombination by crossing over.

1. DNA from one cell aligns with DNA in the recipient cell. Notice that there is a nick in the donor DNA.

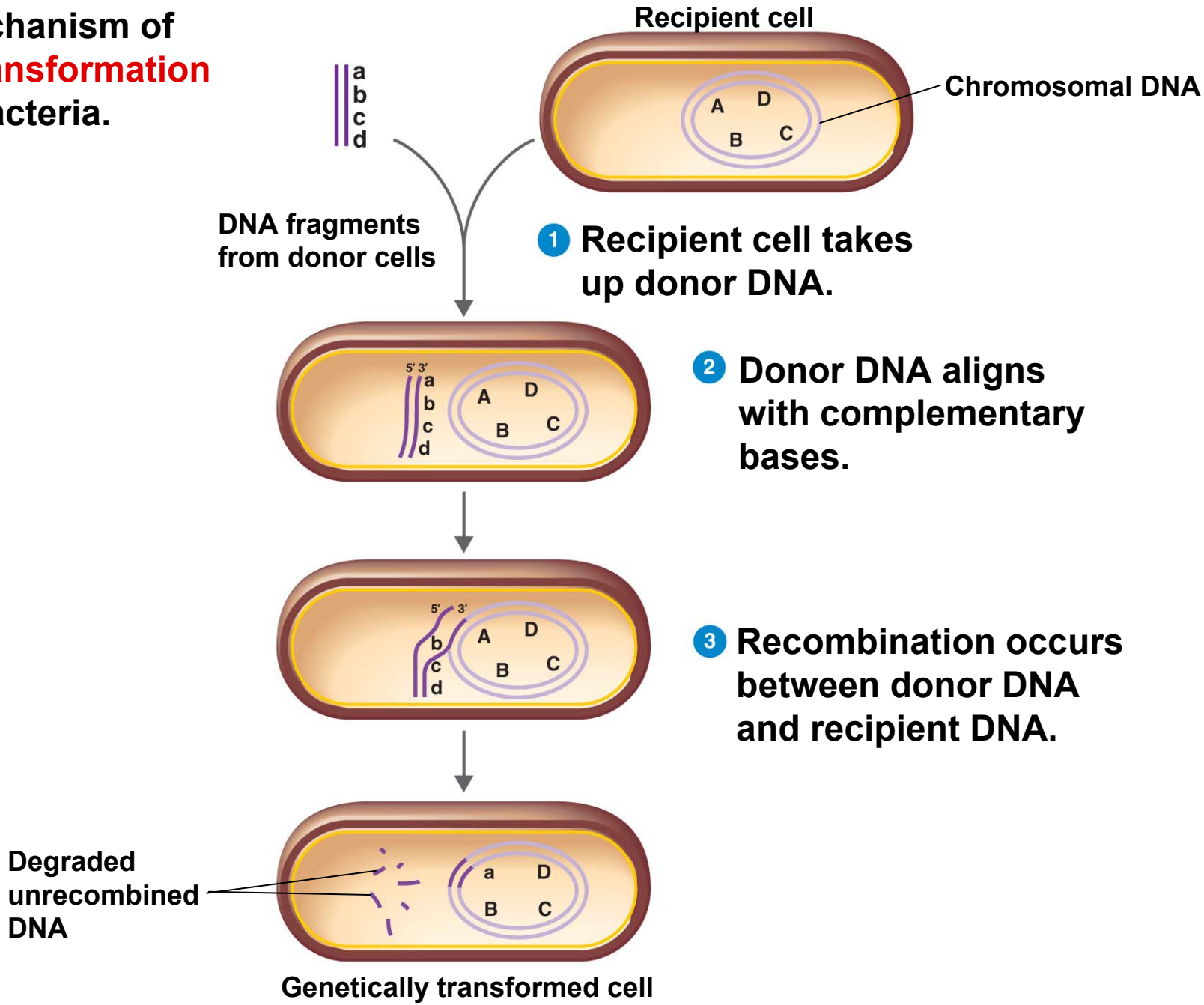
2. DNA from the donor aligns with complementary base pairs in the recipient's chromosome. This can involve thousands of base pairs.

3. RecA protein catalyzes the joining of the two strands.

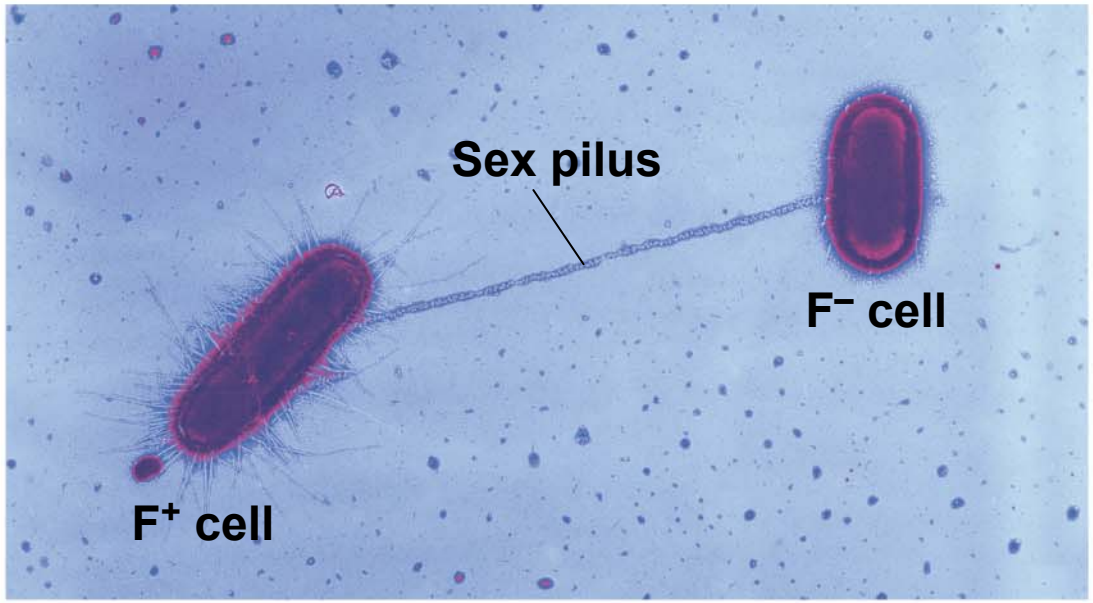
4. The result is that the recipient's chromosome contains new DNA. Complementary base pairs between the two strands will be resolved by DNA polymerase and ligase. The donor DNA will be destroyed. The recipient may now have one or more new genes.



The mechanism of genetic transformation in bacteria.



Bacterial conjugation.

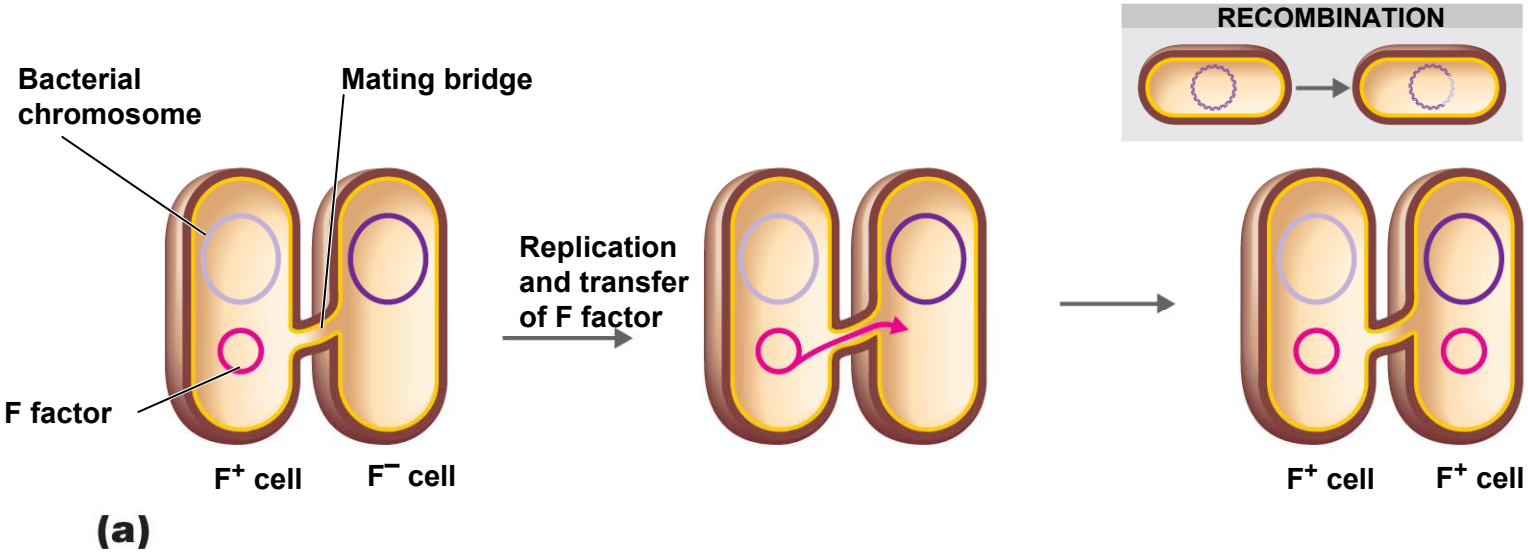


(a) Sex pilus

TEM 1 μm

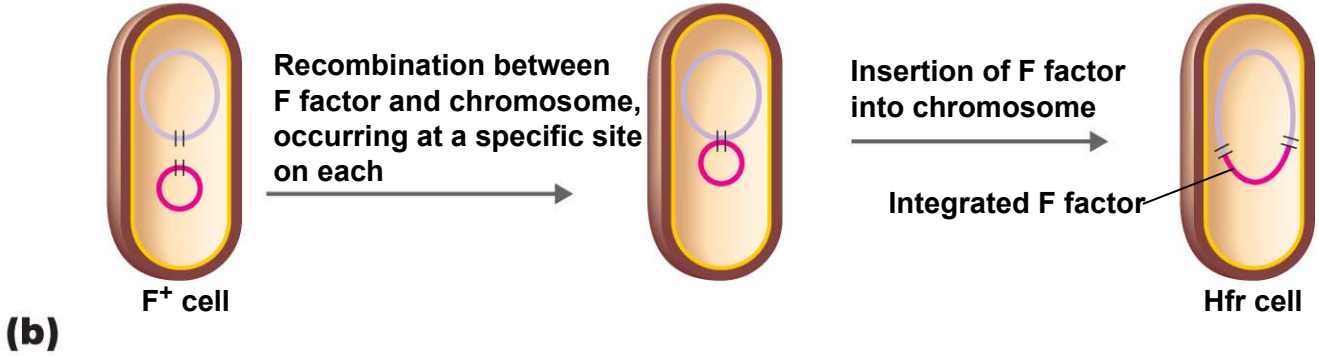
(b) Mating bridge TEM 0.3 μm

Conjugation in *E. coli*.



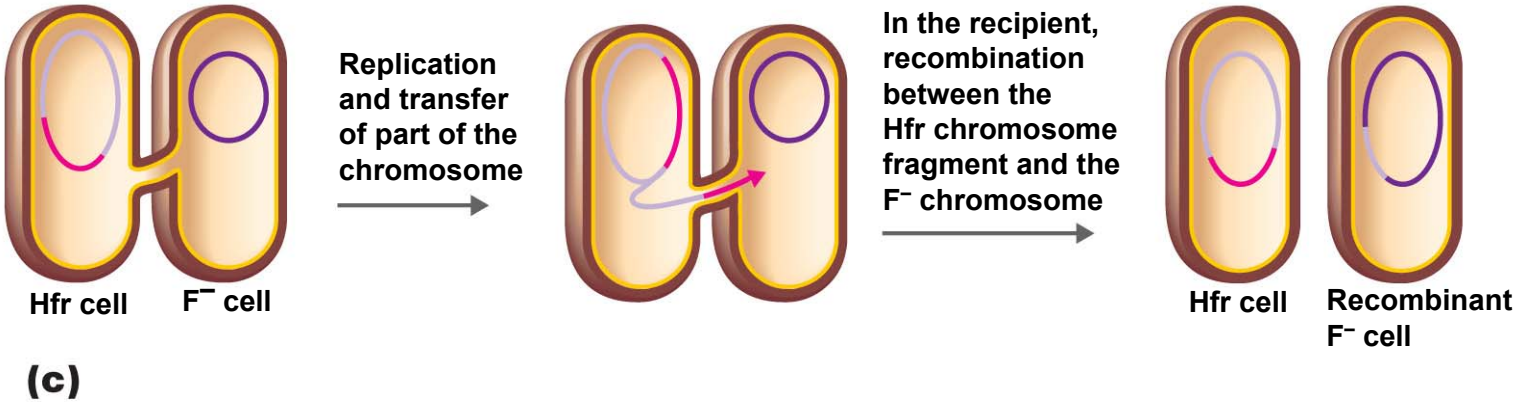
When an F factor (a plasmid) is transferred from a donor (F^+) to a recipient (F^-), the F^- cell is converted to an F^+ cell.

Conjugation in *E. coli*.



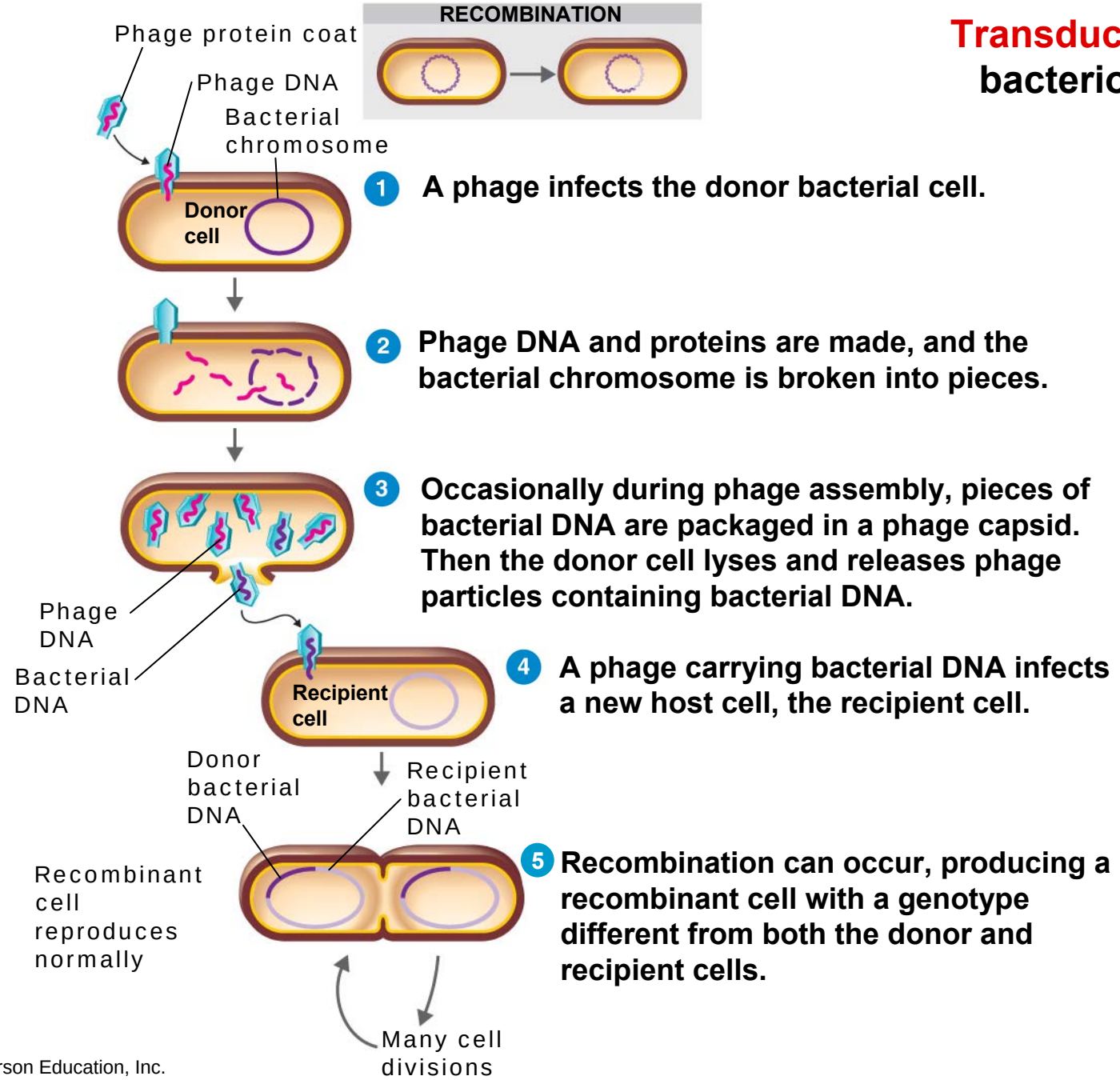
When an F factor becomes integrated into the chromosome of an F^+ cell, it makes the cell a high frequency of recombination (Hfr) cell.

Conjugation in *E. coli*.

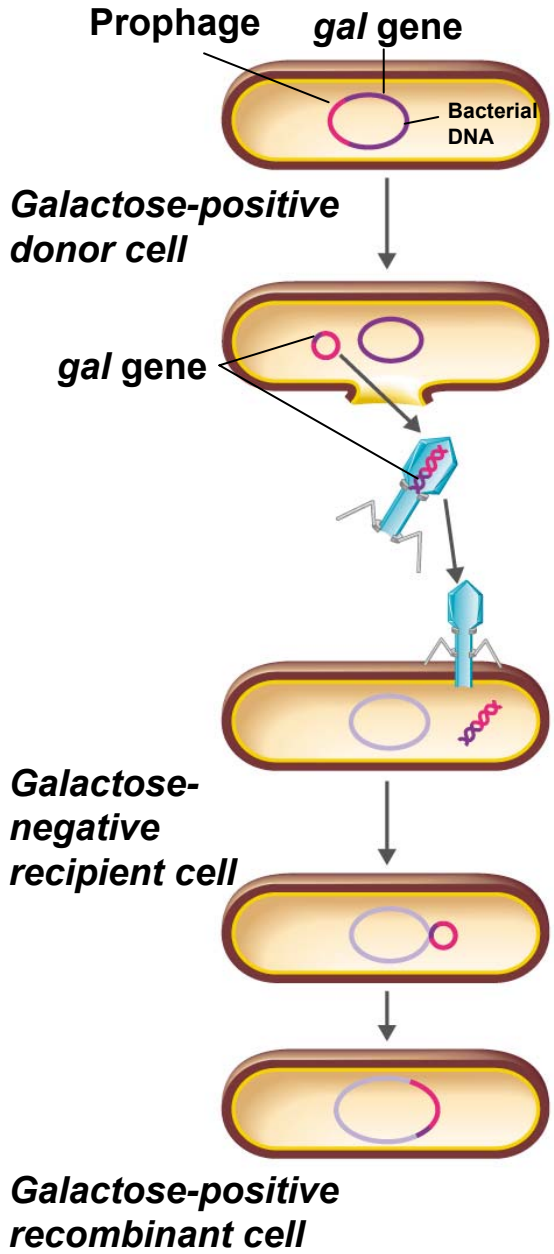


When an Hfr donor passes a portion of its chromosome into an F^- recipient, a recombinant F^- cell results.

Transduction by a bacteriophage.



Specialized transduction.

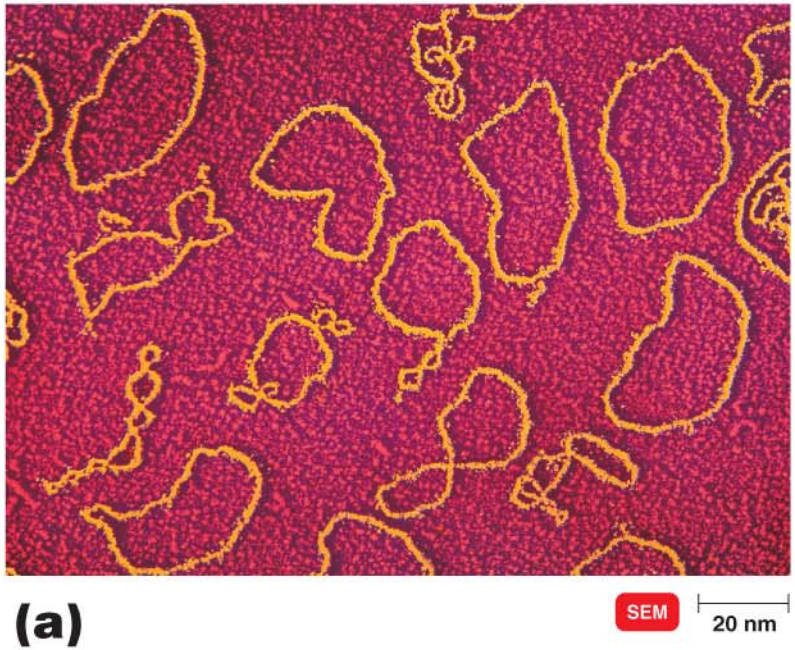


1. Prophage exists in galactose-using host (containing the *gal* gene).
2. Phage genome excises, carrying with it the adjacent *gal* gene from the host.
3. Phage matures and cell lyses, releasing phage carrying *gal* gene.
4. Phage infects a cell that cannot utilize galactose (lacking *gal* gene).
5. Along with the prophage, the bacterial *gal* gene becomes integrated into the new host's DNA.
6. Lysogenic cell can now metabolize galactose.

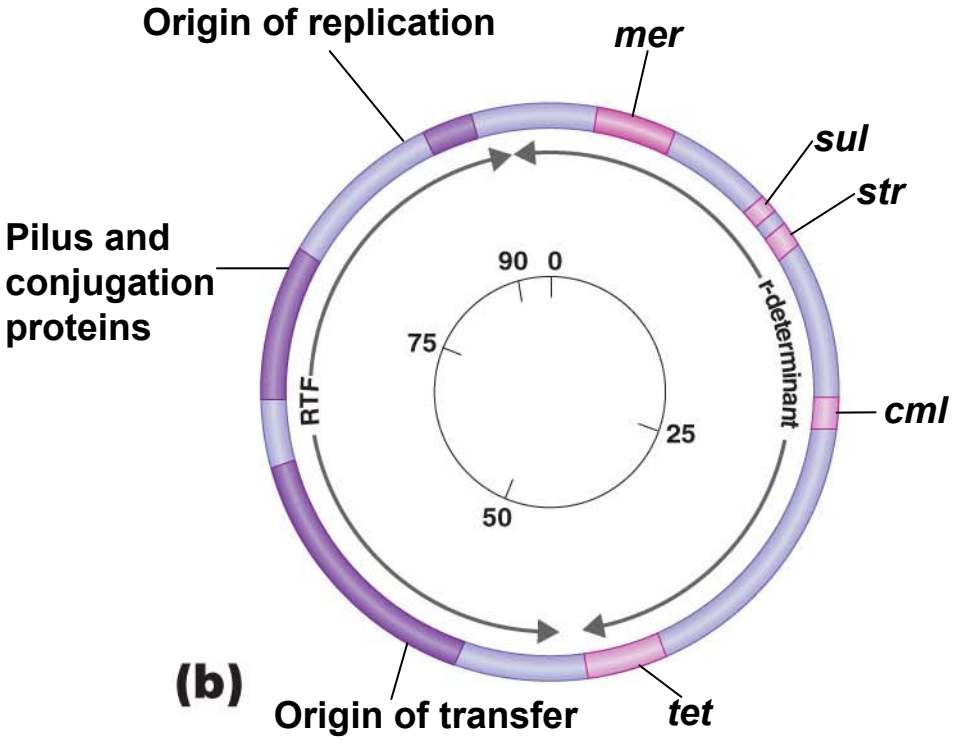
Plasmids

- **Conjugative plasmid**: carries genes for sex pili and transfer of the plasmid
- **Dissimilation plasmids**: encode enzymes for catabolism of unusual compounds
- **R factors**: encode antibiotic resistance

R factor, a type of plasmid.



(a)

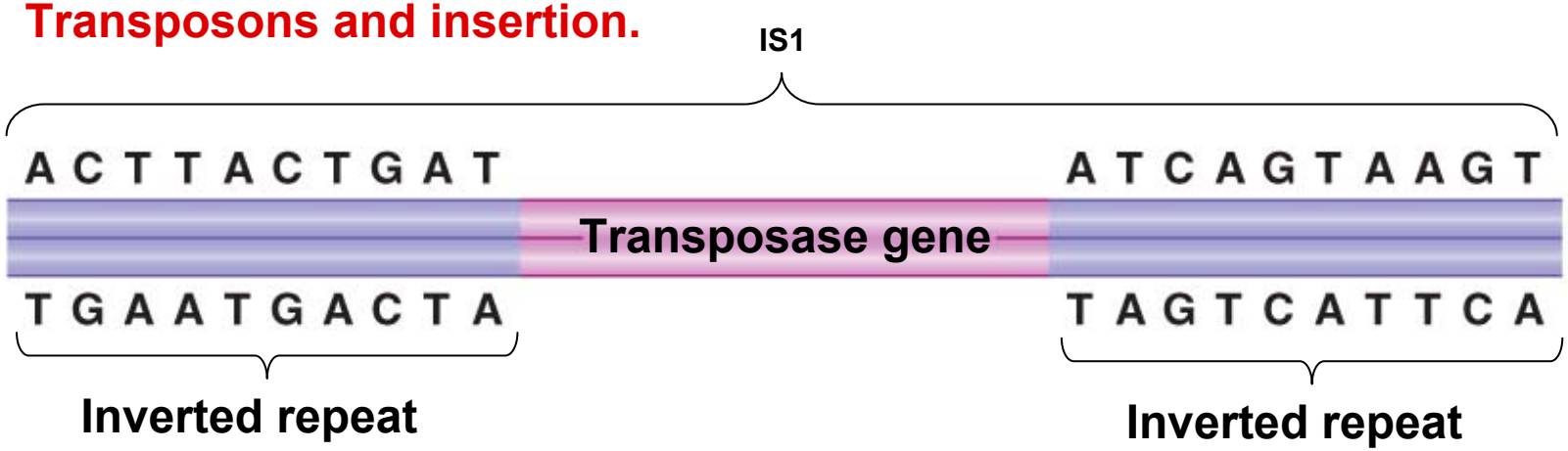


(b)

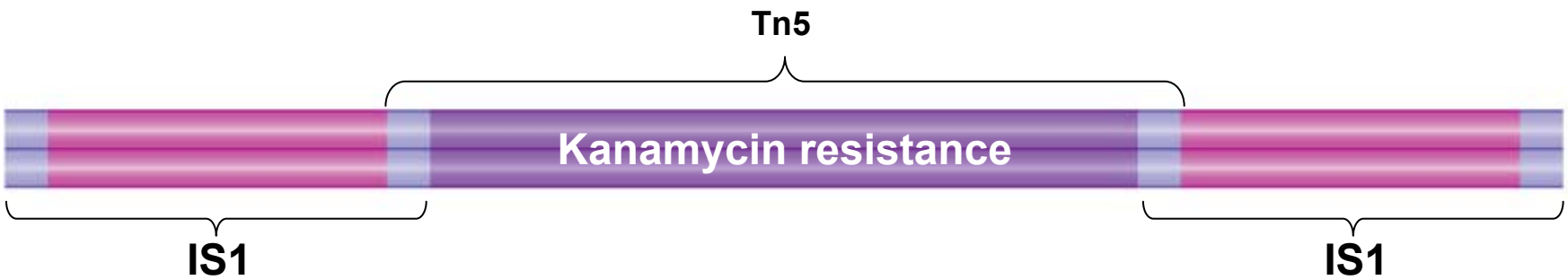
Transposons

- Segments of DNA that can move from one region of DNA to another
- Contain insertion sequences for cutting and resealing DNA (transposase)
- Complex transposons carry other genes

Transposons and insertion.



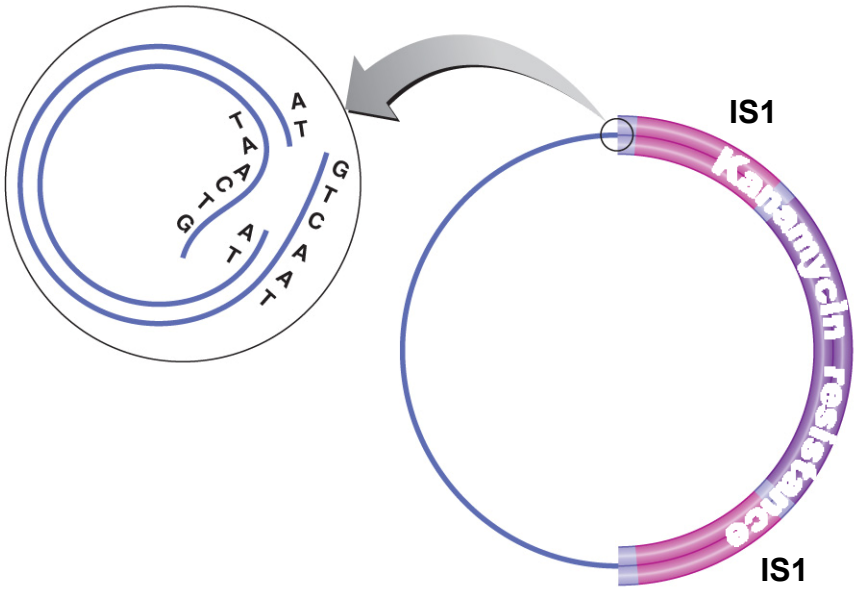
(a) **An insertion sequence (IS), the simplest transposon, contains a gene for transposase, the enzyme that catalyzes transposition. The tranposase gene is bounded at each end by inverted repeat sequences that function as recognition sites for the transposon. IS1 is one example of an insertion sequence, shown here with simplified IR sequences.**



(b) **Complex transposons carry other genetic material in addition to transposase genes. The example shown here, Tn5, carries the gene for kanamycin resistance and has complete copies of the insertion sequence IS1 at each end.**

Transposons and insertion.

1 **Transposase cuts DNA, leaving sticky ends.**



2 **Sticky ends of transposon and target DNA anneal.**

(c) **Insertion of the transposon Tn5 into R100 plasmid**

Genes and Evolution

- Mutations and recombination provide diversity
- A change in the genetic code (evolution) – which alters the proteins of the organism
- Fittest organisms for any given environment are then selected by natural selection