Chapter 9

Topics

- Genetics
- Flow of Genetics
- Regulation
- Mutation
- Recombination

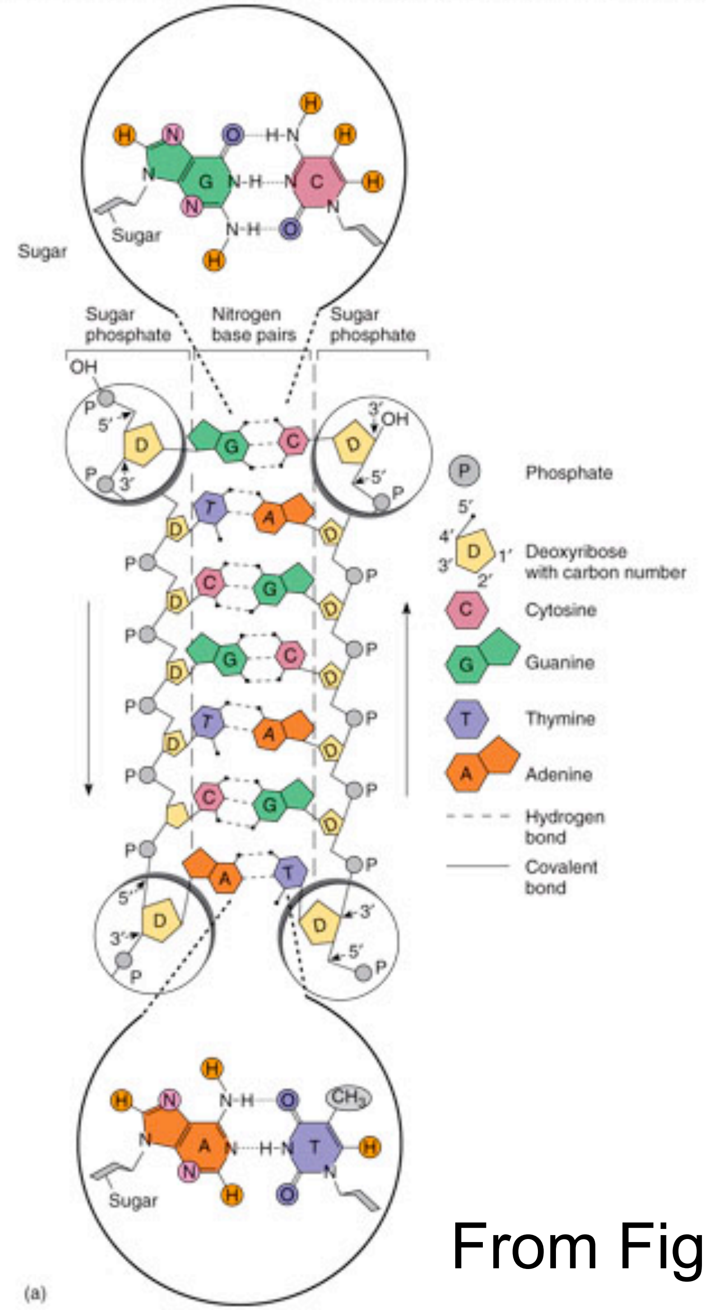
Flow of Genetics

- NA replication (DNA => DNA; RNA => RNA)
  - Replication
- Reverse transcription (RNA => DNA)
- Gene Expression (DNA =>RNA=>Protein)
  - Transcription
  - Translation
  - Post-translational modification

Storing hereditary information in a (double-stranded) DNA molecule:

- Sequence of nucleotides

- Hydrogen bonds between
  A & T and G & C



From Fig. 9.4

Replication

- Nature: Semi-conservative & selfish
- Leading strand
  - Initiation at ORI of the 5’ to 3’ synthesis of DNA in a continuous manner
- Lagging strand
  - is synthesized (5’ to 3’) in form of multiple DNA (Okazaki) fragments
    - Primer synthesis performed by RNA polymerase (Primase),
    - DNA synthesis performed by DNA polymerase III
    - RNA primer removal and fill-in with DNA by DNA polymerase I
    - Connection of two Okazaki fragments by DNA ligase.

Reactants: DNA template and dNTP

Products: DNA and water

Involved Enzymes (Tab. 9.1):

- *DNA-dependent polymerases:* DNA-polymerases I & III, Primase (RNA-polymerase),
- Ligase,
- *Topoisomerases:* Helicase, Gyrase

3 phases:

- Initiation: ORI and priming in fork
- Elongation: asymmetric polymerization of dNTPs
- Termination: Once the entire DNA is replicated; “ter” sequences

Transcription

- Nature: Conservative & selfish
- A single strand of RNA is transcribed from a double strand of DNA
- The strand complementary to the coding (plus, sense) strand is called template (minus, non-sense) strand of DNA
- One RNA polymerase catalyzes the reaction
  - (forming phosphodiester bonds)
- Synthesis occurs always in 5’ to 3’ direction!

Reactants: DNA template and NTP

Products: RNA and water

(Thymine is replaced by uracil)

Involved Enzymes: RNP [α,β,β‘ & σ]

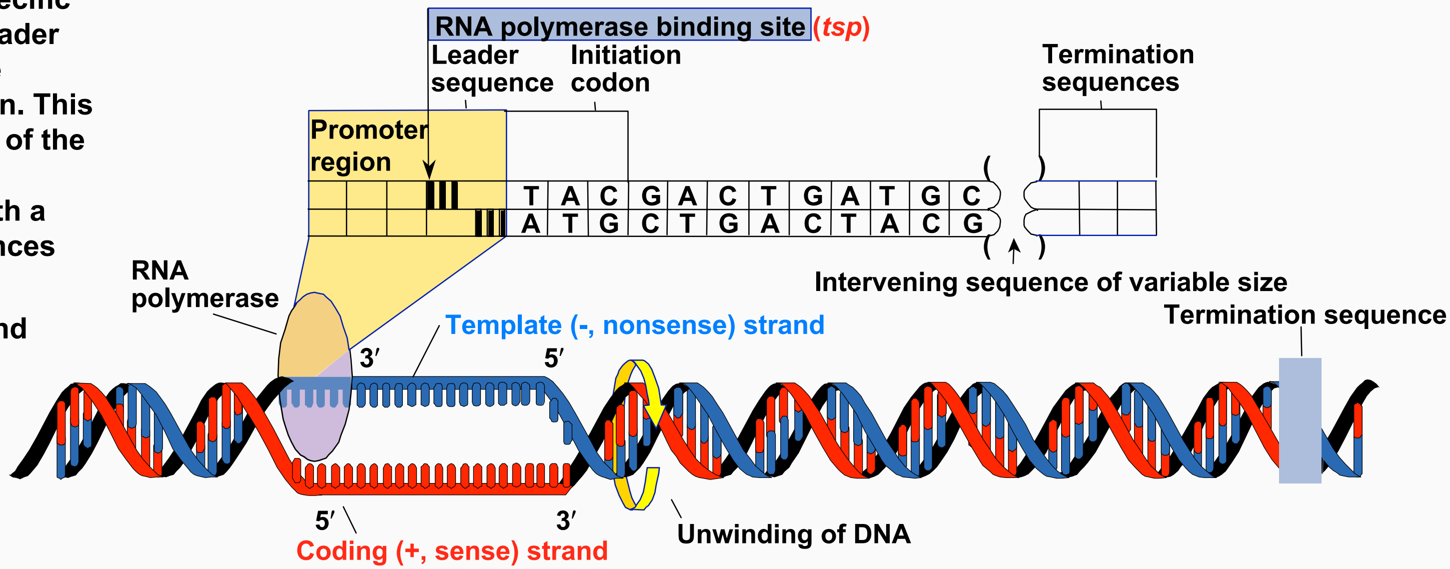
3 phases:

- Initiation: Open complex formation at promoter
- Elongation: polymerization of NTPs
- Termination: palindromic sequence or Rho-protein

Fig. 9.12 The major events in mRNA synthesis

(a) **Overview of a gene (t unit).** Each gene contains a specific promoter region and a leader sequence for guiding the beginning of transcription. This is followed by the region of the gene that codes for a polypeptide and ends with a series of terminal sequences that stop translation.

(b) **Initiation:** DNA is unwound at the promoter by RNA polymerase. Only one strand of DNA, called the template strand, is copied by the RNA polymerase. This strand runs in the 3′ to 5′ direction.



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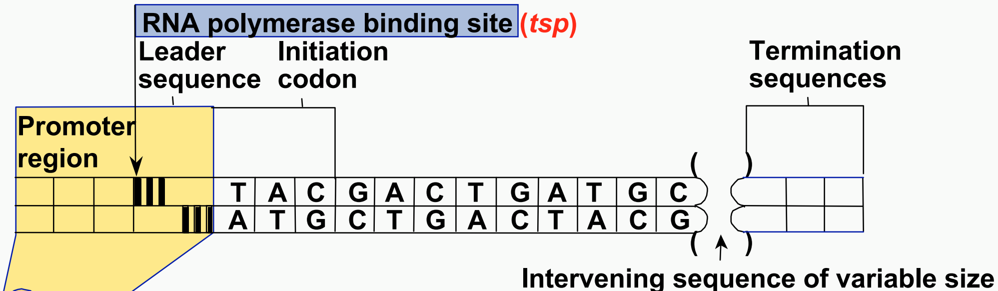

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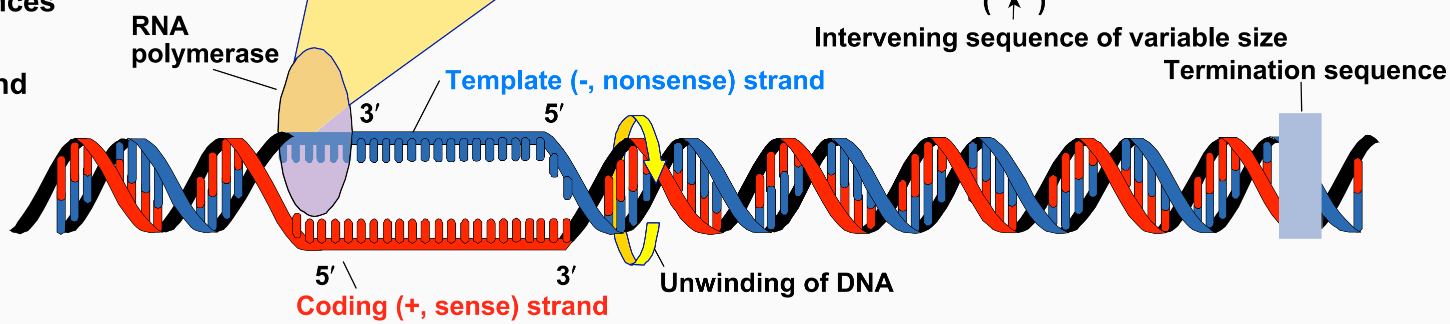
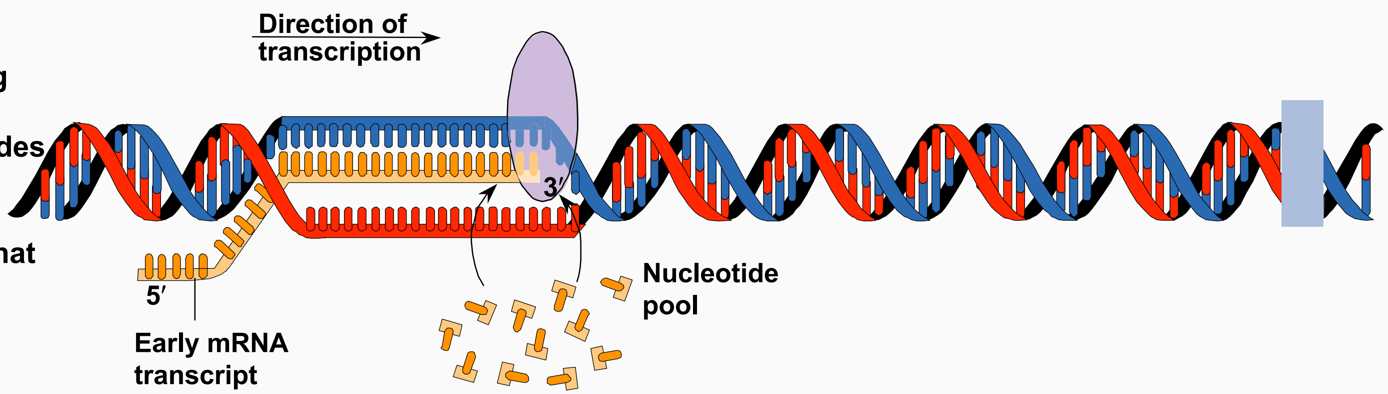


(c) **Elongation:** As the RNA polymerase moves along the strand, it adds complementary nucleotides as dictated by the DNA template, forming the single-stranded mRNA that reads in the 5′ to 3′ direction.



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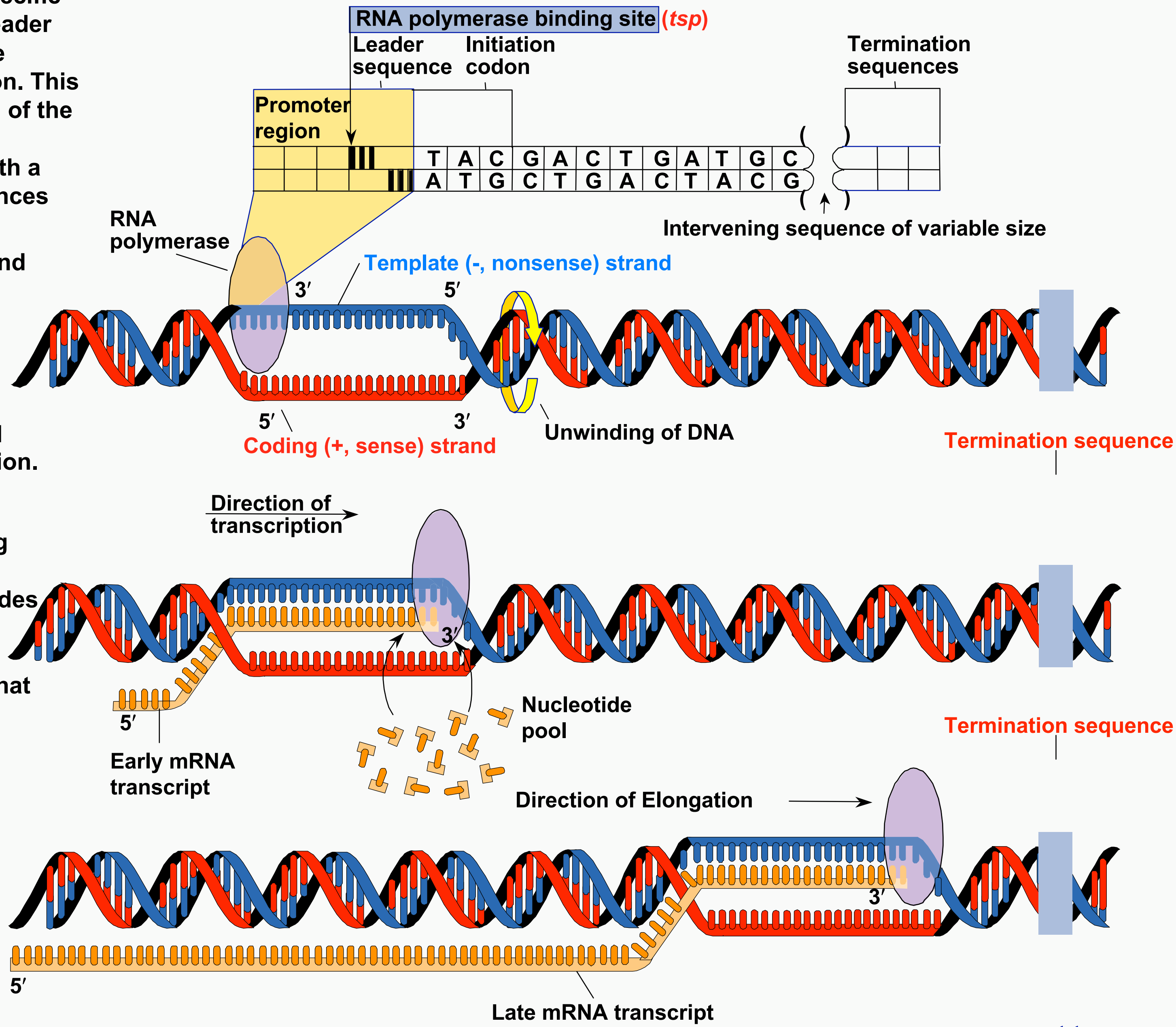
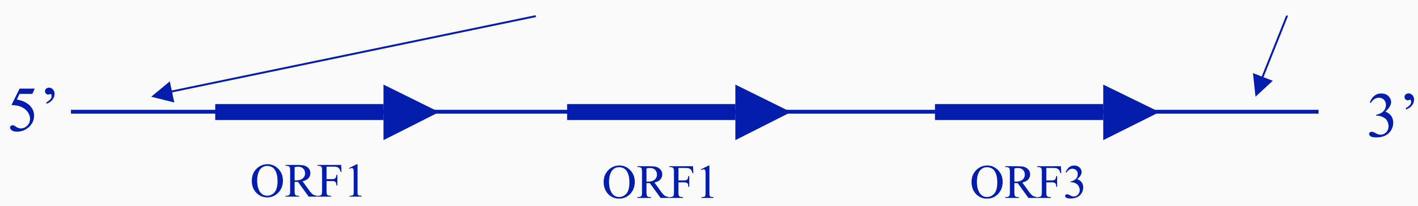
(d) **Termination:** The polymerase continues transcribing until it reaches a termination site and the mRNA transcript is released for translation. Note that the section of the DNA that has been transcribed is rewound into its original configuration.



1. mRNA

- "Message"; contains (a) segment(s) that "code", make "sense", which can be translated into protein(s). These segments are also called Open Reading Frame(s).
  -> Can encode multiple proteins (polygenic message)
- The ORF(s) contain(s) codons (base triplets).
- The ORFs are flanked by non-translated sequences
  (the first one being the "leader" and the last one being the "trailer").



2. rRNA

- All 3 prokaryotic rRNAs are contained in one message, which needs to be processed to release the mature rRNAs

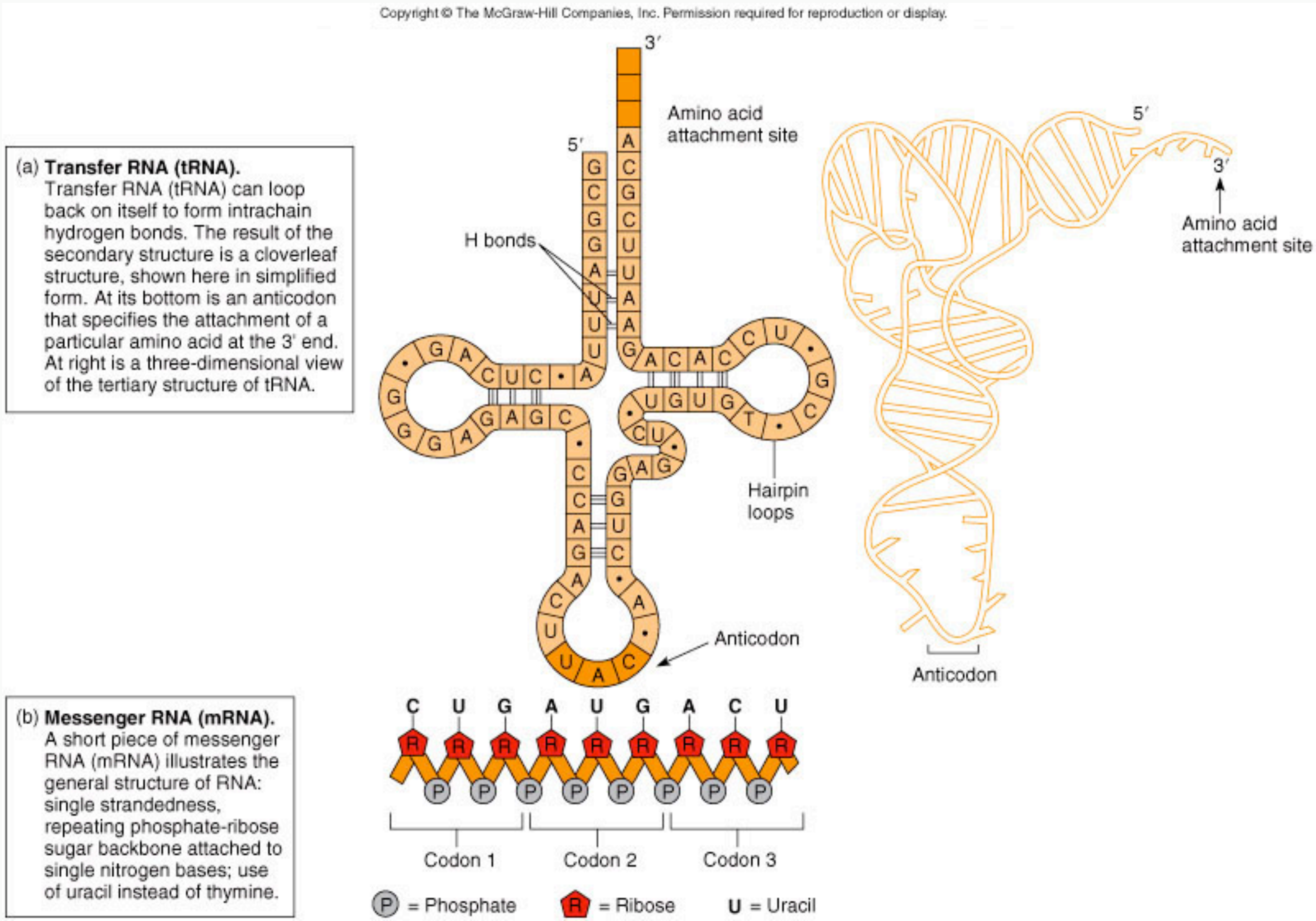
- rRNA together with r-proteins make up the small and large subunits of the ribosome
- (70S - prokaryote, 80S - eukaryote)

- Ribosomes carry out translation (protein synthesis)

tRNA

- Certain segments of DNA contain the information for tRNA, some of which are in proximity to genes for rRNA, r-proteins or polymerase-encoding genes in the genome.
- The many palindromic (complementary) sequence segments form hairpin or stem loop structures
  - Amino acid attachment site: the 3’ end of the RNA
  - Anticodon: triplet of ribonucleotides in the stemloop that interacts with mRNA during translation (protein synthesis).
- Amino acids and tRNAs are connected by enzyme “amino acyl-tRNA synthetase.”

Important structural characteristics for tRNA and mRNA.



(a) **Transfer RNA (tRNA).** Transfer RNA (tRNA) can loop back on itself to form intrachain hydrogen bonds. The result of the secondary structure is a cloverleaf structure, shown here in simplified form. At its bottom is an anticodon that specifies the attachment of a particular amino acid at the 3′ end. At right is a three-dimensional view of the tertiary structure of tRNA.

(b) **Messenger RNA (mRNA).** A short piece of messenger RNA (mRNA) illustrates the general structure of RNA: single strandedness, repeating phosphate-ribose sugar backbone attached to single nitrogen bases; use of uracil instead of thymine.

Fig. 9.11 Characteristics of transfer and message RNA

Codons

- Triplet of nucleotides ("codon") that specifies ("encodes") a given amino acid
- Multiple codons for one amino acid
- 20 amino acids
- Start codon of translation
- Stop (non-sense) codons of translation

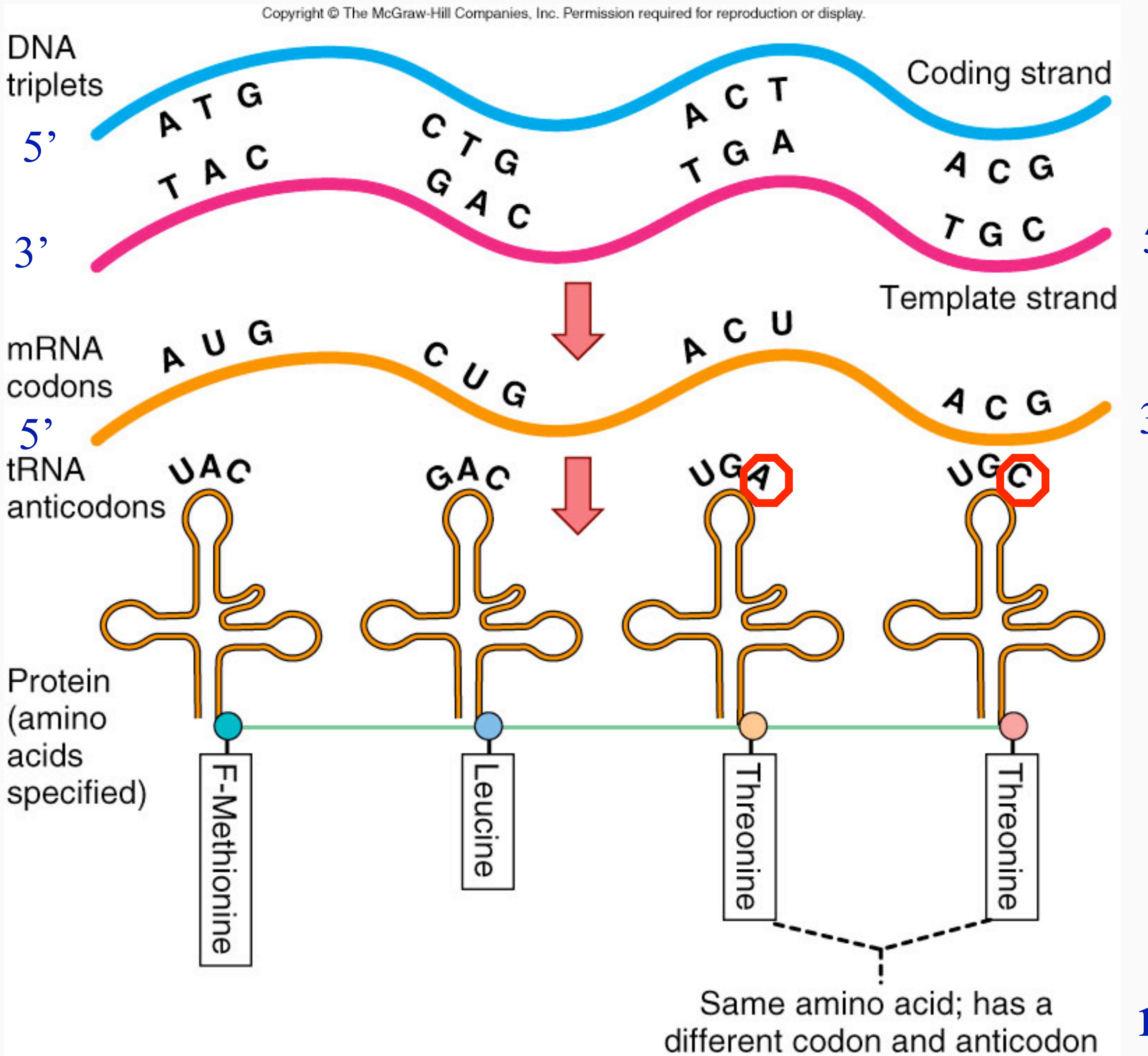
The codons from mRNA specify a given amino acid.

First Base Position	Second Base Position: U		C		A		G		Third Base Position
U	UUU	Phenylalanine	UCU	Serine	UAU	Tyrosine	UGU	Cysteine	U
	UUC		UCC		UAC		UGC		C
	UUA	Leucine	UCA		UAA	STOP**	UGA	STOP**	A
	UUG		UCG		UAG		UGG	Tryptophan	G
C	CUU	Leucine	CCU	Proline	CAU	Histidine	CGU	Arginine	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	Glutamine	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	Isoleucine	ACU	Threonine	AAU	Asparagine	AGU	Serine	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	Lysine	AGA	Arginine	A
	AUG	START Methionine*	ACG		AAG		AGG		G
G	GUU	Valine	GCU	Alanine	GAU	Aspartic acid	GGU	Glycine	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	Glutamic acid	GGA		A
	GUG		GCG		GAG		GGG		G

* This codon initiates translation.
**For these codons, which give the orders to stop translation, there are no corresponding tRNAs and no amino acids.

Fig. 9.14 The Genetic Code

Representation of the codons and their corresponding amino acids.



64 combinations

20 AA

redundancy

Fig. 9.15 Interpreting the genetic code

Protein synthesis

- Translation at the ribosome
  - Protein synthesis has the following participating macromolecules:
    - mRNA
    - tRNA with attached amino acid
    - rRNA and r-protein (Ribosome)
    - Protein (Initiation factors)

Participants involved in the translation process.

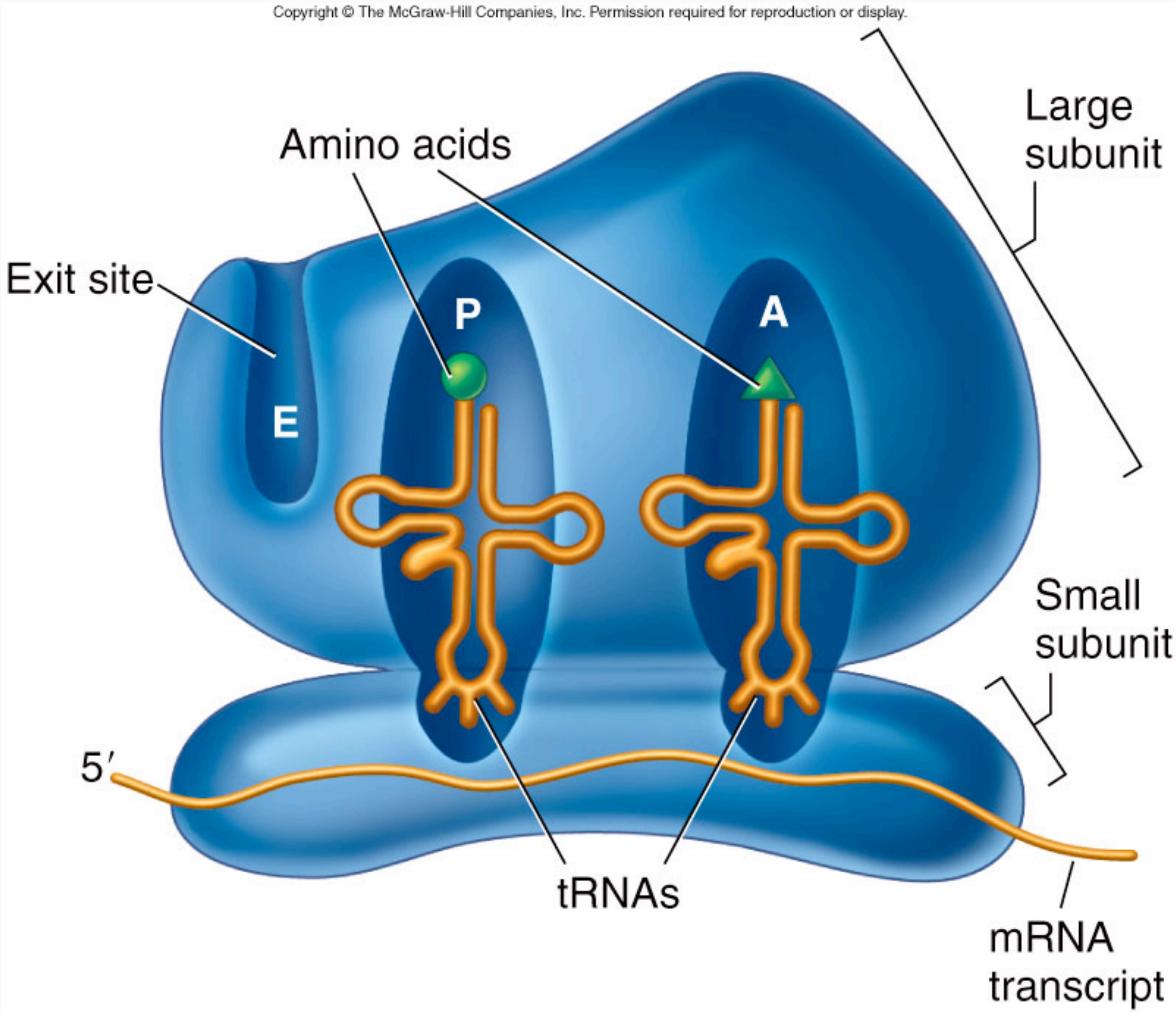
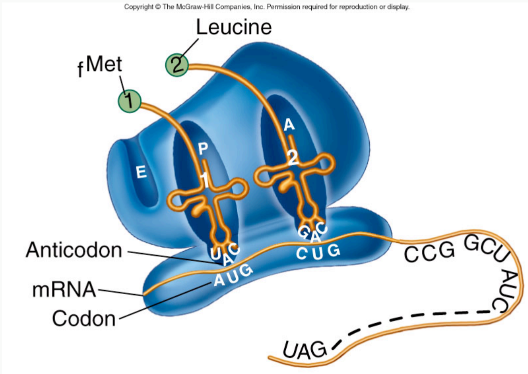


Fig. 9.13 The “players” in translation

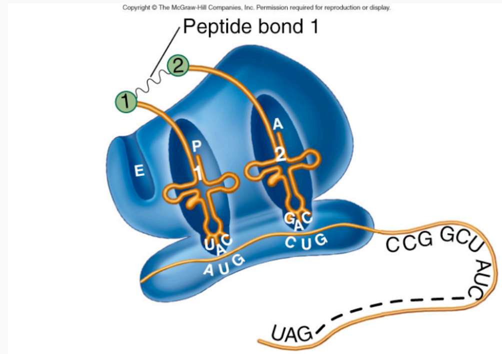
Translation

- Ribosomes bind mRNA near the start codon (mostly AUG); => assembly of complex @ P-site
- tRNA with attached amino acid binds to the start codon with its complementary anticodon.
- a new AA-tRNA enters the A-site and bonds via a peptide bond to the amino acid from the P-site.
- Ribosome translocates (move along mRNA to the next codon), allowing a new AA-tRNA to bind to the A-site and kicking out the uncharged tRNA in E-site.
- Amino acids are connected by peptide bonds
- Stop (non-sense) codon terminates translation.

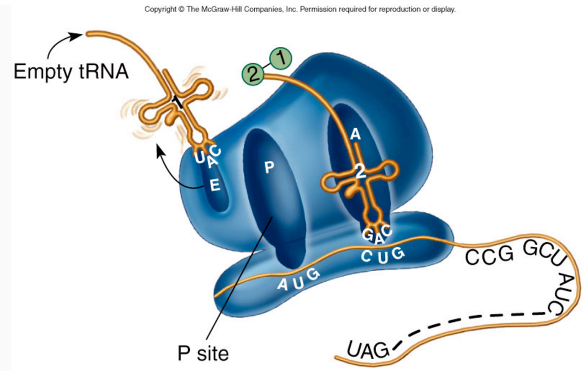
The process of translation.



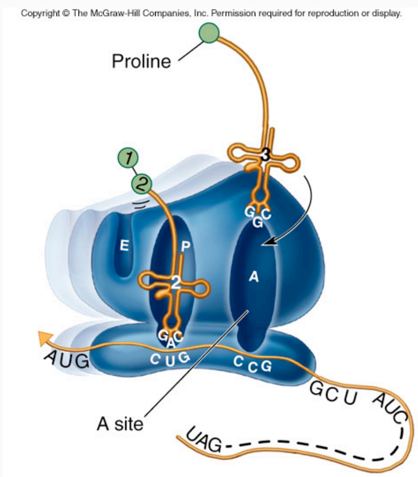
(a) Entrance of tRNAs 1 and 2



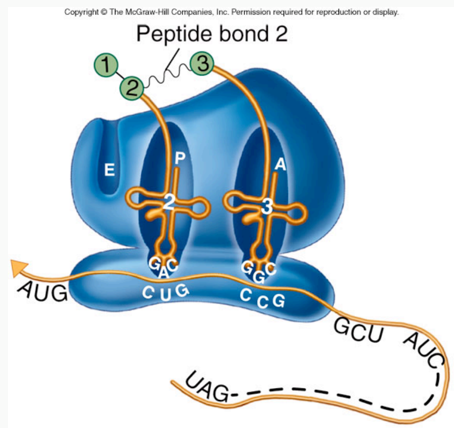
(b) Formation of peptide bond



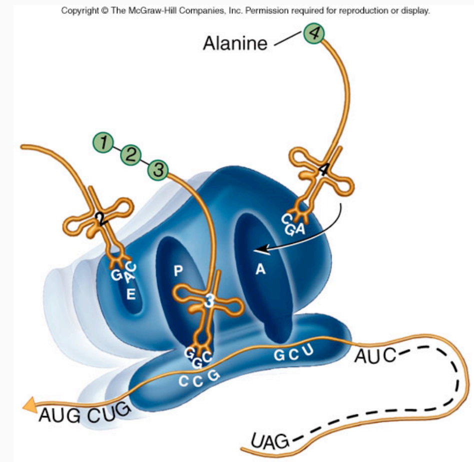
(c) Discharge of tRNA 1 at E site



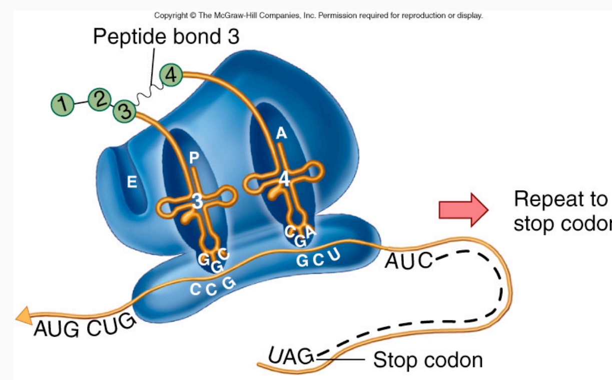
(d) First translocation; tRNA 2 shifts into P site; enter tRNA 3 by ribosome



(e) Formation of peptide bond



(f) Discharge of tRNA 2; second translocation; enter tRNA 4



(g) Formation of peptide bond

Fig. 9.16 The events in protein synthesis

In prokaryotes, translation can occur at multiple sites on the mRNA while the message is still being transcribed.

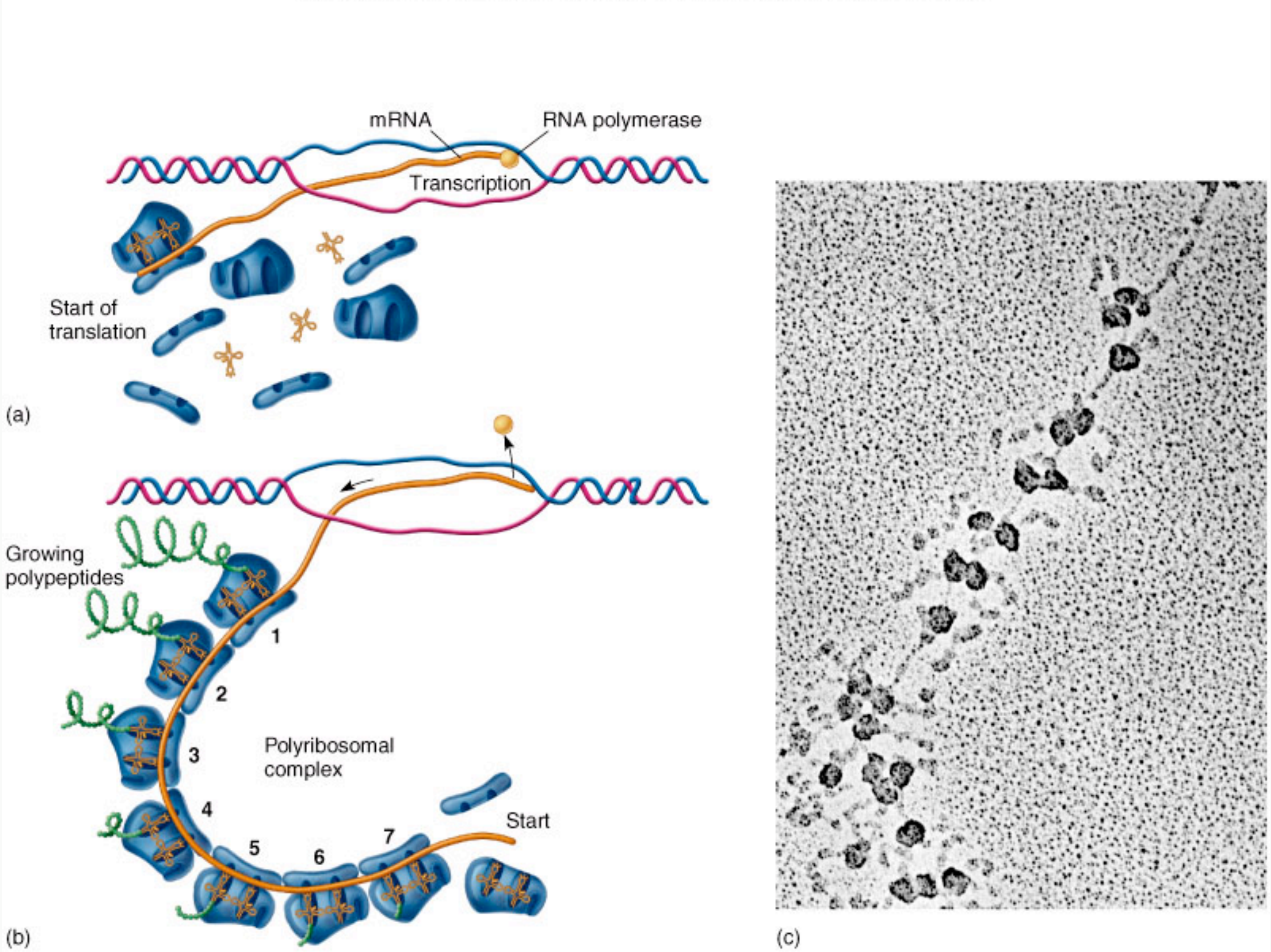


Fig. 9.17 Speeding up the protein assembly line in bacteria

Transcription and translation in eukaryotes

- Similar to prokaryotes except
  - AUG encodes for a different form of methionine; always AUG
  - mRNAs always code only for one protein
  - Transcription and translation are not simultaneous (separated in space and time)
  - Pre-mRNA ("hn-RNA") needs to be processed
    - Introns
    - Exons

The processing of pre-mRNA into mRNA involves the removal of introns.

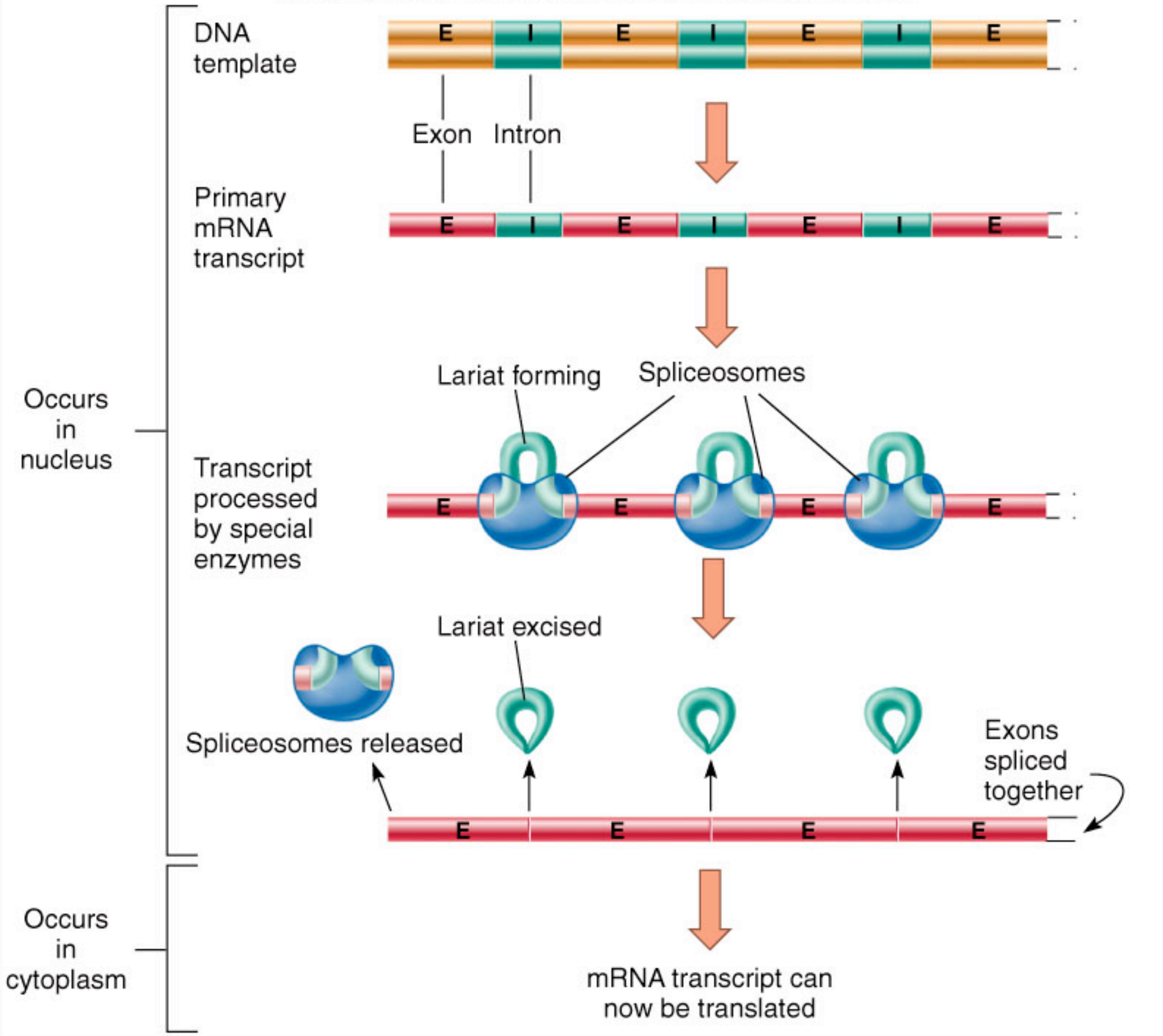


Fig. 9.18 The split gene of eukaryotes

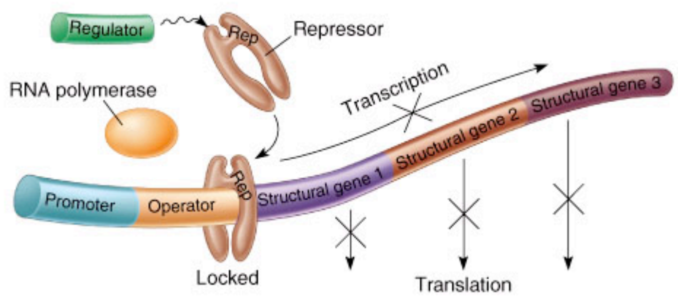
Regulation

- Internal:
  - Inducible operon
    - Switching to new tasks (Catabolism, defense)
  - Repressible operon
    - Saving energy, enough product (Amino acids, nucleotides)

- External:
  - Antimicrobials
  - Signal transduction (chemical, physical, biological)

The regulation of sugar metabolism such as lactose involves repression in the absence of lactose, and induction when lactose is present.

(a) Operon Off. In the absence of lactose, a repressor protein (the product of a regulatory gene located elsewhere on the bacterial chromosome) attaches to the operator of the operon. This effectively locks the operator and prevents any transcription of structural genes downstream (to its right). Suppression of transcription (and consequently, of translation) prevents the unnecessary synthesis of enzymes for processing lactose.



De-repression
(by inducer)

(b) Operon On. Upon entering the cell, the substrate (lactose) becomes a genetic inducer by attaching to the repressor, which loses its grip and falls away. The RNA polymerase is now free to bind to the promoter and initiate transcription, and the enzymes produced by translation of the mRNA perform the necessary reactions on their lactose substrate.

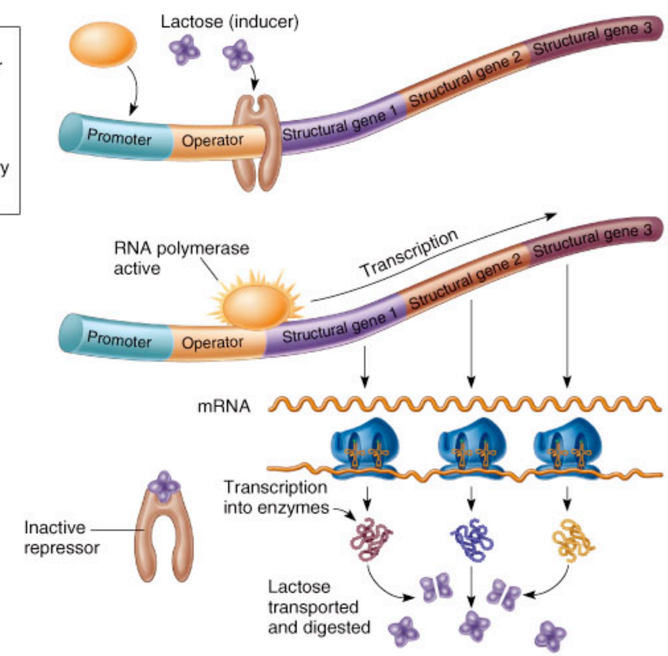
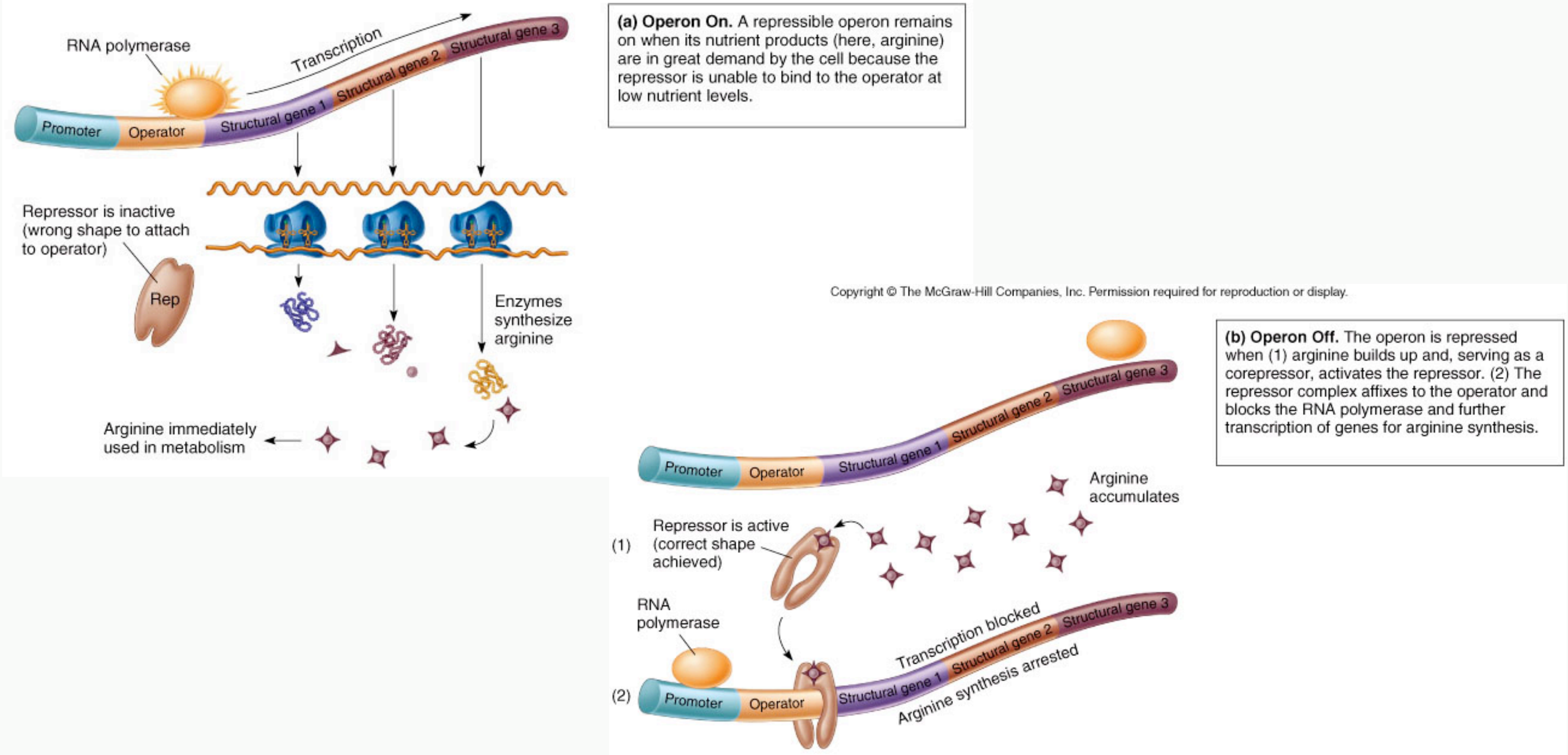


Fig. 9.19 The lactose operon in bacteria

The regulation of amino acids such as arginine involves repression when arginine accumulates, and no repression when arginine is being used.



(a) Operon On. A repressible operon remains on when its nutrient products (here, arginine) are in great demand by the cell because the repressor is unable to bind to the operator at low nutrient levels.

(b) Operon Off. The operon is repressed when (1) arginine builds up and, serving as a corepressor, activates the repressor. (2) The repressor complex affixes to the operator and blocks the RNA polymerase and further transcription of genes for arginine synthesis.

Co-repression

Fig. 9.20 Repressible operon

Antimicrobials

- Ex. Antibiotics and drugs can inhibit the enzymes involved in transcription and translation

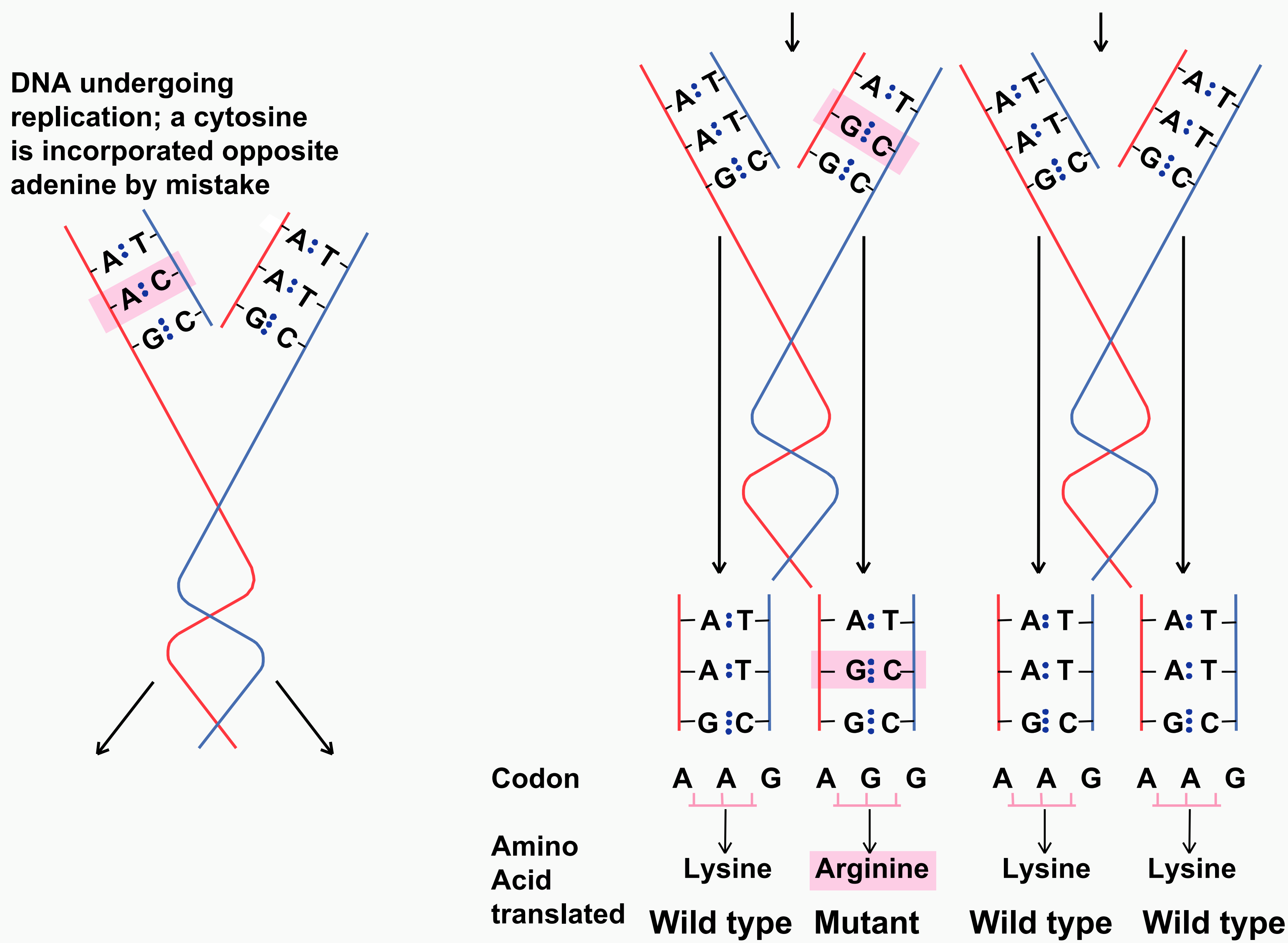
Mutations

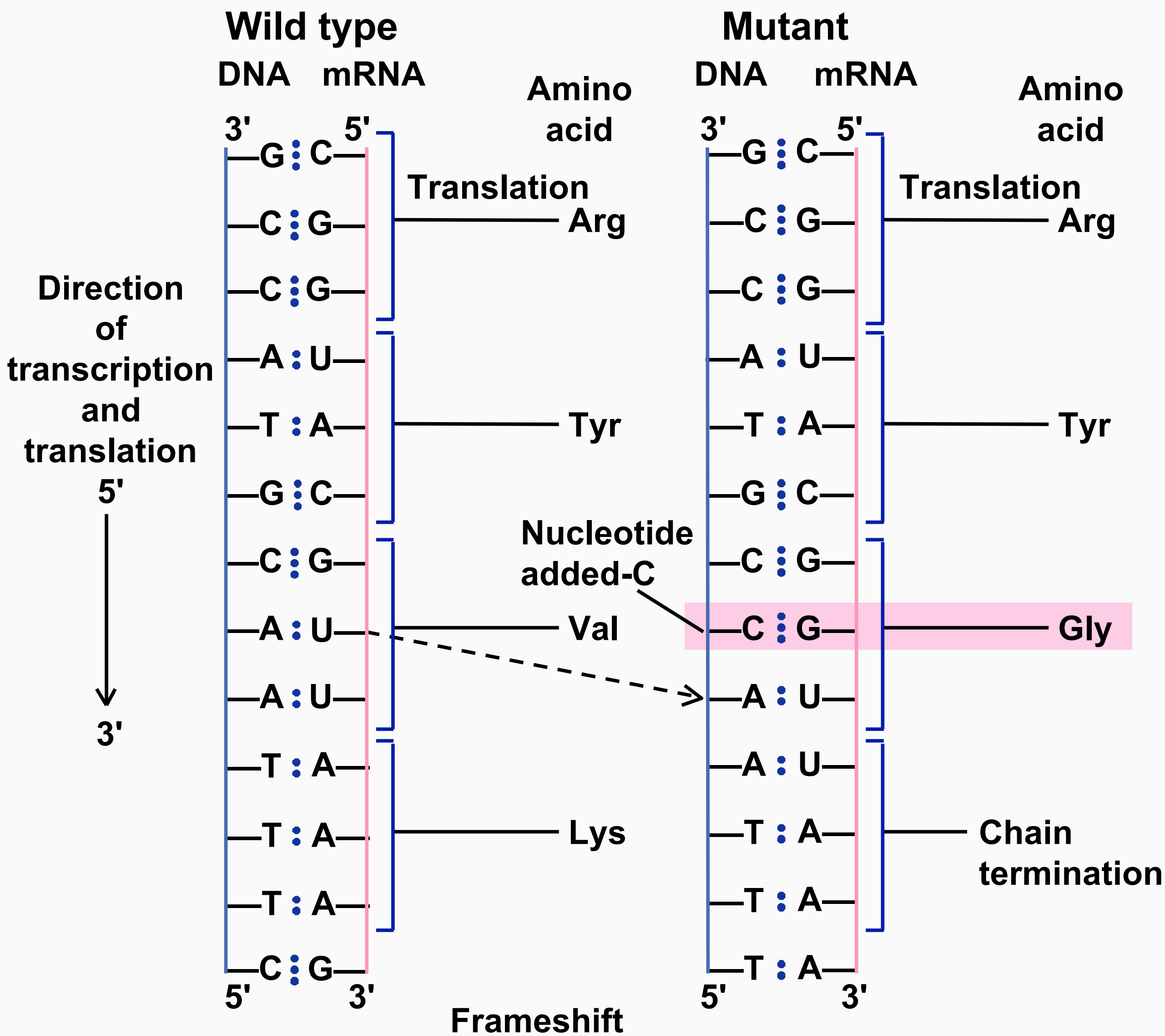
- **“Heritable Changes in the DNA Sequence”**
  - Sources of mutations
  - Kinds of mutations

Mutations (kinds of mutations)

- Spontaneous – random change (e.g., error in replication)
- Induced – chemical, radiation

- Point – change a single base
  - Substitution
  - Insertion or deletion
- Nonsense – change a coding triplet into a stop codon (no respective AA-tRNA present).
- Mis-sense - change a codon into a codon that encodes a different amino acid.
- Silent - change a codon whereby the new mutant codon encodes the same amino acid.
- Frameshift – reading frame of the mRNA changes
- Back-mutation – mutation is reversed (genotype or phenotype)


DNA undergoing replication; a cytosine is incorporated opposite adenine by mistake
A : T
A : C
G : C
A : T
A : T
G : C
Codon
A A G
A G G
A A G
A A G
Amino Acid translated
Lysine
Arginine
Lysine
Lysine
Wild type
Mutant
Wild type
Wild type



Wild type
DNA mRNA
Amino acid
3' 5'
G C
C G
C G
A U
T A
G C
C G
A U
A U
T A
T A
T A
C G
5' 3'
Translation
Arg
Tyr
Val
Lys
Direction of transcription and translation
5'
3'
Mutant
DNA mRNA
Amino acid
3' 5'
G C
C G
C G
A U
T A
G C
C G
C G
A U
A U
T A
T A
T A
5' 3'
Translation
Arg
Tyr
Gly
Chain termination
Nucleotide added-C
Frameshift

Examples of chemical and radioactive mutagens, and their effects.

TABLE 9.3 Selected Mutagenic Agents and Their Effects

Agent	Effect
Chemical	
Nitrous acid, bisulfite	Removes an amino group from some bases
Ethidium bromide	Inserts between the paired bases
Acridine dyes	Cause frameshifts due to insertion between base pairs
Nitrogen base analogs	Compete with natural bases for sites on replicating DNA
Radiation	
Ionizing (gamma rays, X rays)	Form free radicals that cause single or double breaks in DNA
Ultraviolet	Causes cross-links between adjacent pyrimidines

Table 9.3 Selected mutagenic agents and their effects

Repair of mutations involves enzymes recognizing, removing, and replacing the bases.

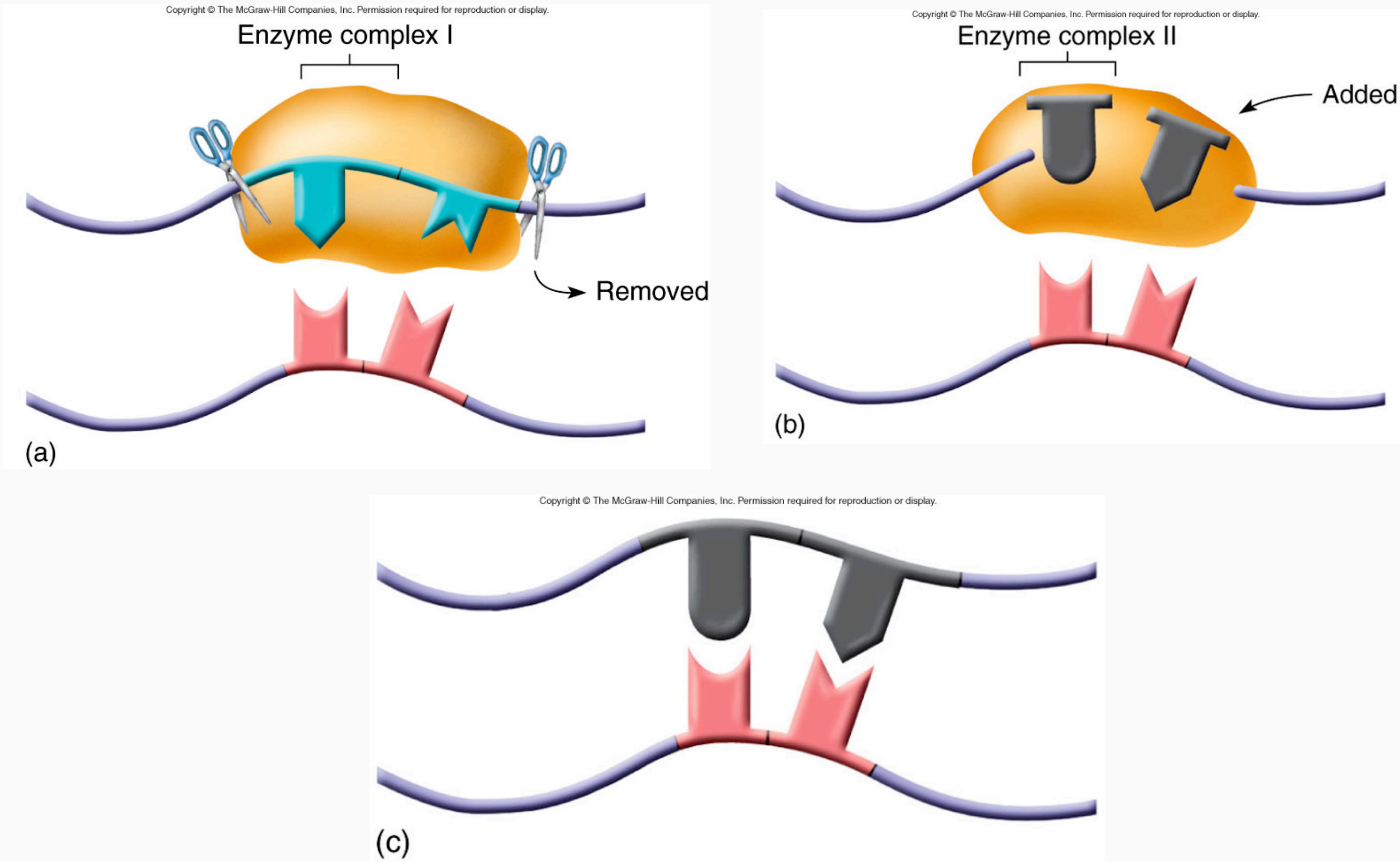


Fig. 9.22 Excision repair of mutation by enzymes

The Ames test is used to screen environmental and dietary chemicals for mutagenicity and carcinogenicity without using animal studies.

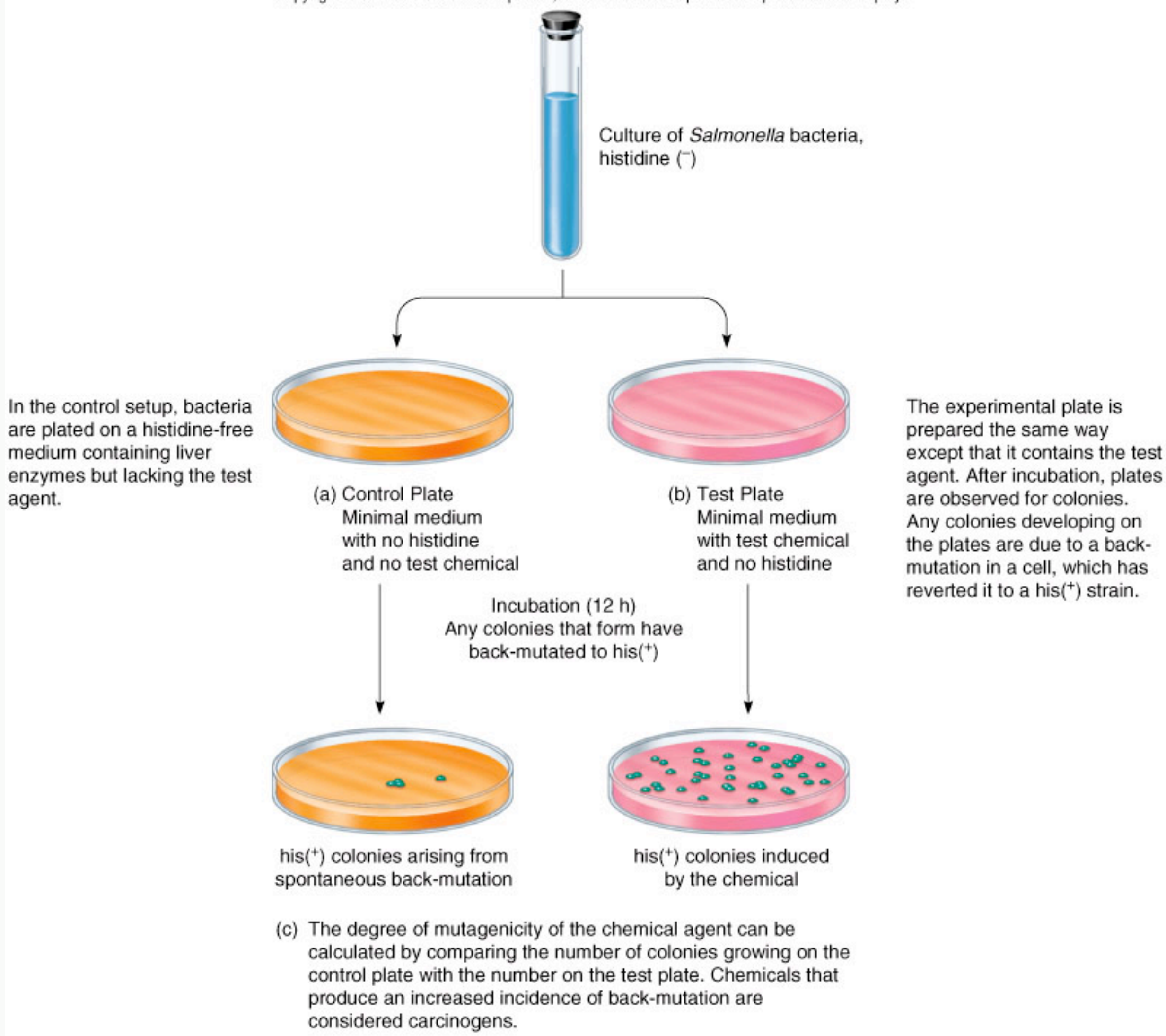


Fig. 9.23 The Ames test.

Effects of mutations

- Positive effects for the cell
  - Allow cells to adapt
- Negative effects for the cell
  - Loss of function
  - Cells cannot survive

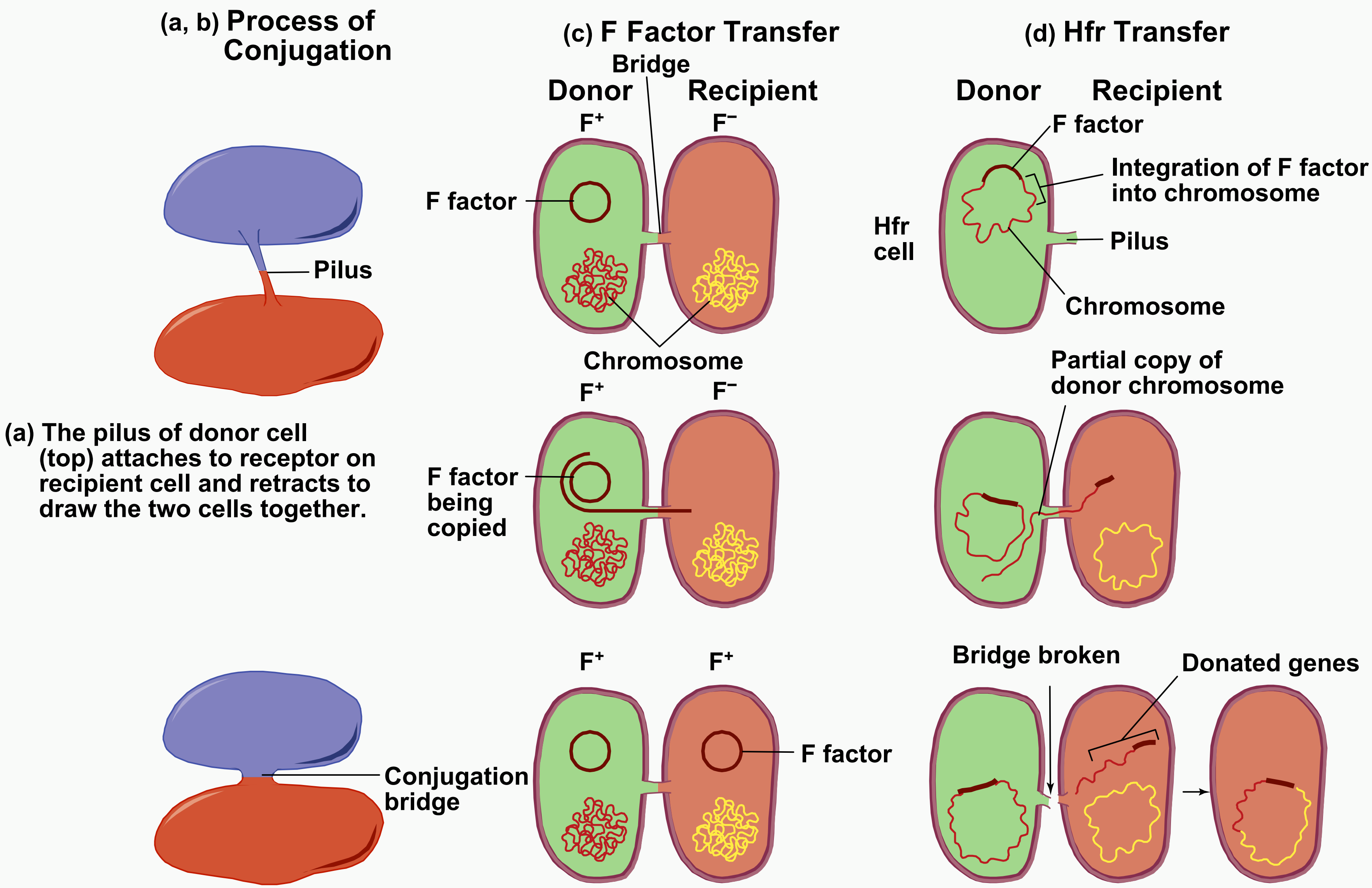
Recombination

- Sharing or recombining parts of the genome with foreign DNA
- DNA needs to be taken up:
  - Conjugation
  - Transformation
  - Transduction

Conjugation

- Transfer of plasmid DNA from a F^+ (F factor) cell (donor) to a F^- cell (recipient)
- An F^+ bacterium possesses a pilus
- Pilus attaches to the recipient cell and creates pore for the transfer DNA
- High frequency recombination (Hfr) donors contain the F factor in the chromosome

Fig. 9.24 Conjugation: genetic transmission from cell to cell



(a) The pilus of donor cell (top) attaches to receptor on recipient cell and retracts to draw the two cells together.

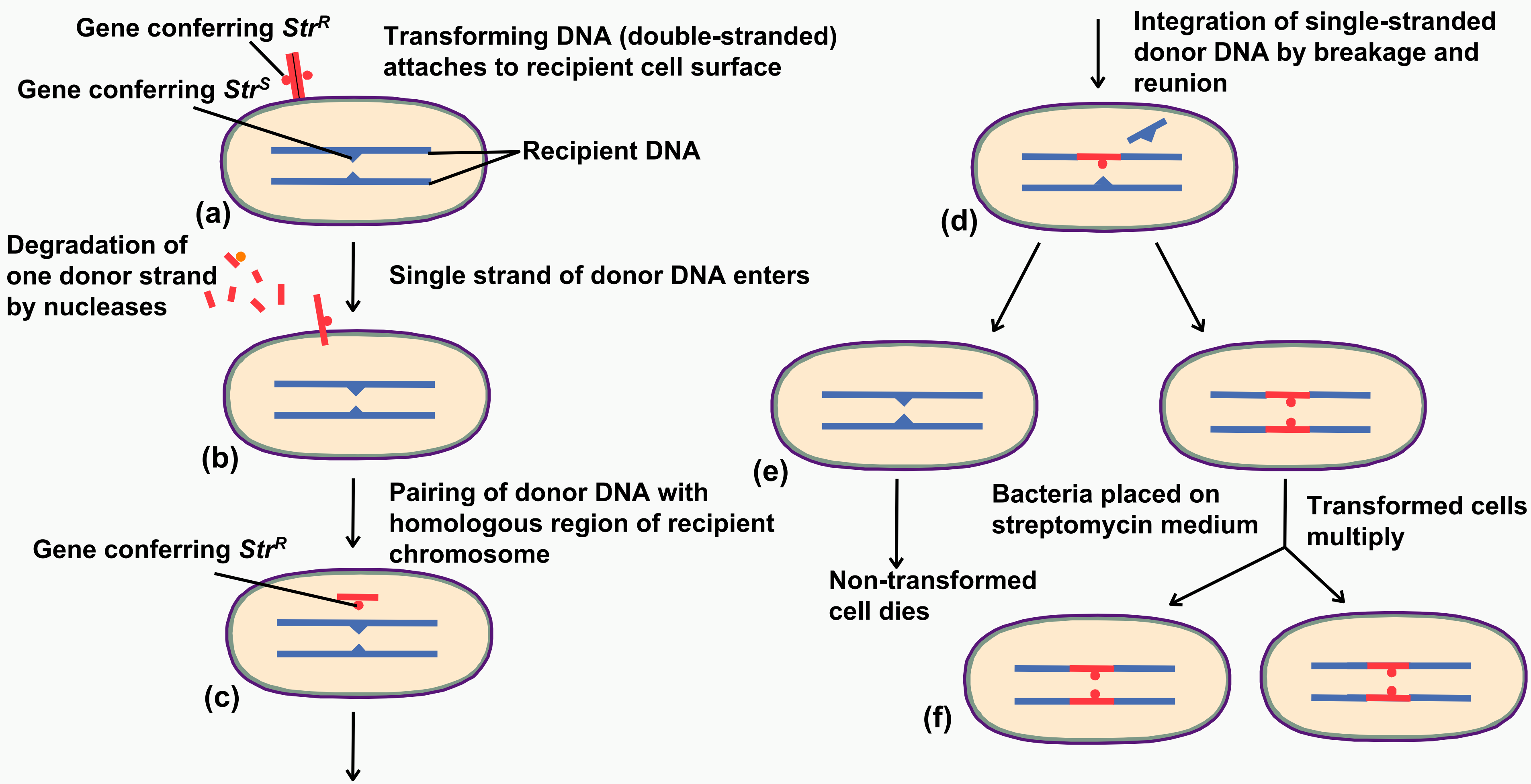
(b) An opening or pore forms between the cell walls, thereby creating a bridge to transmit genetic material.

(c) Transfer of the F factor, or conjugative plasmid. A cell must have this plasmid to transfer chromosomal genes.

(d) High-frequency (Hfr) transfer involves transmission of chromosomal genes from a donor cell to a recipient cell. The donor chromosome is duplicated and transmitted in part to a recipient cell, where it is integrated into the chromosome.

Transformation

- Nonspecific acceptance of free DNA by the cell (ex. DNA fragments, plasmids)
- DNA can be inserted into the chromosome (by recombination)
- "Competent" cells readily accept DNA


Gene conferring Str^R
Transforming DNA (double-stranded) attaches to recipient cell surface
Gene conferring Str^S
Recipient DNA
(a)
Degradation of one donor strand by nucleases
Single strand of donor DNA enters
(b)
Pairing of donor DNA with homologous region of recipient chromosome
Gene conferring Str^R
(c)
Integration of single-stranded donor DNA by breakage and reunion
(d)
(e)
Bacteria placed on streptomycin medium
Transformed cells multiply
Non-transformed cell dies
(f)

DNA released from a killed cell can be accepted by a live competent cell, expressing a new phenotype.

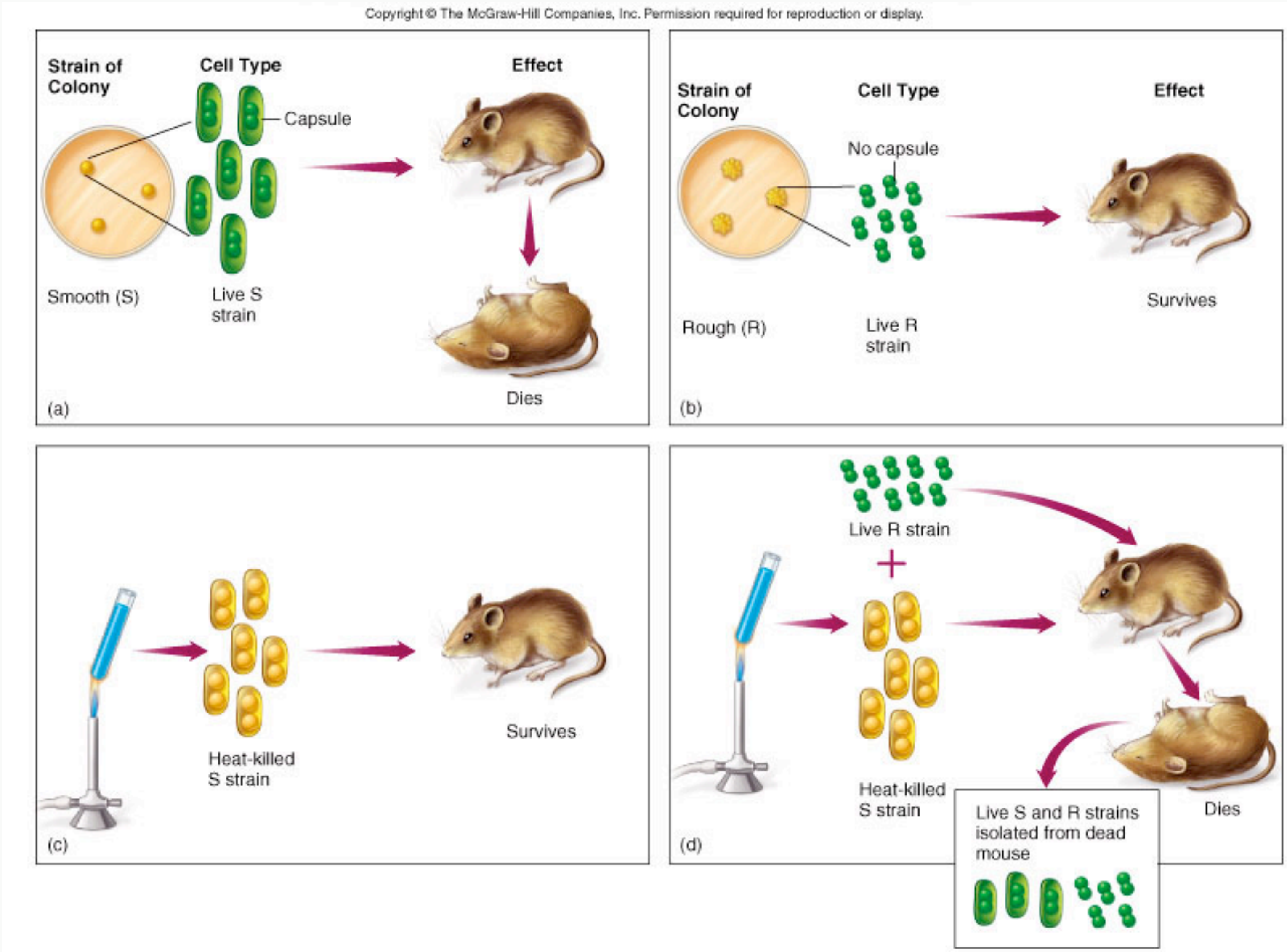
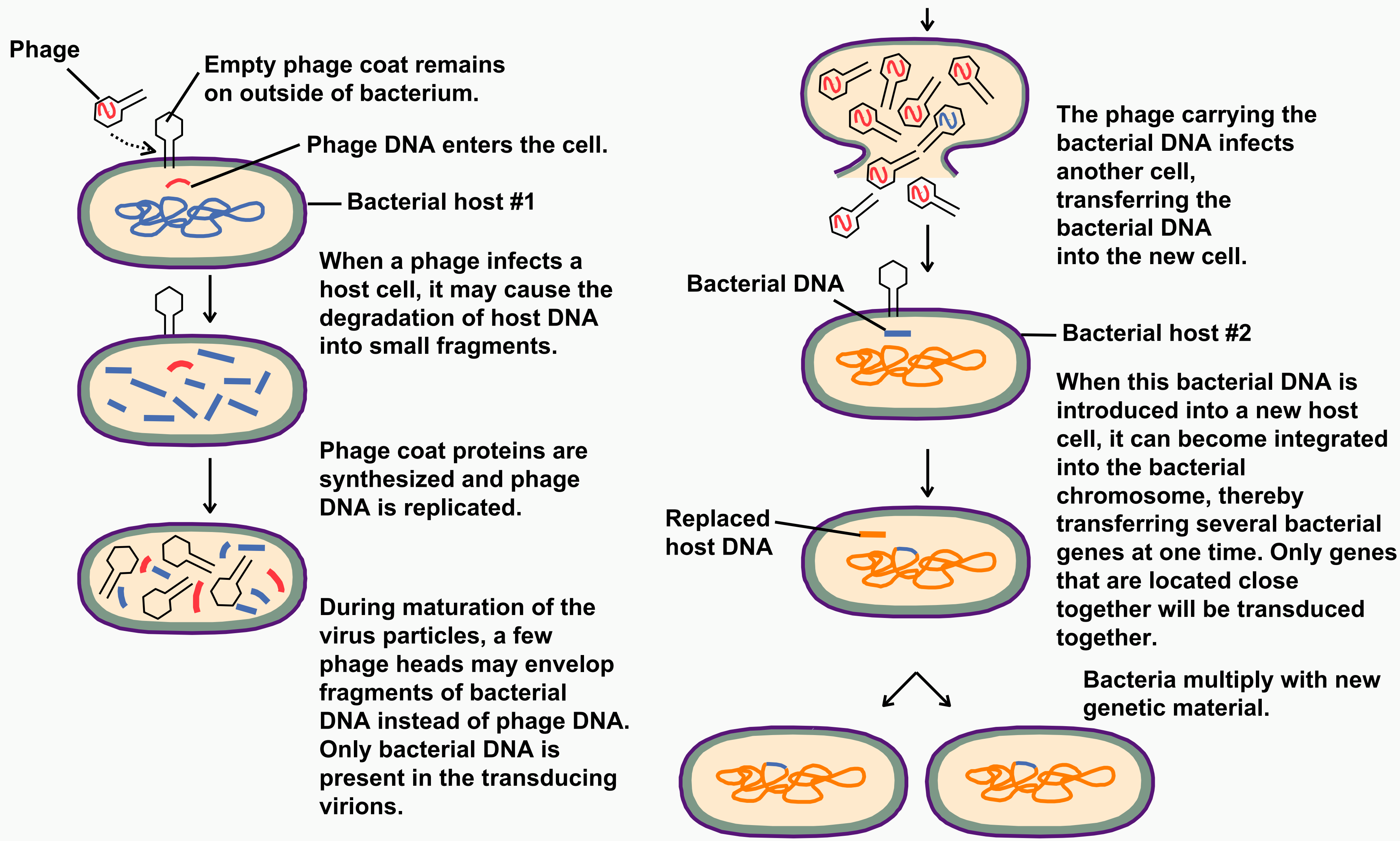


Fig. 9.25 Griffith's classic experiment in transformation

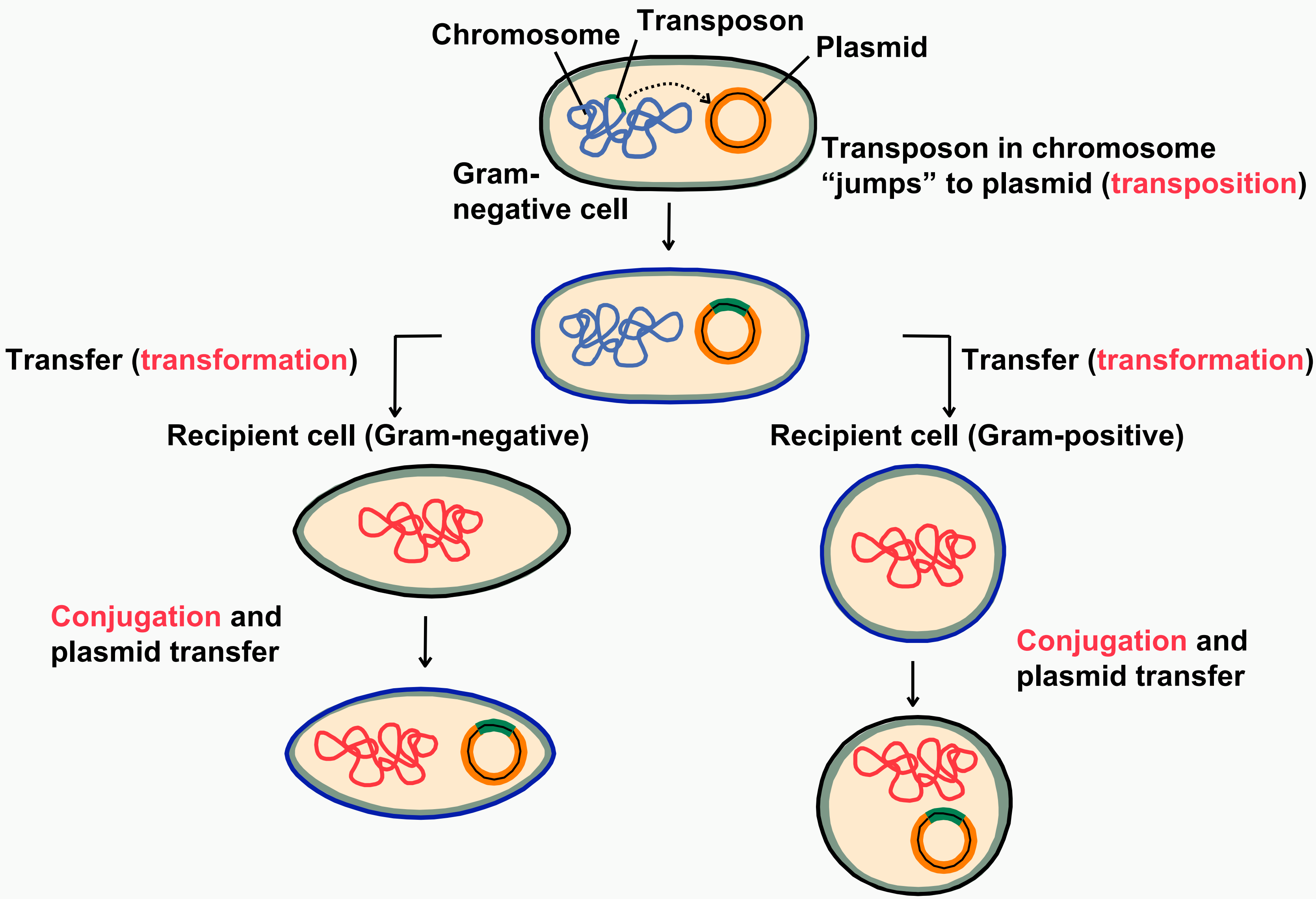
Transduction

- Bacterial virus (bacteriophage) infects bacterial host cells
- Bacteriophage can serve as the carrier of DNA from a bacterial donor cell to a recipient cell


Phage
Empty phage coat remains on outside of bacterium.
Phage DNA enters the cell.
Bacterial host #1
When a phage infects a host cell, it may cause the degradation of host DNA into small fragments.
Phage coat proteins are synthesized and phage DNA is replicated.
During maturation of the virus particles, a few phage heads may envelop fragments of bacterial DNA instead of phage DNA. Only bacterial DNA is present in the transducing virions.
The phage carrying the bacterial DNA infects another cell, transferring the bacterial DNA into the new cell.
Bacterial DNA
Bacterial host #2
When this bacterial DNA is introduced into a new host cell, it can become integrated into the bacterial chromosome, thereby transferring several bacterial genes at one time. Only genes that are located close together will be transduced together.
Replaced host DNA
Bacteria multiply with new genetic material.

Transposon

- “Jumping genes”
- Exist in plasmids and chromosomes
- Contains genes that encode for enzymes that excise and reintegrate the transposon
- Small transposons are called insertion elements


Chromosome
Transposon
Plasmid
Transposon in chromosome
“jumps” to plasmid (transposition)
Gram-
negative cell
Transfer (transformation)
Transfer (transformation)
Recipient cell (Gram-negative)
Recipient cell (Gram-positive)
Conjugation and
plasmid transfer
Conjugation and
plasmid transfer