Chapter 13

Microbial Recombination and Plasmids

Eucaryotic recombination

- recombination
  - process in which one or more nucleic acid molecules are rearranged or combined to produce a new nucleotide sequence
- in eucaryotes, usually occurs as the result of crossing-over during meiosis

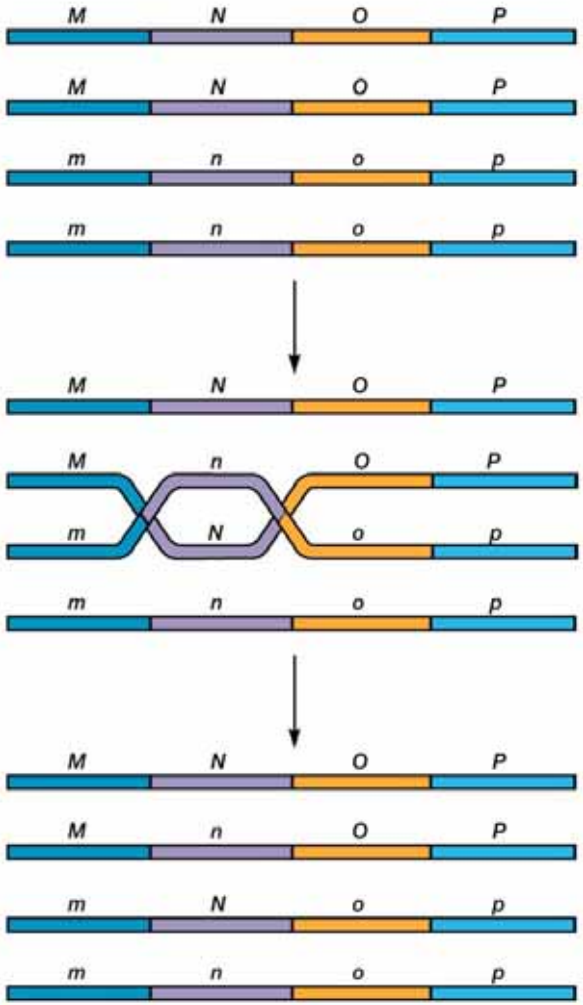


Figure 13.1

Bacterial Recombination: General Principles

- several types of recombination
  - general recombination
    - can be reciprocal or nonreciprocal
  - site-specific recombination
  - replicative recombination

Reciprocal general recombination

- most common type of recombination
- a reciprocal exchange between pair of homologous chromosomes
- results from DNA strand breakage and reunion, leading to crossing-over

Reciprocal general recom-bination

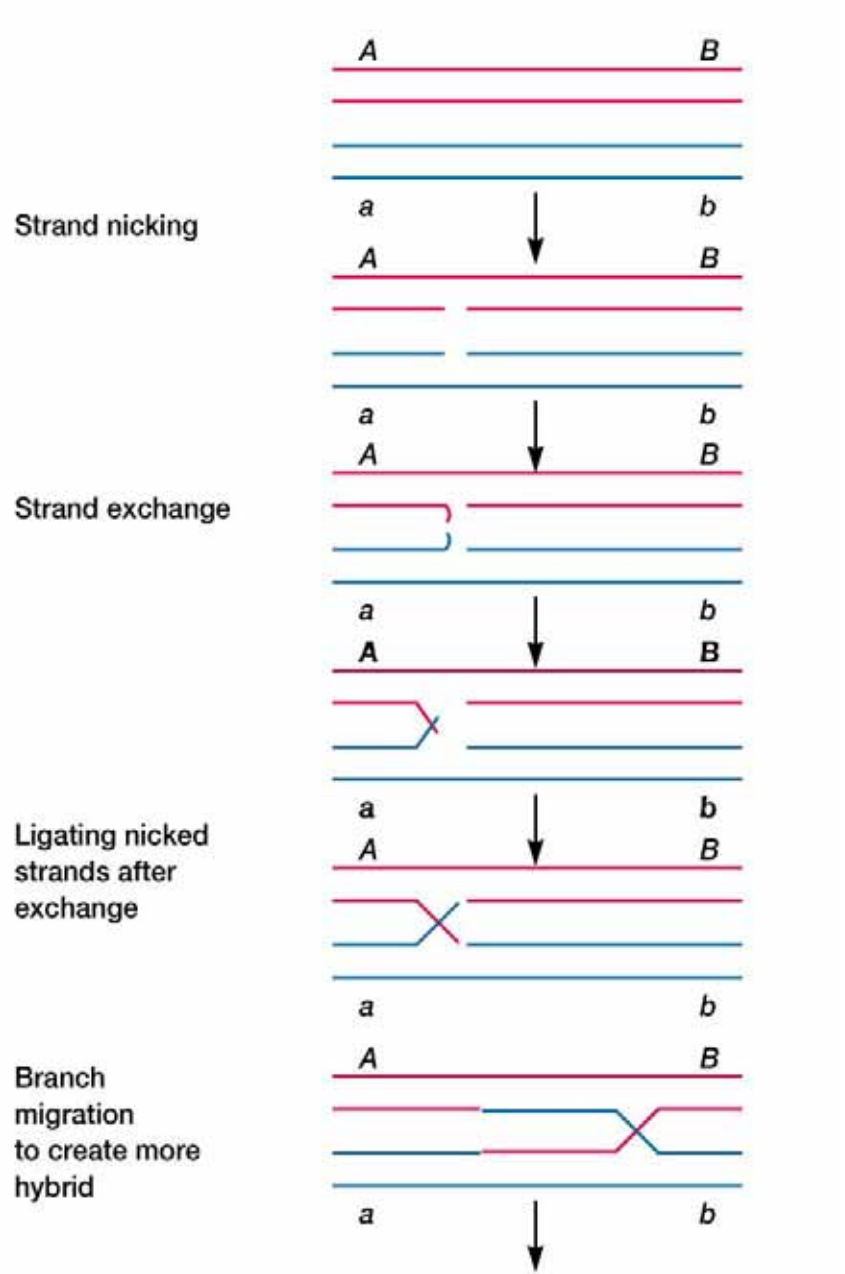


Figure 13.2

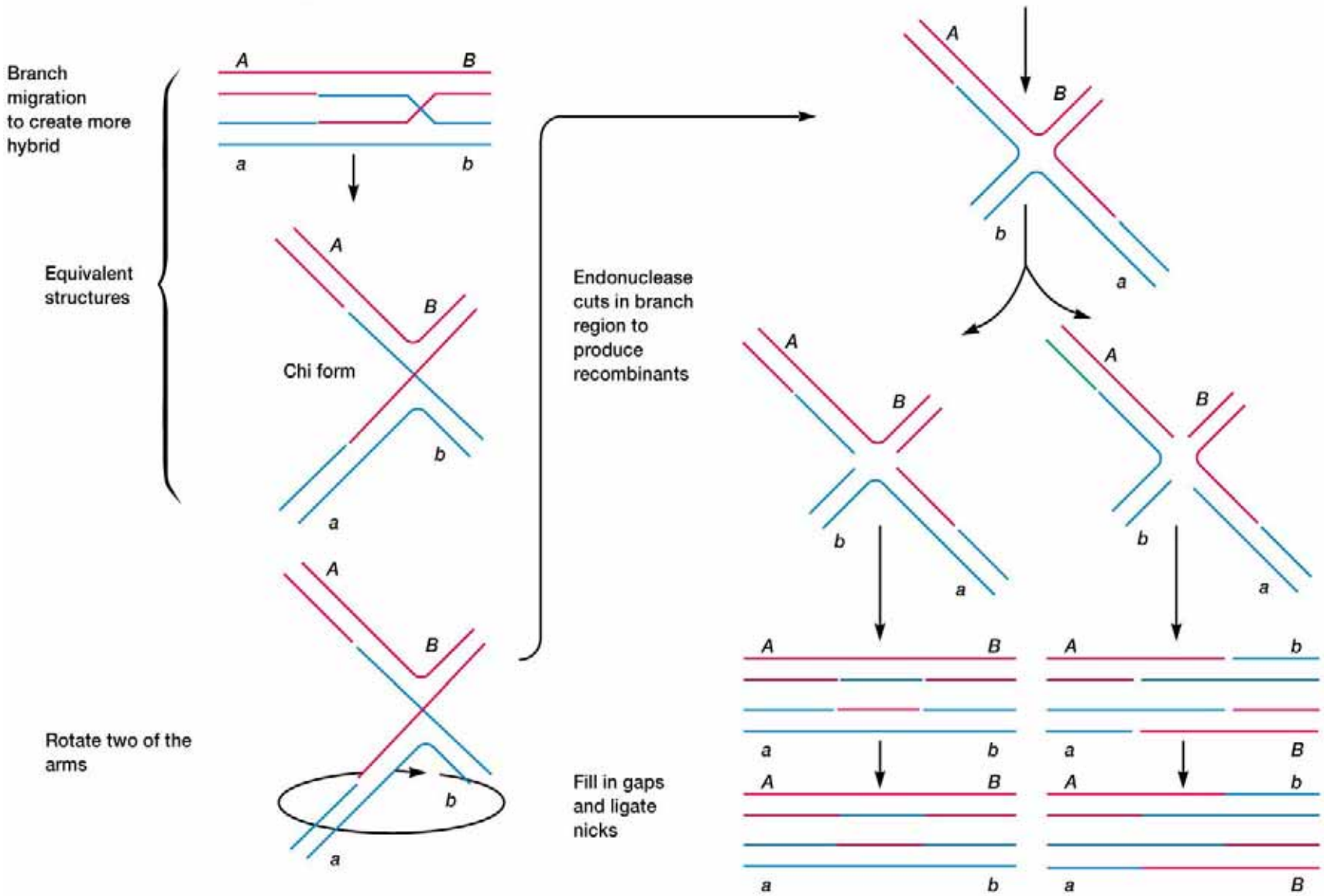

Branch migration to create more hybrid
Equivalent structures
Chi form
Rotate two of the arms
Endonuclease cuts in branch region to produce recombinants
Fill in gaps and ligate nicks
A
B
a
b

Figure 13.2

Nonreciprocal general recombination

- incorporation of single strand of DNA into chromosome, forming a stretch of heteroduplex DNA
- proposed to occur during bacterial transformation

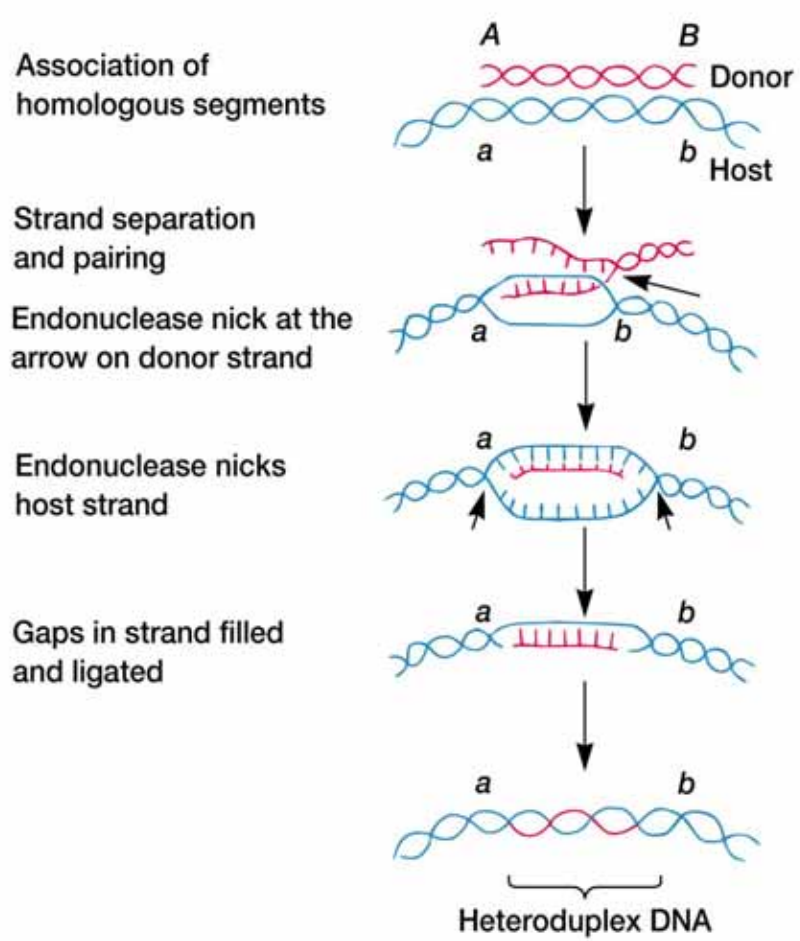


Figure 13.3

Site-specific recombination

- insertion of nonhomologous DNA into a chromosome
- often occurs during viral genome integration into host chromosome
  - enzymes responsible are specific for virus and its host

Replicative recombination

- accompanies replication of genetic material
- used by genetic elements that move about the genome

Horizontal gene transfer

- transfer of genes from one mature, independent organism (donor) to another (recipient)
- exogenote
  - DNA that is transferred to recipient
- endogenote
  - genome of recipient
- merozyogote
  - recipient cell that is temporarily diploid as result of transfer process

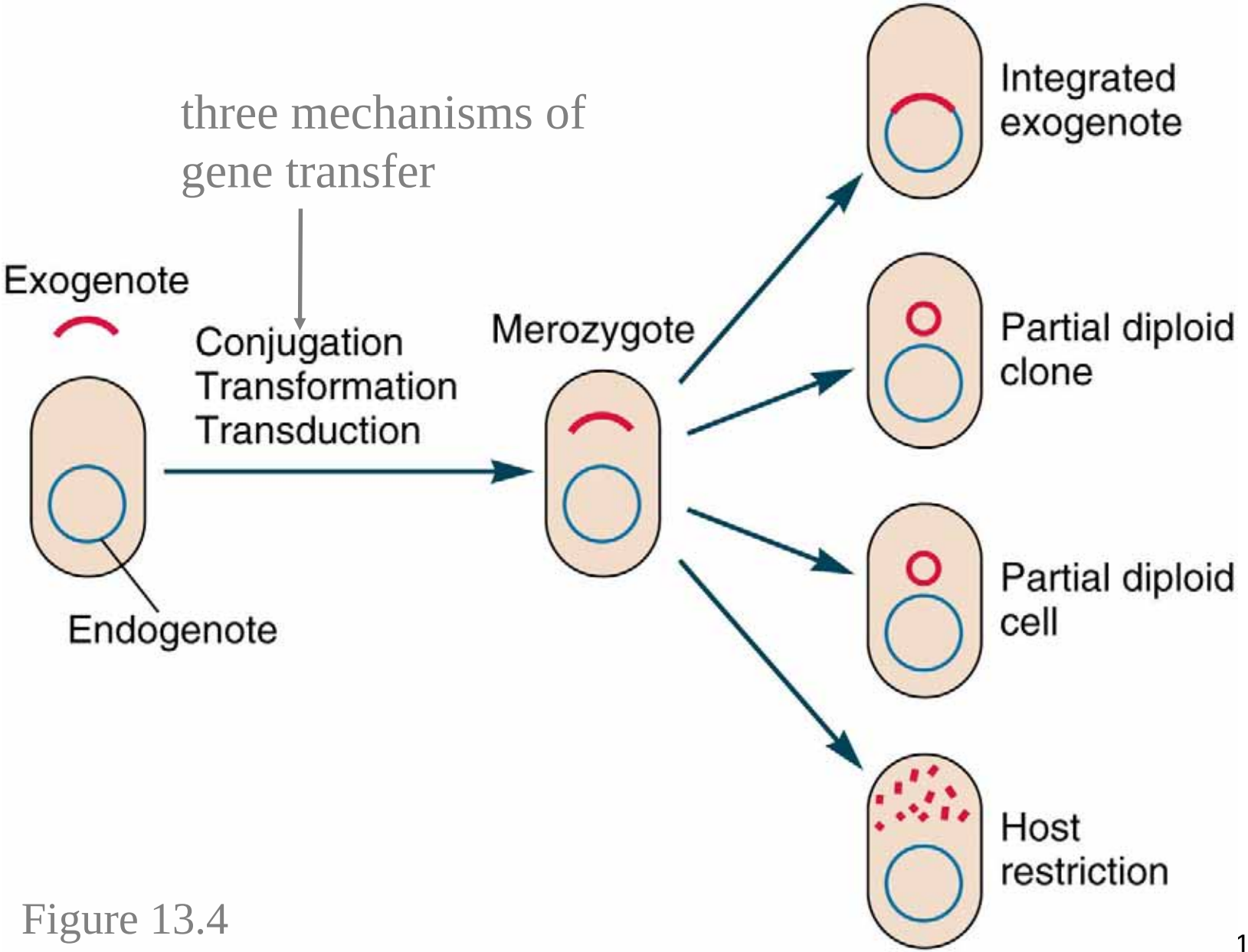

three mechanisms of
gene transfer
Exogenote
Conjugation
Transformation
Transduction
Endogenote
Merozygote
Integrated
exogenote
Partial diploid
clone
Partial diploid
cell
Host
restriction

Figure 13.4

Bacterial Plasmids

- small, double-stranded, usually circular DNA molecules
- are replicons
  - have their own origin of replication
  - can exist as single copies or as multiple copies
- curing
  - elimination of plasmid
  - can be spontaneous or induced by treatments that inhibit plasmid replication but not host cell reproduction

Bacterial plasmids…

- episomes
  - plasmids that can exist either with or without integrating into chromosome
- conjugative plasmids
  - have genes for pili
  - can transfer copies of themselves to other bacteria during conjugation

Table 13.1 Major Types of Plasmids

Type	Representatives	Approximate Size (kbp)	Copy Number (Copies/Chromosome)	Hosts	Phenotypic Features[a]
Fertility Factor[b]	F factor	95–100	1–3	*E. coli, Salmonella, Citrobacter*	Sex pilus, conjugation
R Plasmids	RP4	54	1–3	*Pseudomonas* and many other gram-negative bacteria	Sex pilus, conjugation, resistance to Ap, Km, Nm, Tc
	R1	80	1–3	Gram-negative bacteria	Resistance to Ap, Km, Su, Cm, Sm
	R6	98	1–3	*E. coli, Proteus mirabilis*	Su, Sm, Cm, Tc, Km, Nm
	R100	90	1–3	*E. coli, Shigella, Salmonella, Proteus*	Cm, Sm, Su, Tc, Hg
	pSH6	21		*Staphylococcus aureus*	Gm, Tm, Km
	pSJ23a	36		*S. aureus*	Pn, Asa, Hg, Gm, Km, Nm, Em, etc.
	pAD2	25		*Enterococcus faecalis*	Em, Km, Sm
Col Plasmids	ColE1	9	10–30	*E. coli*	Colicin E1 production
	ColE2		10–15	*Shigella*	Colicin E2
	CloDF13			*Enterobacter cloacae*	Cloacin DF13
Virulence Plasmids	Ent (P307)	83		*E. coli*	Enterotoxin production
	K88 plasmid			*E. coli*	Adherence antigens
	ColV-K30	2		*E. coli*	Siderophore for iron uptake; resistance to immune mechanisms
	pZA10	56		*S. aureus*	Enterotoxin B
	Ti	200	ted into it	*Agrobacter tumefaciens*	Tumor induction
Metabolic Plasmids	CAM	230		*Pseudomonas*	Camphor degradation
	SAL	56		*Pseudomonas*	Salicylate degradation
	TOL	75		*Pseudomonas putida*	Toluene degradation
	pJP4			*Pseudomonas*	2,4-dichlorophenoxyacetic acid degradation
				E. coli, Klebsiella, Salmonella	Lactose degradation
				Providencia	Urease
	sym			*Rhizobium*	Nitrogen fixation and symbiosis

Fertility Factors

- conjugative plasmids
- e.g., F factor of *E. coli*
- many are also episomes

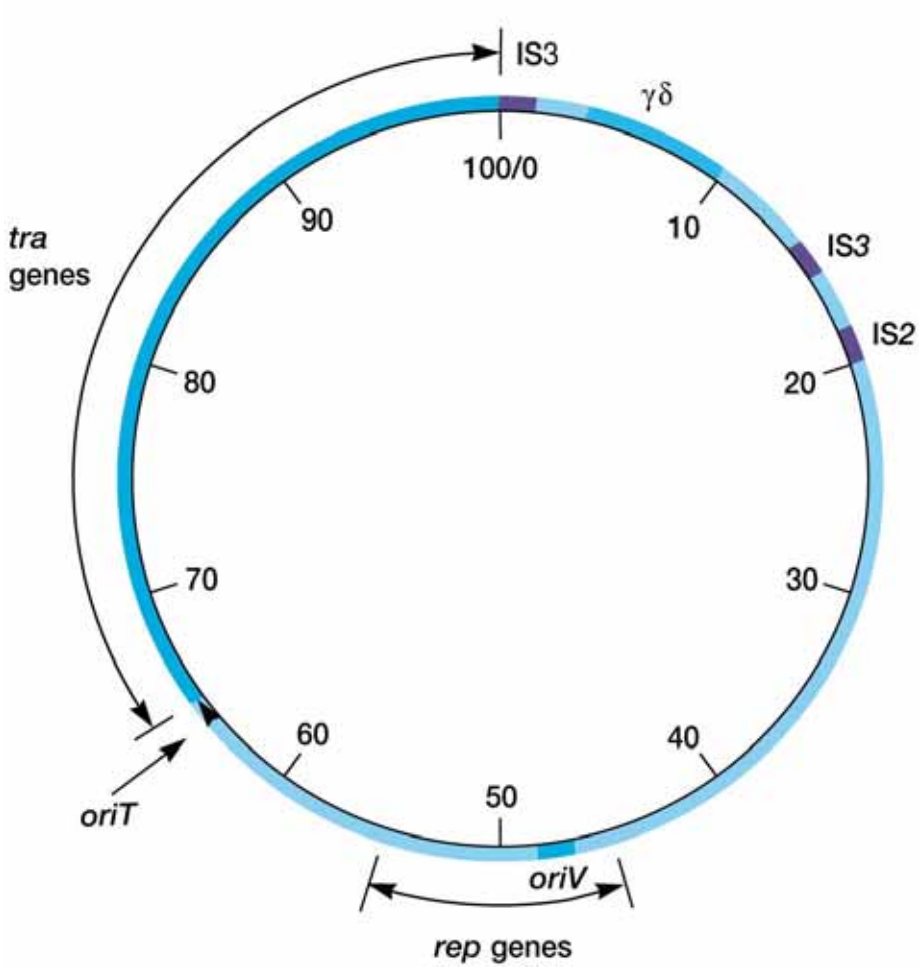


Figure 13.5

F plasmid integration

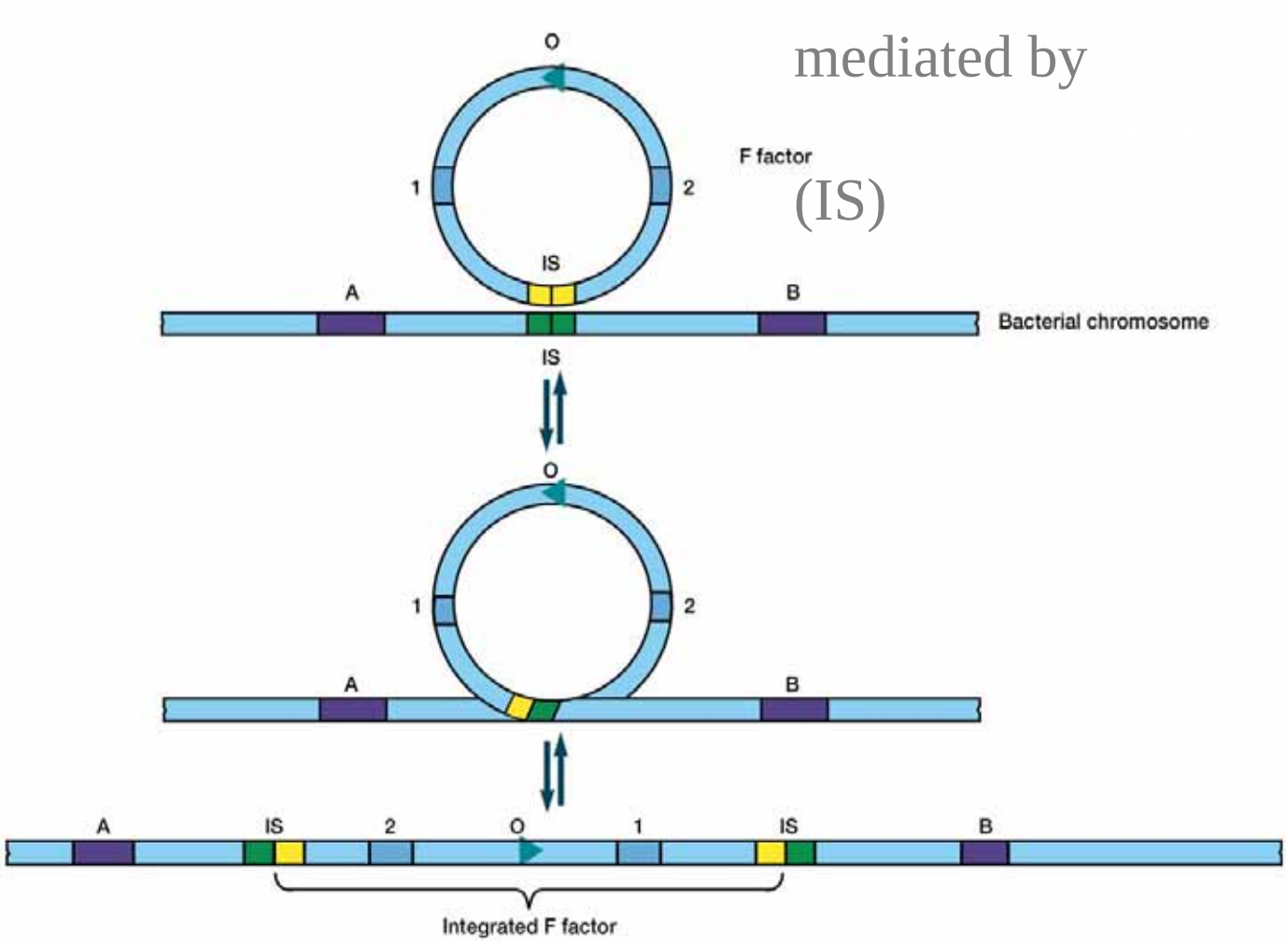


Figure 13.7

Resistance Factors

- R factors (plasmids)
- have genes for resistance to antibiotics
- some are conjugative
- usually do not integrate into chromosome

Col plasmids

- encode colicin
  - kills *E. coli*
  - a type of bacteriocin
    - protein that destroys other bacteria, usually closely related species
- some are conjugative
- some carry resistance genes

Other Types of Plasmids

- virulence plasmids
  - carry virulence genes
    - e.g., genes that confer resistance to host defense mechanisms
    - e.g., genes that encode toxins
- metabolic plasmids
  - carry genes for metabolic processes
    - e.g., genes encoding degradative enzymes for pesticides
    - e.g., genes for nitrogen fixation

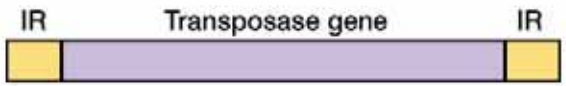
Transposable Elements

- transposition
  - the movement of pieces of DNA around the genome
- transposable elements (transposons)
  - segments of DNA that carry genes for transposition
- widespread in bacteria, eucaryotes and archaea

Types of transposable elements

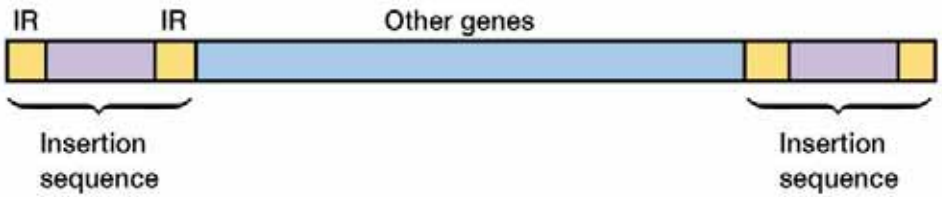
- insertion sequences (IS elements)
  - contain only genes encoding enzymes required for transposition
    - e.g., transposase
- composite transposons
  - carry genes in addition to those needed for transposition
  - conjugative transposons
    - carry transfer genes in addition to transposition genes

Insertion sequence



IR = inverted repeats

A composite transposon



A target site for the Tn*3* transposon

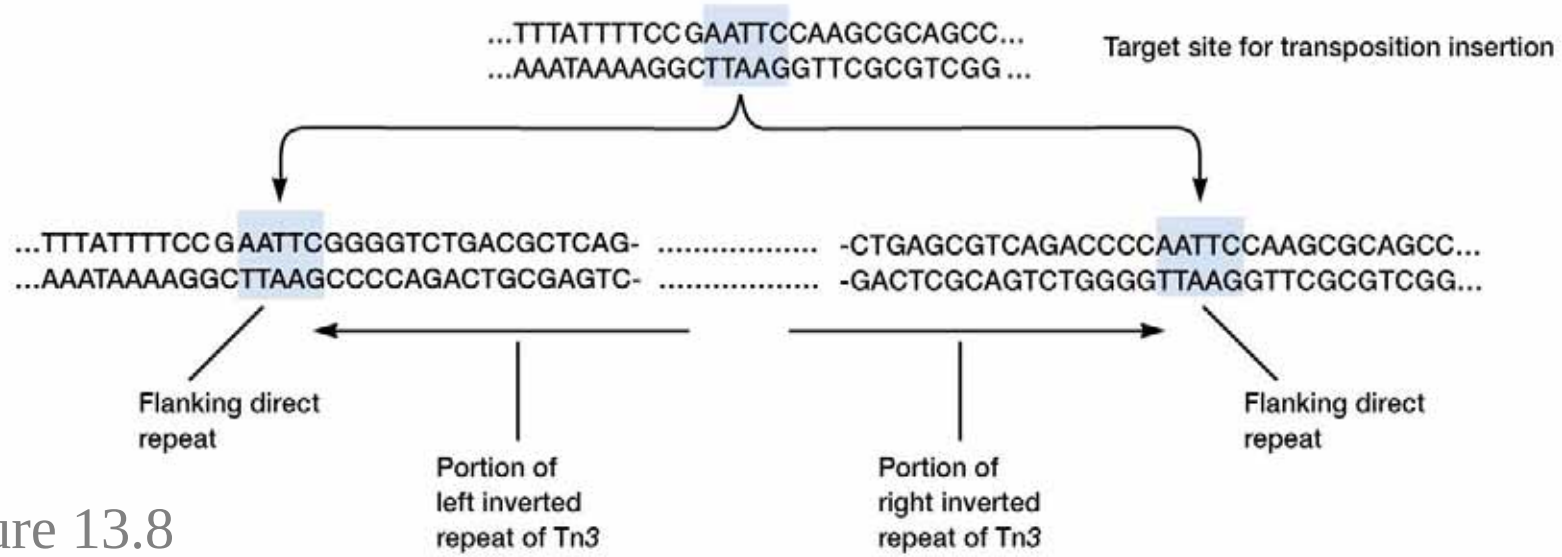


Figure 13.8

Table 13.2 The Properties of Selected Insertion Sequences

Insertion Sequence	Length (bp)	Inverted Repeat (Length in bp)	Target Site (Length in bp)	Number of Copies on *E. coli* Chromosome
IS*1*	768	23	9 or 8	6–10
IS*2*	1,327	41	5	4–13(1)[a]
IS*3*	1,400	38	3–4	5–6(2)
IS*4*	1,428	18	11 or 12	1–2
IS*5*	1,195	16	4	10–11

[a]The value in parentheses indicates the number of IS elements on the F factor.

Table 13.3 The Properties of Selected Composite Transposons

Transposon	Length (bp)	Terminal Repeat Length	Terminal Module	Genetic Markers[a]
Tn*3*	4,957	38		Ap
Tn*501*	8,200	38		Hg
Tn*951*	16,500	Unknown		Lactose utilization
Tn*5*	5,700		IS*50*	Km
Tn*9*	2,500		IS*1*	Cm
Tn*10*	9,300		IS*10*	Tc
Tn*903*	3,100		IS*903*	Km
Tn*1681*	2,061		IS*1*	Heat-stable enterotoxin
Tn*2901*	11,000		IS*1*	Arginine biosynthesis

[a]Abbreviations for antibiotics and metals same as in table 13.1.

The transposition event

- usually transposon replicated, remaining in original site, while duplicate inserts at another site
- insertion generates direct repeats of flanking host DNA

Tn3 trans-position

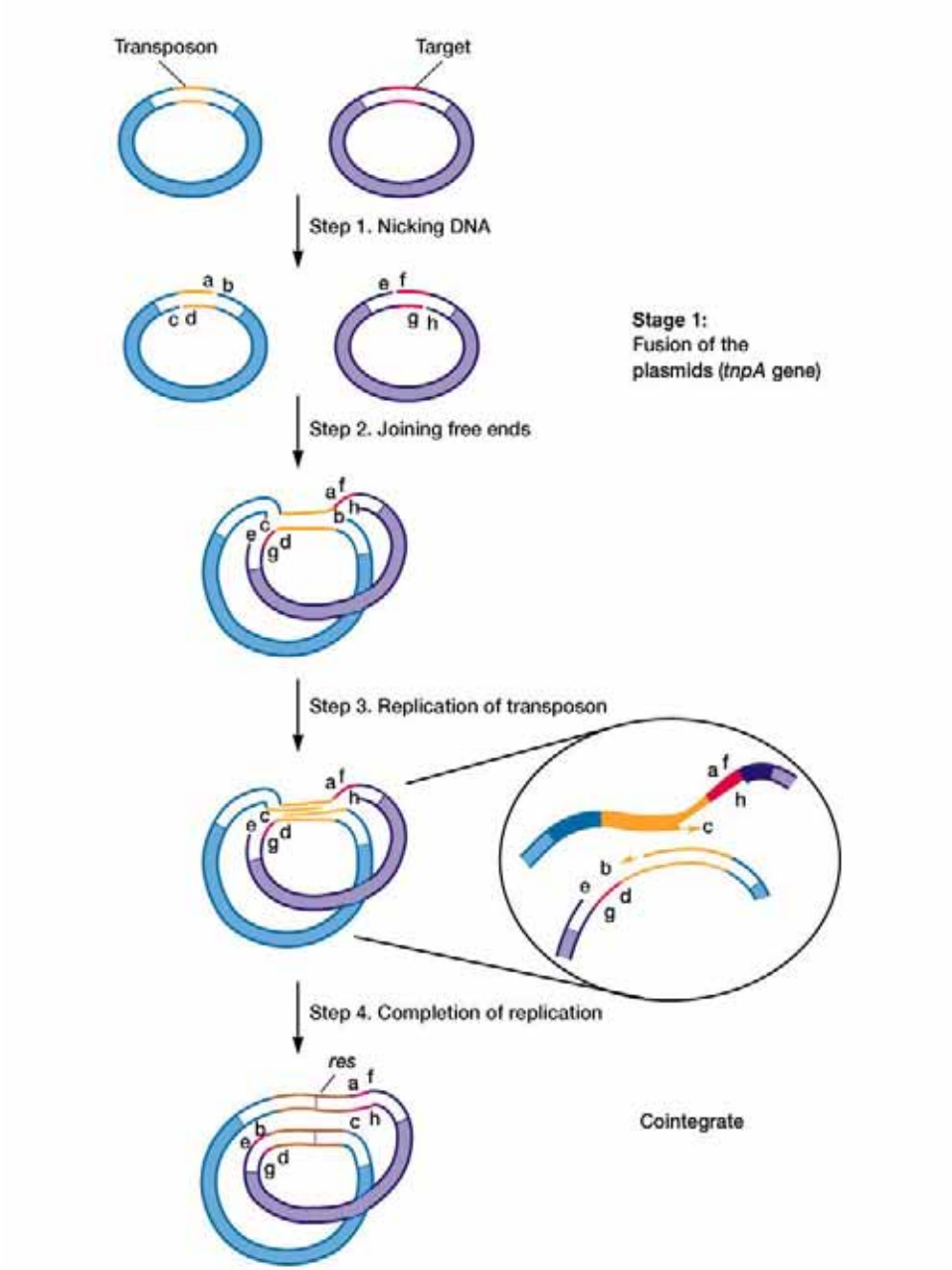


Figure 13.10

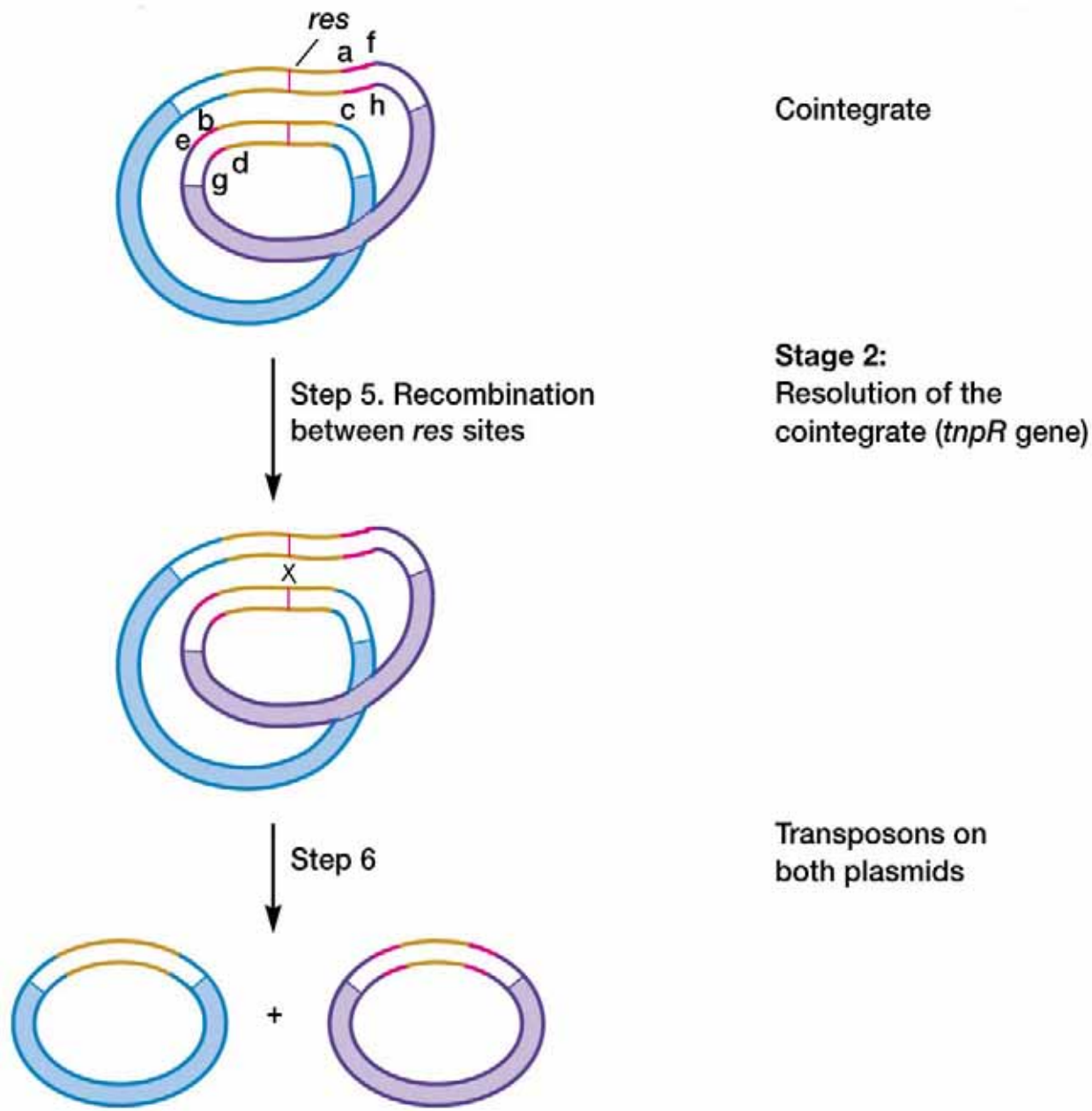

res
a
f
c
h
b
e
d
g
Cointegrate
Step 5. Recombination between res sites
Stage 2:
Resolution of the cointegrate (tnpR gene)
X
Step 6
Transposons on both plasmids
+

Figure 13.10

Generation of direct repeats

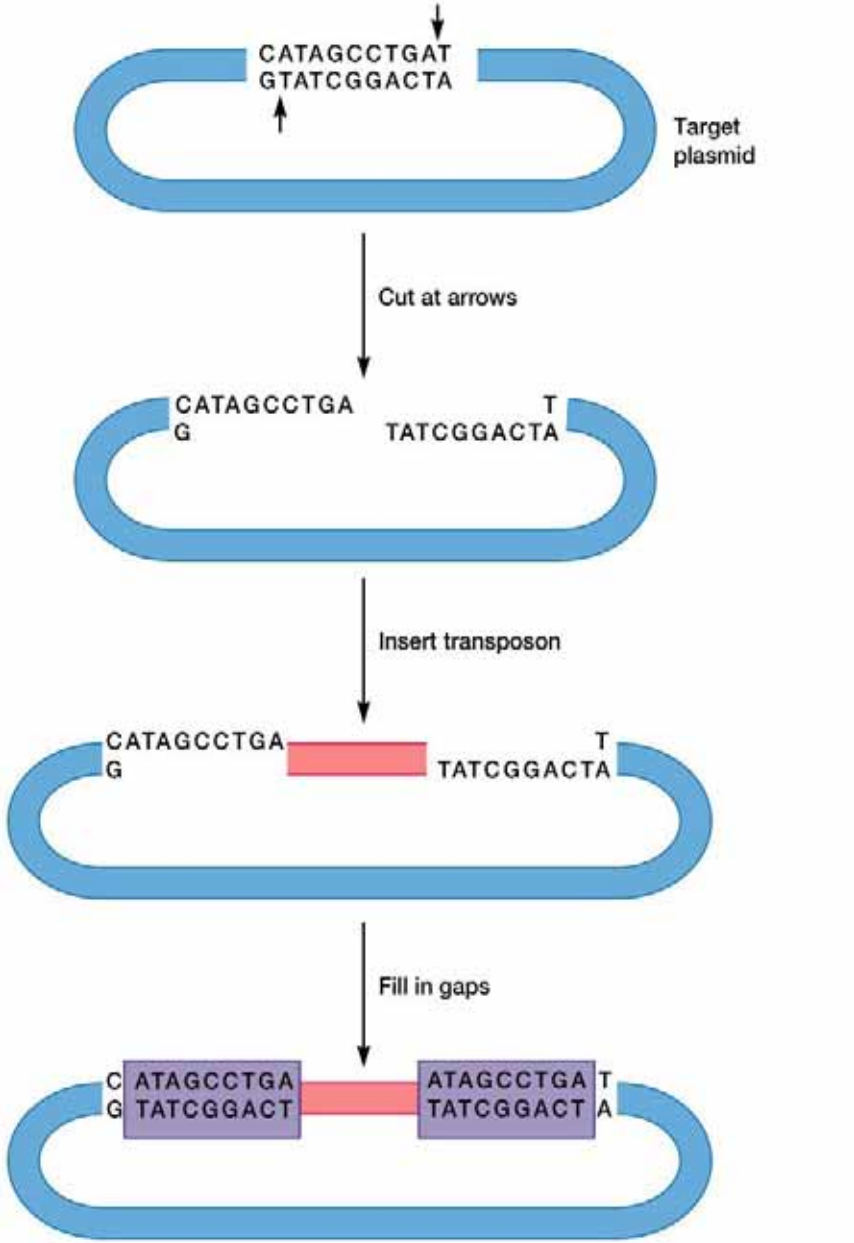


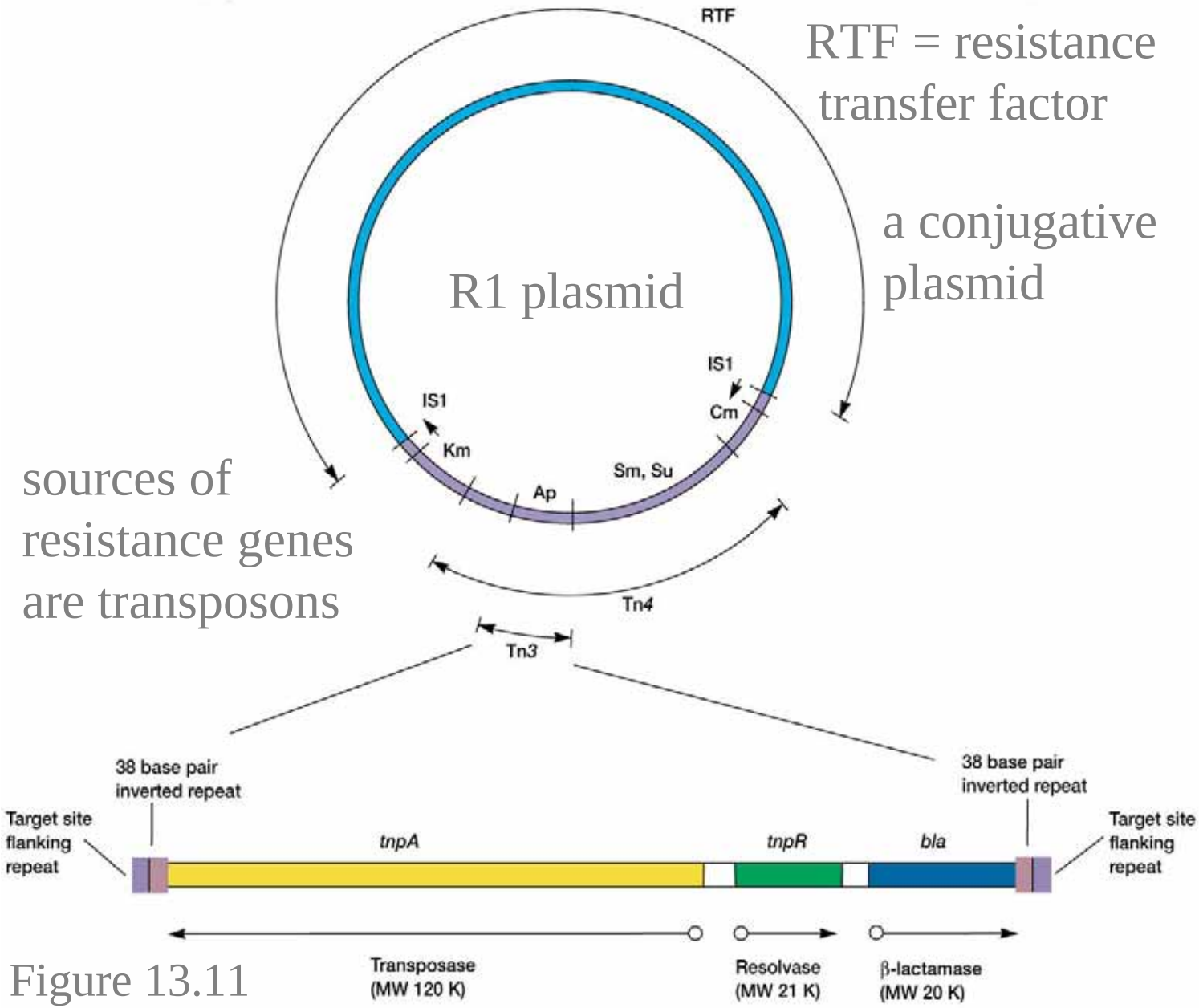
Figure 13.9

Effects of transposition

- mutation in coding region
  - e.g., deletion of genetic material
- arrest of translation or transcription
- activation of genes
- generation of new plasmids
  - e.g., resistance plasmids

RTF = resistance transfer factor

a conjugative plasmid



sources of resistance genes are transposons

Figure 13.11

Bacterial Conjugation

- transfer of DNA by direct cell to cell contact
- discovered 1946 by Lederberg and Tatum

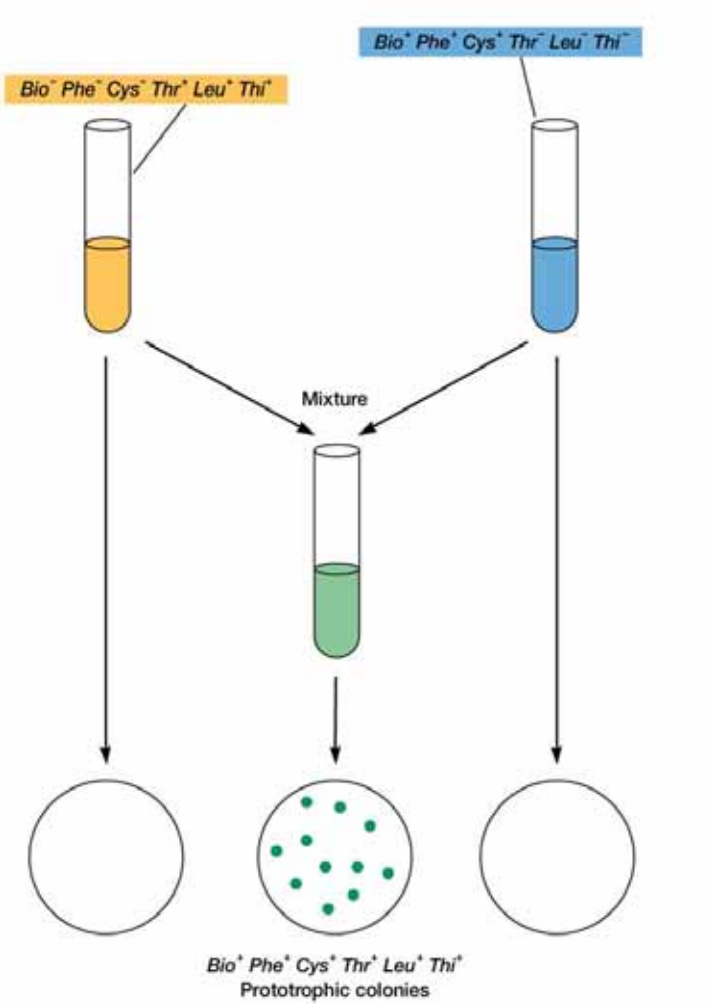
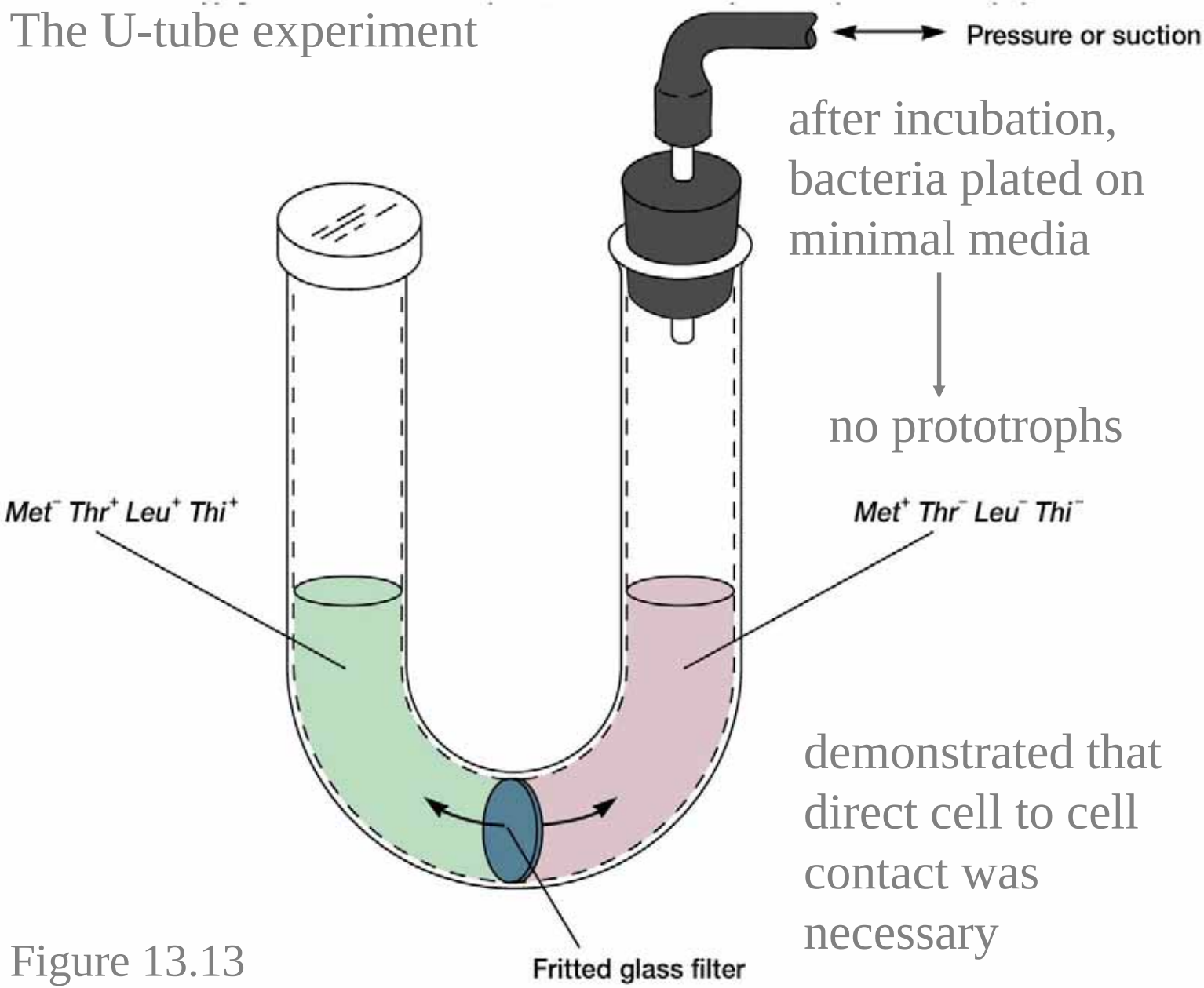


Figure 13.12

The U-tube experiment



after incubation, bacteria plated on minimal media

no prototrophs

demonstrated that direct cell to cell contact was necessary

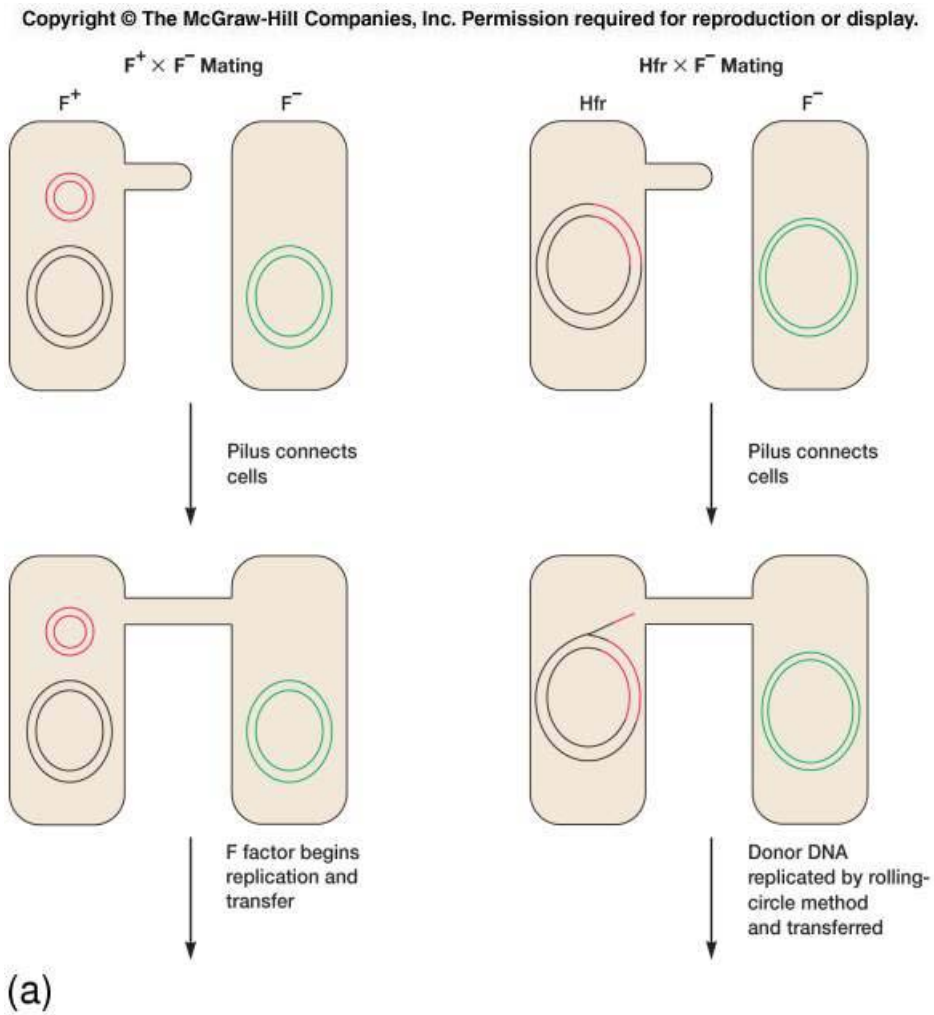
Figure 13.13

F^+ x F^- Mating

- F^+ = donor
  - contains F factor
- F^- = recipient
  - does not contain F factor
- F factor replicated by rolling-circle mechanism and duplicate is transferred
- recipients usually become F^+
- donor remains F^+

F^+ x F^- mating

Figure 13.14a



Hfr Conjugation

- Hfr strain
  - donor having F factor integrated into its chromosome
- both plasmid genes and chromosomal genes are transferred

Hfr x F⁻ mating

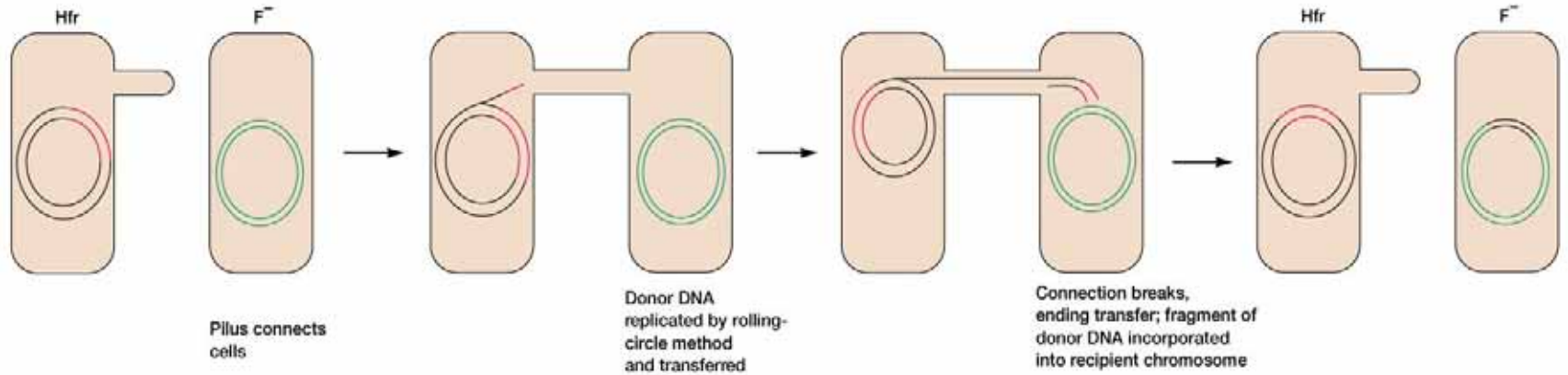


Figure 13.14b

F′ Conjugation

- F′ plasmid
  - formed by incorrect excision from chromosome
  - contains ≥ 1 genes from chromosome
- F′ cell can transfer F′ plasmid to recipient

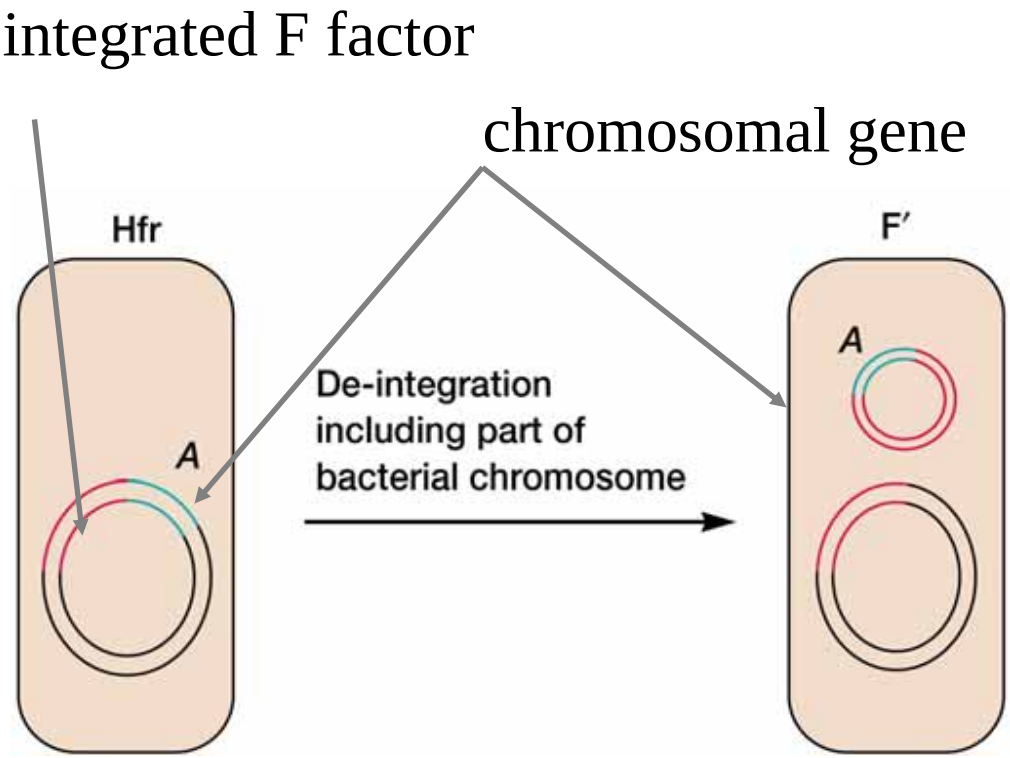


Figure 13.15a

F′ x F⁻ mating

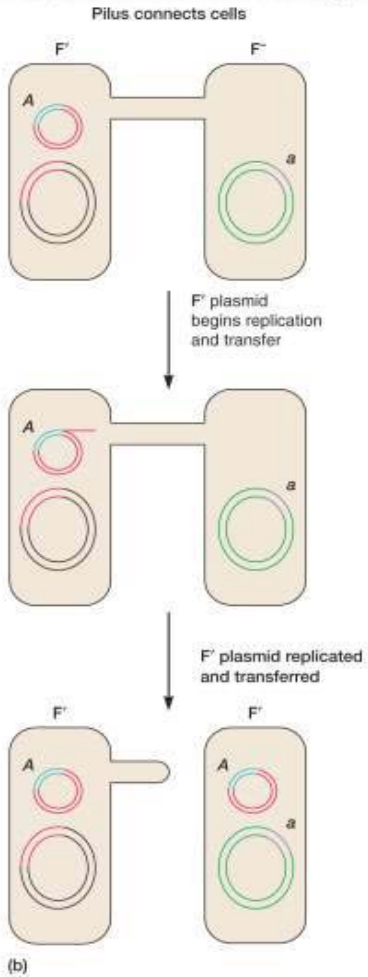


Figure 13.15b

DNA Transformation

- uptake of naked DNA molecule from the environment and incorporation into recipient in a heritable form
- competent cell
  - capable of taking up DNA
- may be important route of genetic exchange in nature

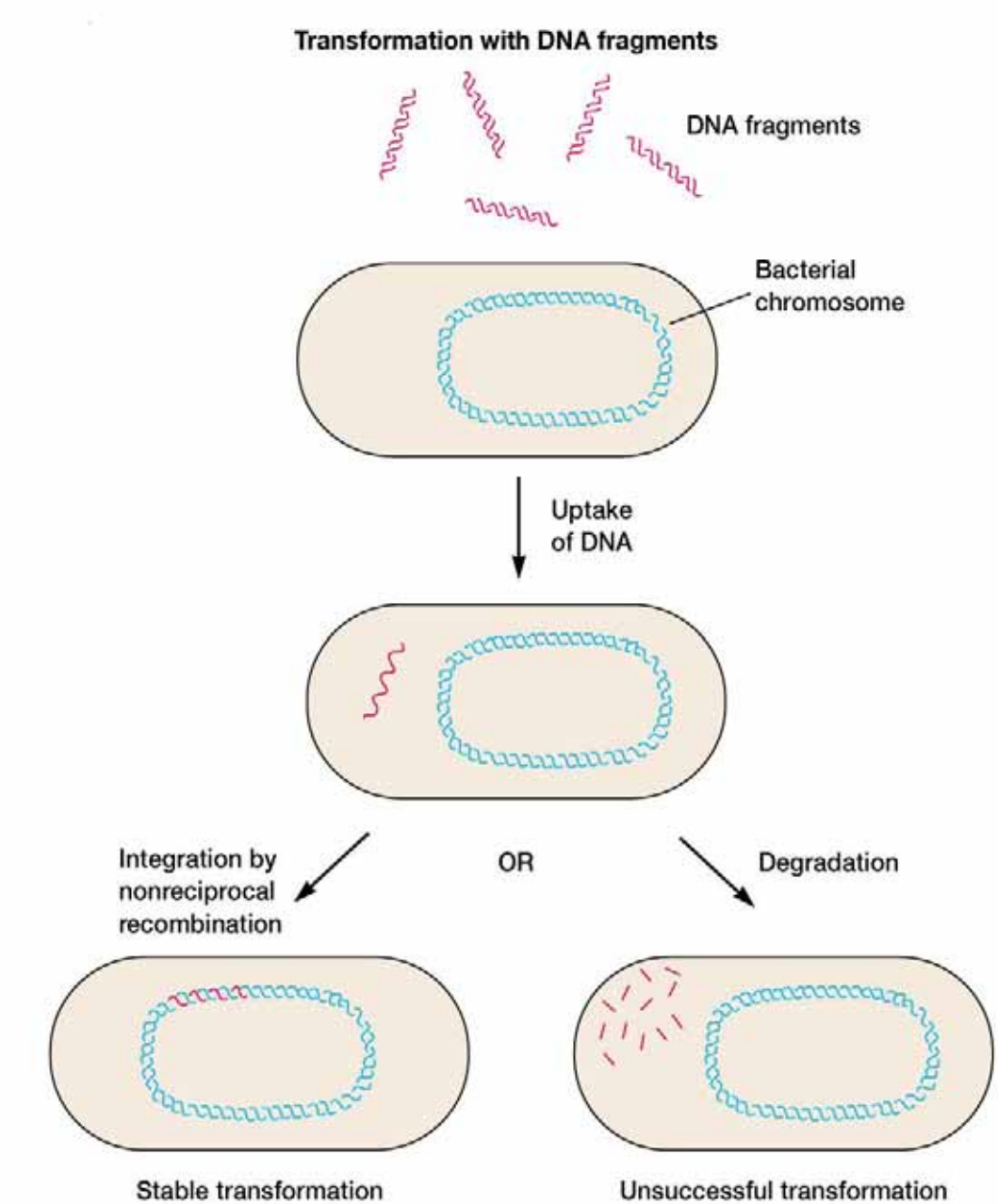


Figure 13.16a

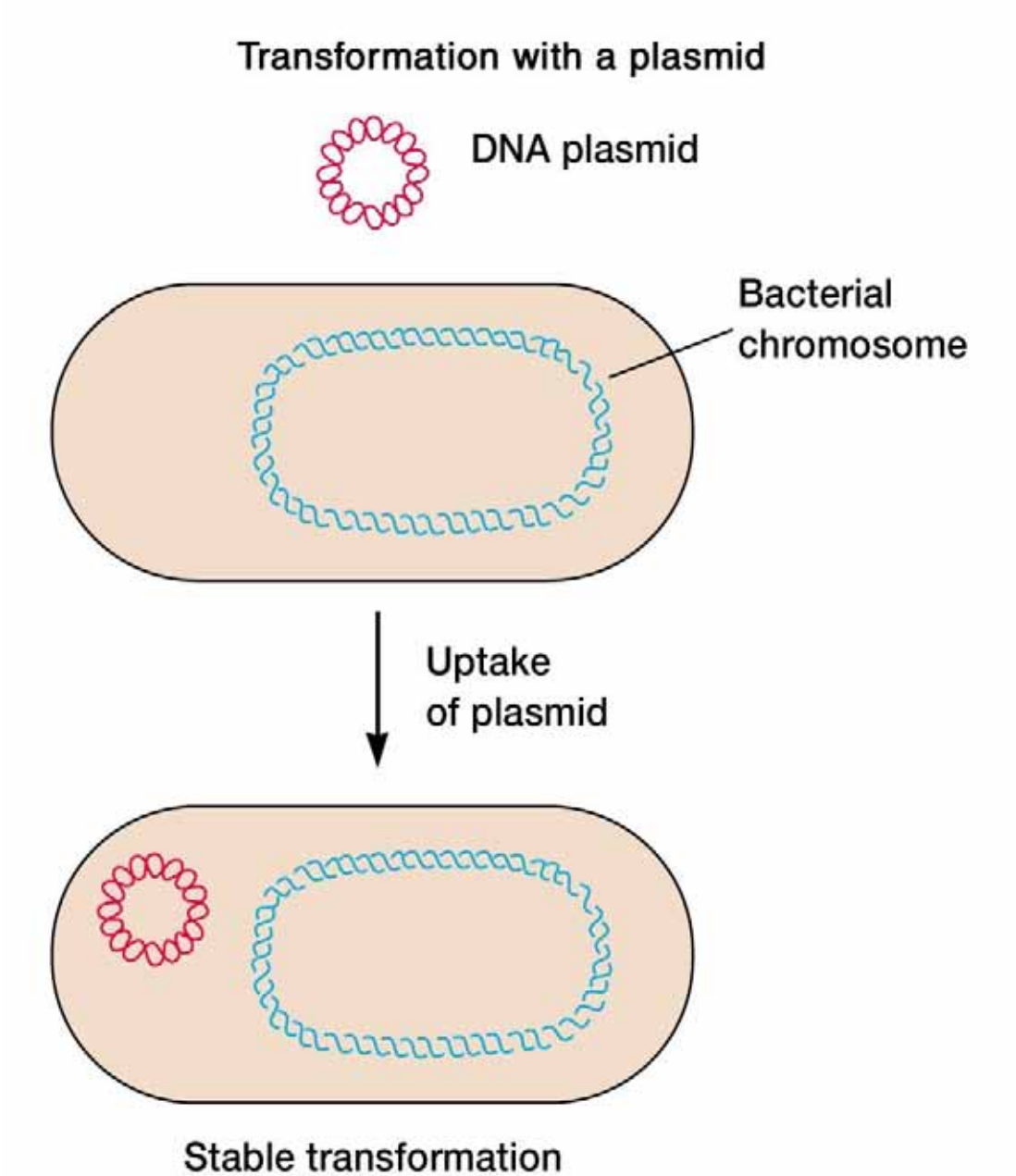


Figure 13.16b

Streptococcus pneumoniae

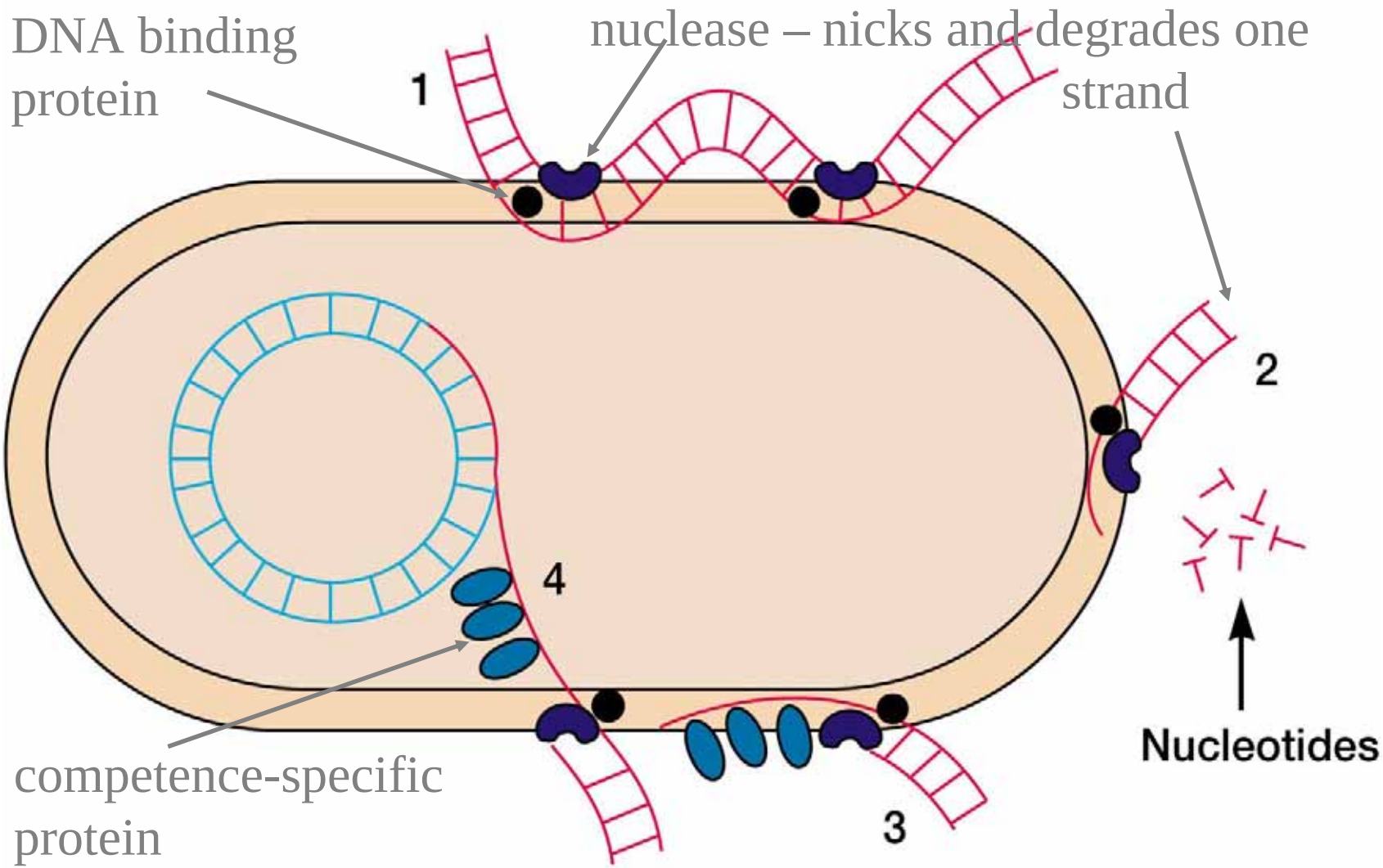


Figure 13.17

Artificial transformation

- transformation done in laboratory with species that are not normally competent (e.g., *E. coli*)
- variety of techniques used to make cells temporarily competent
  - e.g., calcium chloride treatment
    - makes cells more permeable to DNA

Transduction

- transfer of bacterial genes by viruses
- virulent bacteriophages
  - reproduce using lytic life cycle
- temperate bacteriophages
  - reproduce using lysogenic life cycle

Phage lambda life cycles

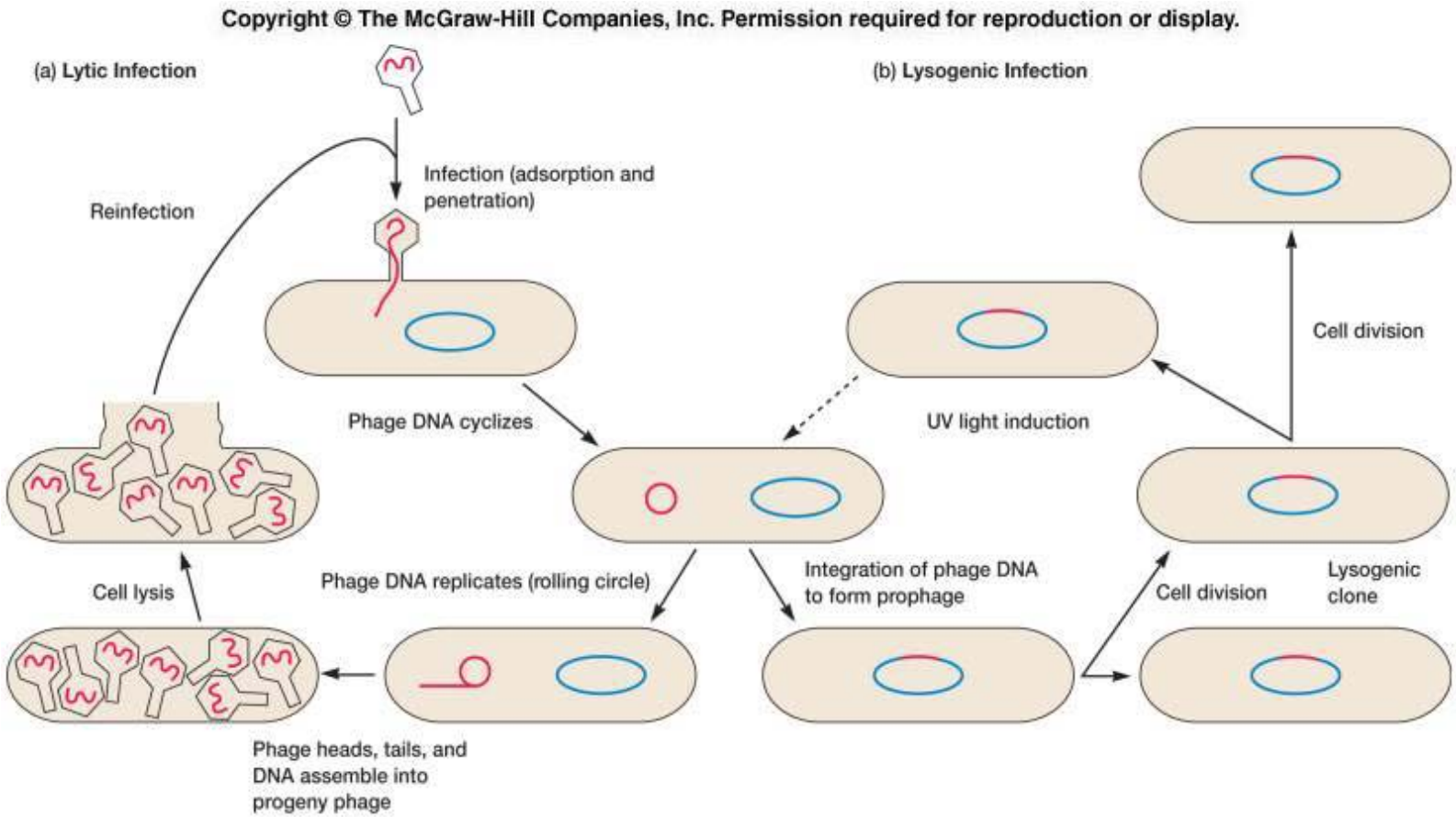


Figure 13.18

prophage=integrated form of viral genome

Generalized Transduction

- any part of bacterial genome can be transferred
- occurs during lytic cycle
- during viral assembly, fragments of host DNA mistakenly packaged into phage head
  - generalized transducing particle

Generalized transduction

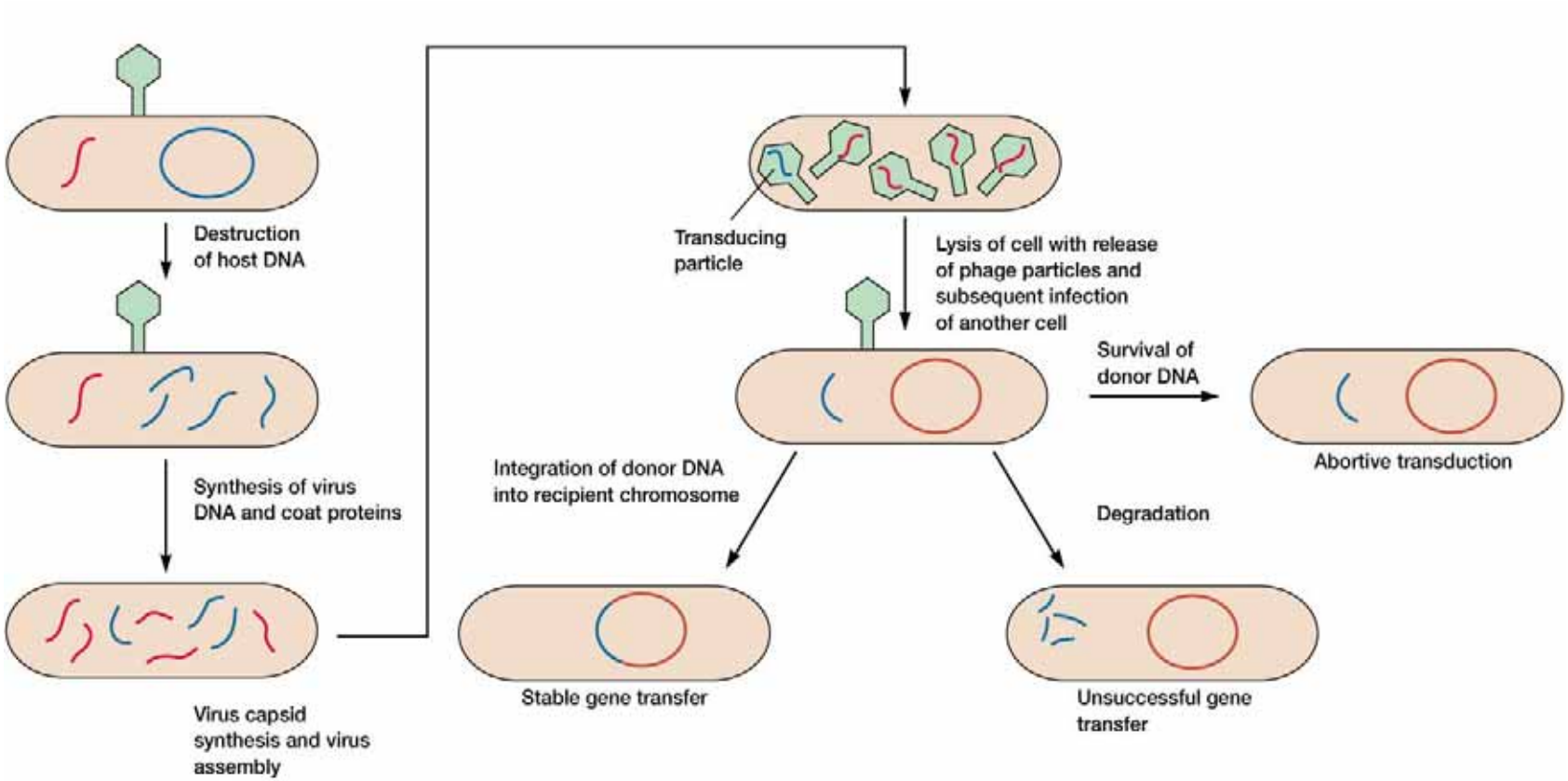


Figure 13.19

abortive transductants- bacteria with nonintegrated transduced DNA

Specialized Transduction

- also called restricted transduction
- carried out only by temperate phages that have established lysogeny
- only specific portion of bacterial genome is transferred
- occurs when prophage is incorrectly excised

Specialized transduction

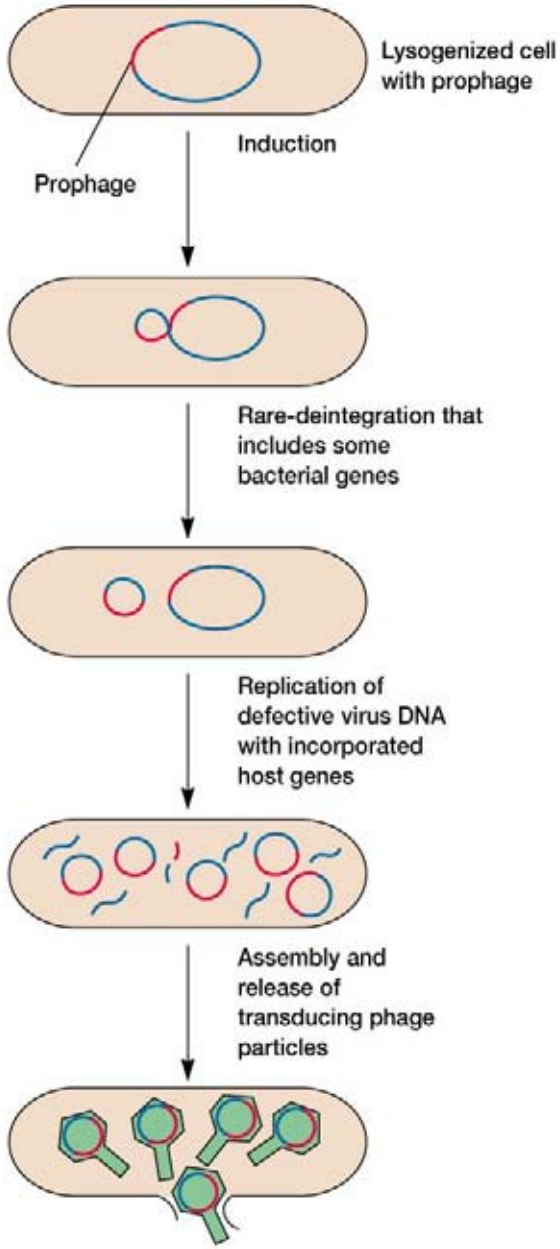


Figure 13.20

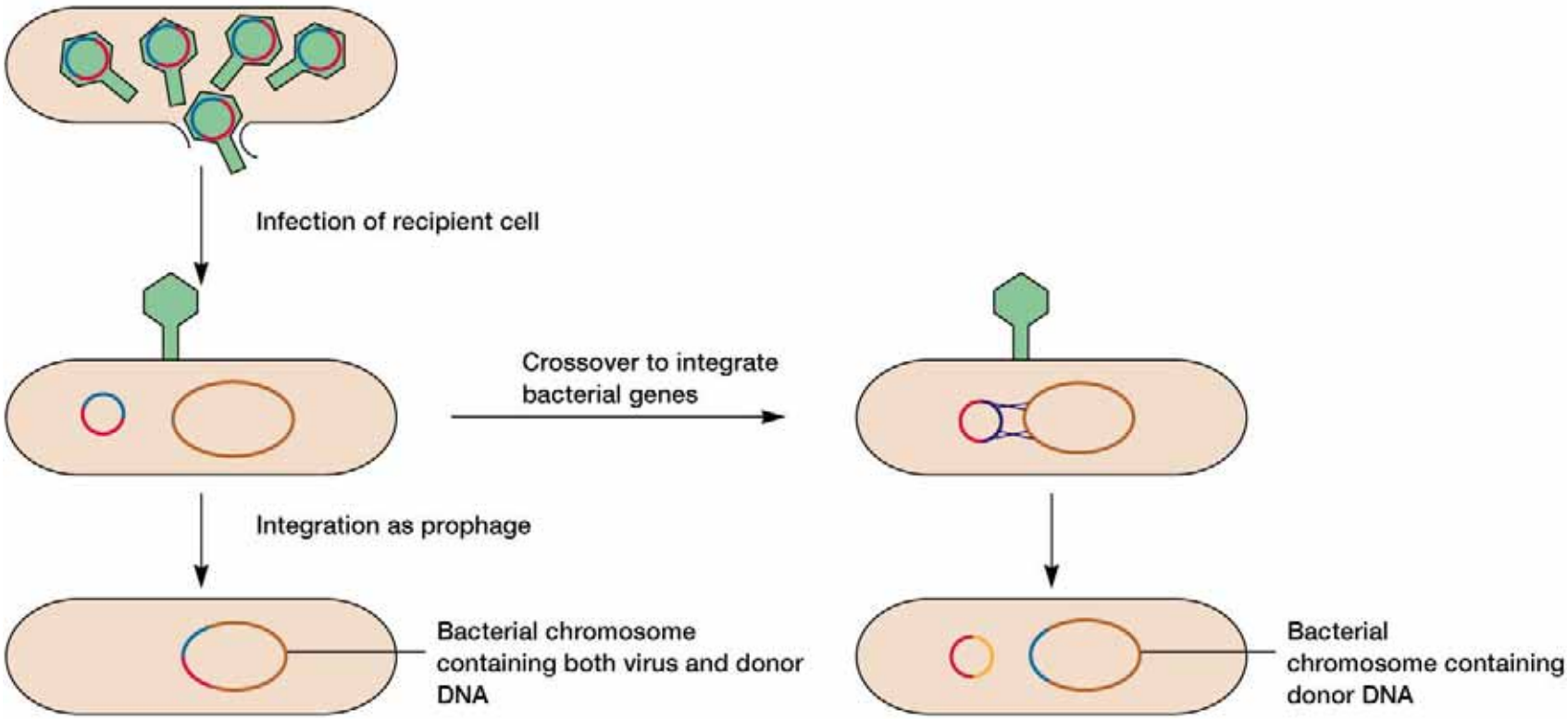

Infection of recipient cell
Crossover to integrate bacterial genes
Integration as prophage
Bacterial chromosome containing both virus and donor DNA
Bacterial chromosome containing donor DNA

Figure 13.20

Phage lambda

low-frequency transducing lysate

insert Figure 13.21

Figure 13.21

insert Figure 13.21

Figure 13.21 high-frequency transduction lysate

Mapping the Genome

- locating genes on an organism's chromosomes
- mapping bacterial genes accomplished using all three modes of gene transfer

Hfr mapping

- used to map relative location of bacterial genes
- based on observation that chromosome transfer occurs at constant rate
- interrupted mating experiment
  - Hfr x F^- mating interrupted at various intervals
  - order and timing of gene transfer determined

Interrupted mating

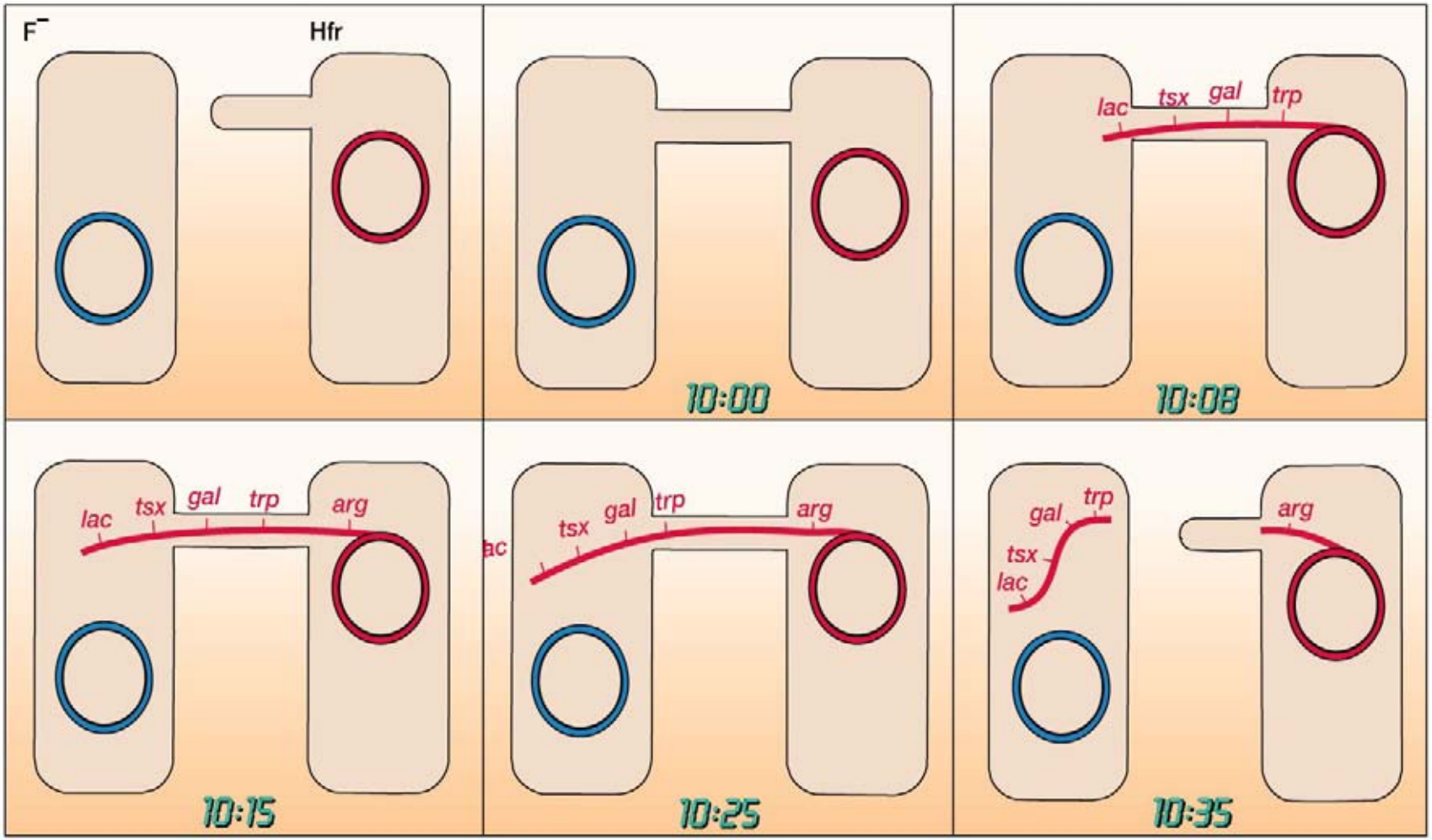


Figure 13.22a

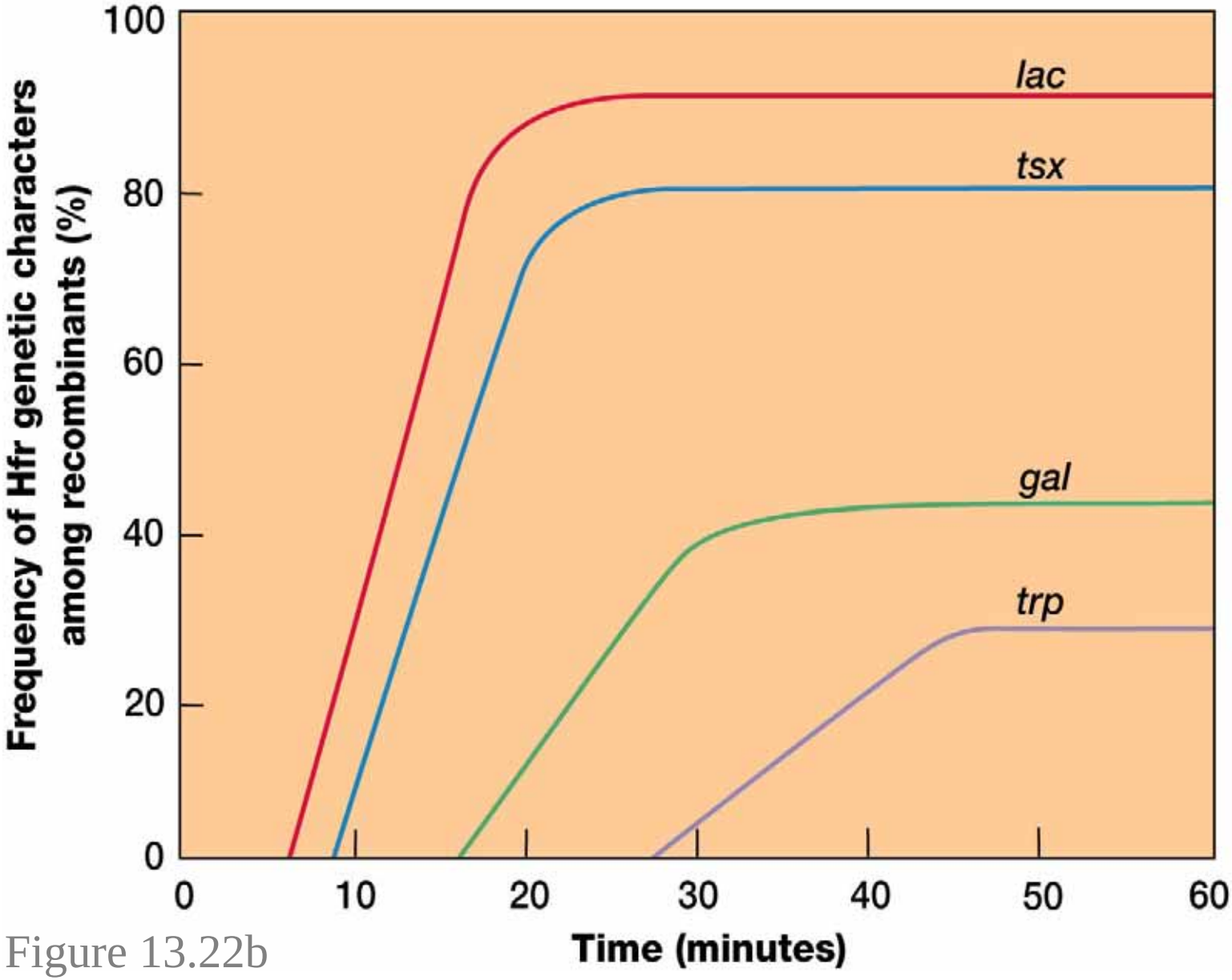


Figure 13.22b

E. coli genetic map

- gene locations expressed in minutes, reflecting time transferred
- made using numerous Hfr strains

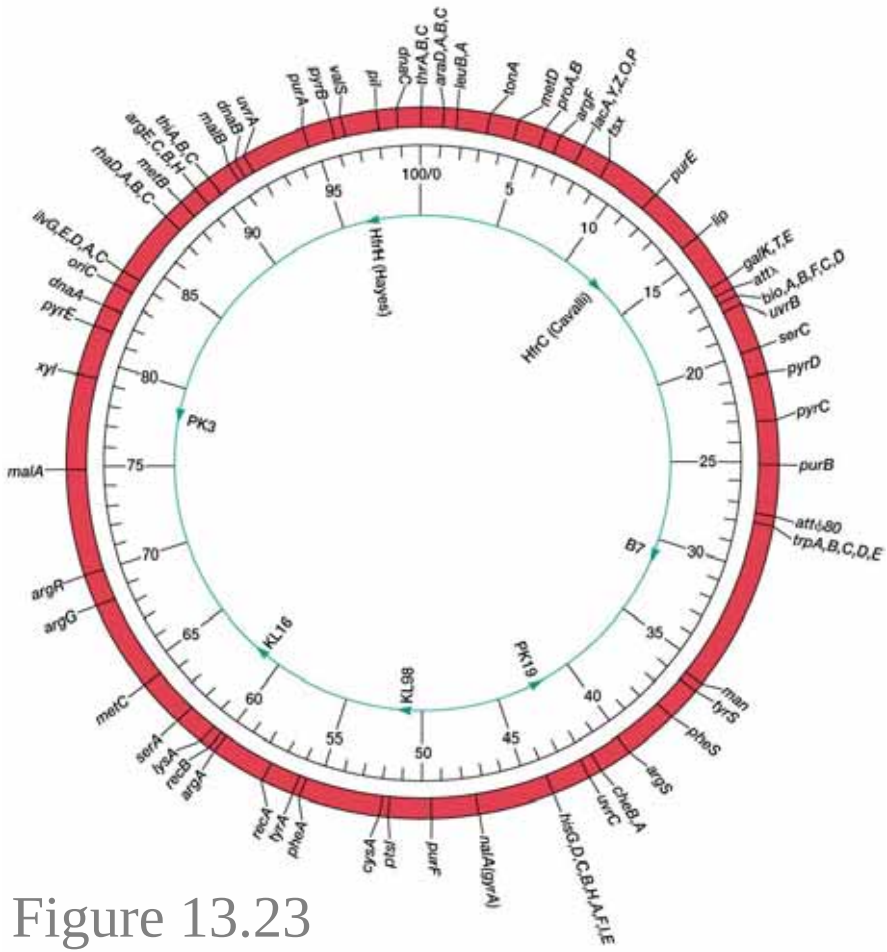


Figure 13.23

Transformation mapping

- used to establish gene linkage
- expressed as frequency of cotransformation
- if two genes close together, greater likelihood will be transferred on single DNA fragment

Generalized transduction mapping

- used to establish gene linkage
- expressed as frequency of cotransduction
- if two genes close together, greater likelihood will be carried on single DNA fragment in transducing particle

Specialized transduction mapping

- provides distance of genes from viral genome integration sites
- viral genome integration sites must first be mapped by conjugation mapping techniques

Recombination and Genome Mapping in Viruses

- viral genomes can also undergo recombination events
- viral genomes can be mapped by determining recombination frequencies
- physical maps of viral genomes can also be constructed using other techniques

Recombination mapping

- recombination frequency determined when cells infected simultaneously with two different viruses

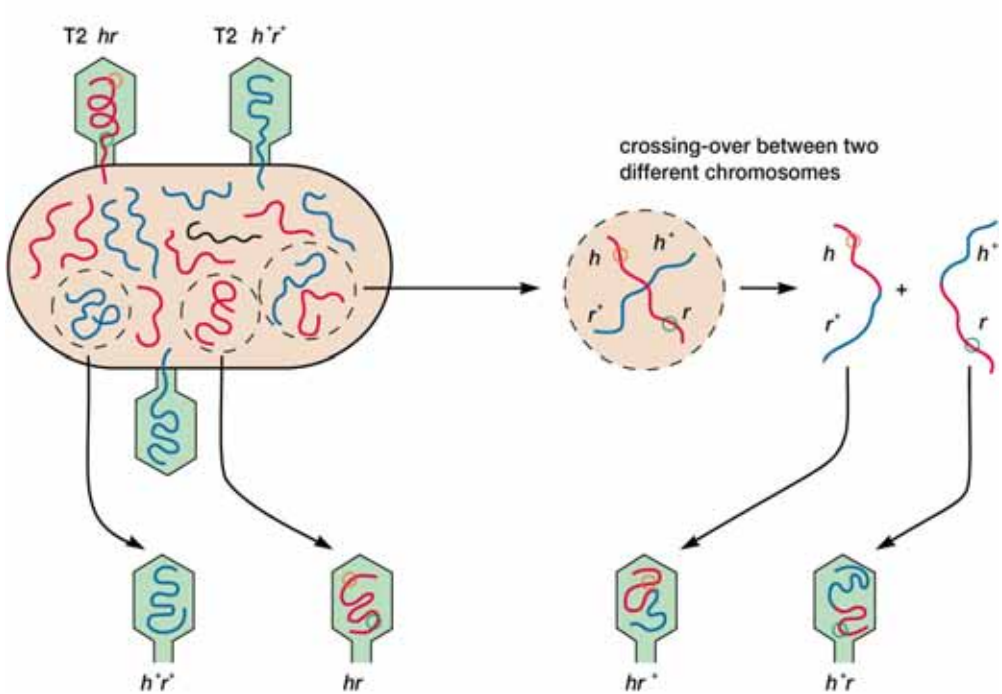


Figure 13.24

Physical maps

- heteroduplex maps
  - genomes of two different viruses denatured, mixed and allowed to anneal
    - regions that are not identical, do not reanneal
  - allows for localization of mutant alleles

Physical maps…

- restriction endonuclease mapping
  - compare DNA fragments from two different viral strains in terms of electrophoretic mobility
- sequence mapping
  - determine nucleotide sequence of viral genome
  - identify coding regions, mutations, etc.