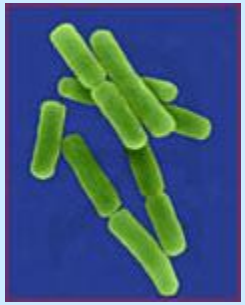


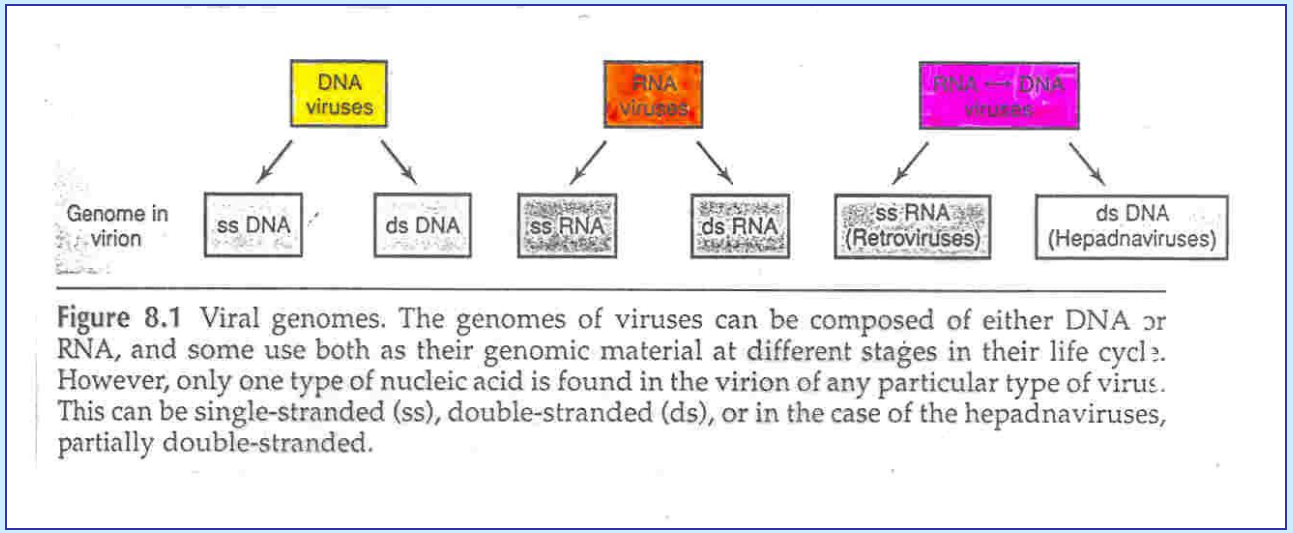
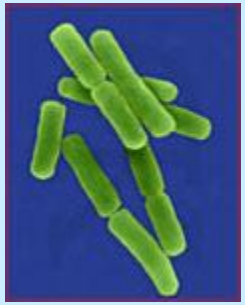



Figure 8.1 Viral genomes. The genomes of viruses can be composed of either DNA or RNA, and some use both as their genomic material at different stages in their life cycle. However, only one type of nucleic acid is found in the virion of any particular type of virus. This can be single-stranded (ss), double-stranded (ds), or in the case of the hepadnaviruses, partially double-stranded.

I VIRUS



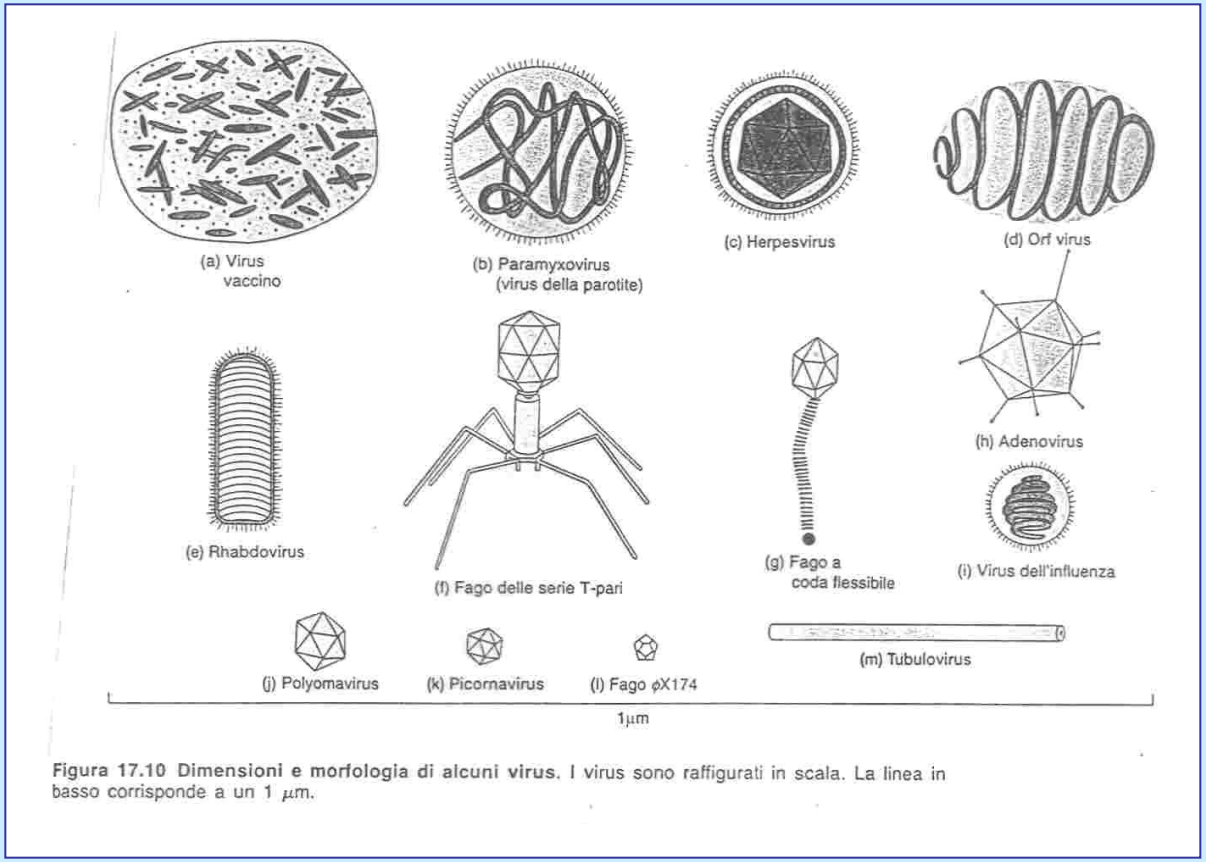
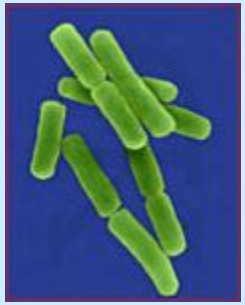


Figura 17.10 Dimensioni e morfologia di alcuni virus. I virus sono raffigurati in scala. La linea in basso corrisponde a un 1 μm.

I VIRUS



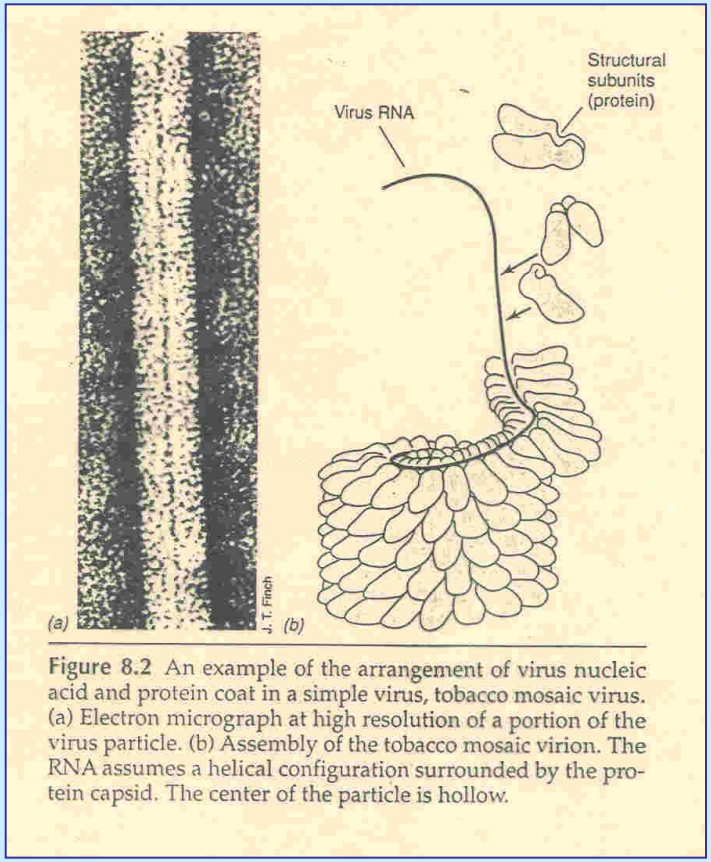
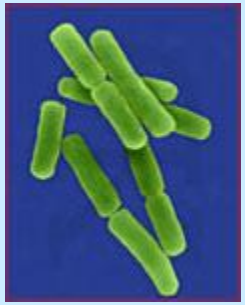


Figure 8.2 An example of the arrangement of virus nucleic acid and protein coat in a simple virus, tobacco mosaic virus. (a) Electron micrograph at high resolution of a portion of the virus particle. (b) Assembly of the tobacco mosaic virion. The RNA assumes a helical configuration surrounded by the protein capsid. The center of the particle is hollow.

I VIRUS



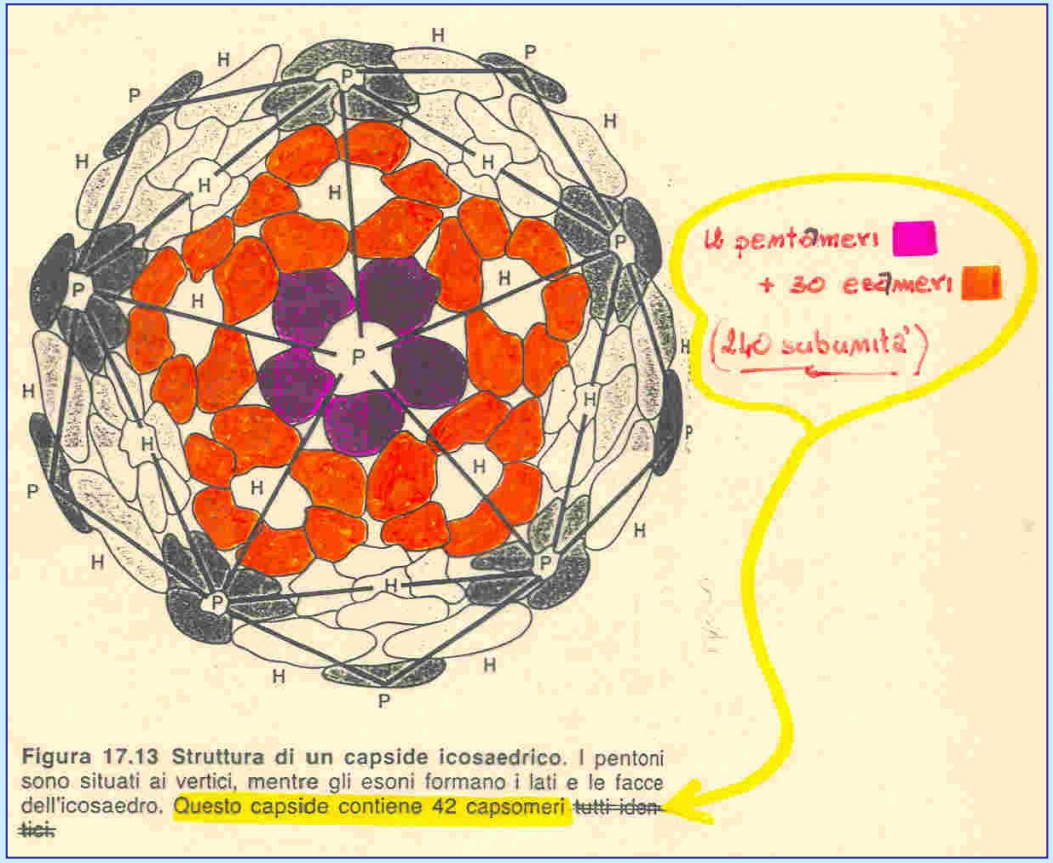
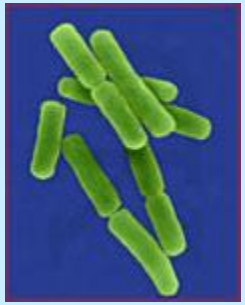


Figura 17.13 Struttura di un capside icosaedrico. I pentoni sono situati ai vertici, mentre gli esoni formano i lati e le facce dell'icosaedro. Questo capside contiene 42 capsomeri ~~tutti identici.~~

I VIRUS



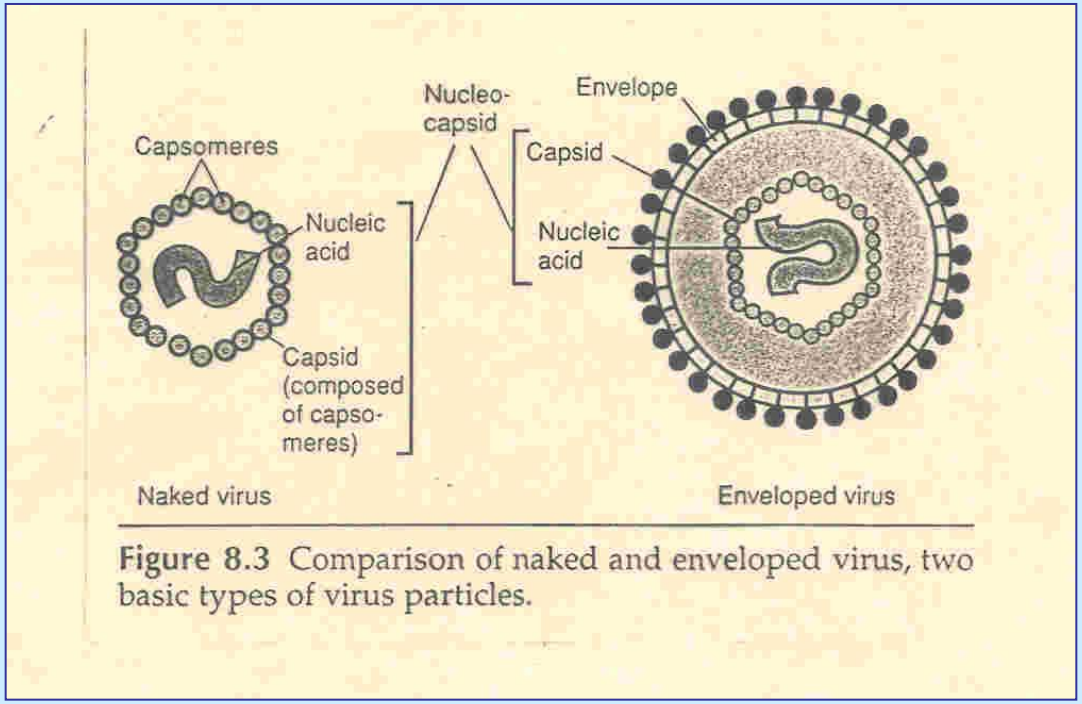
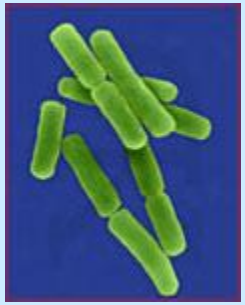
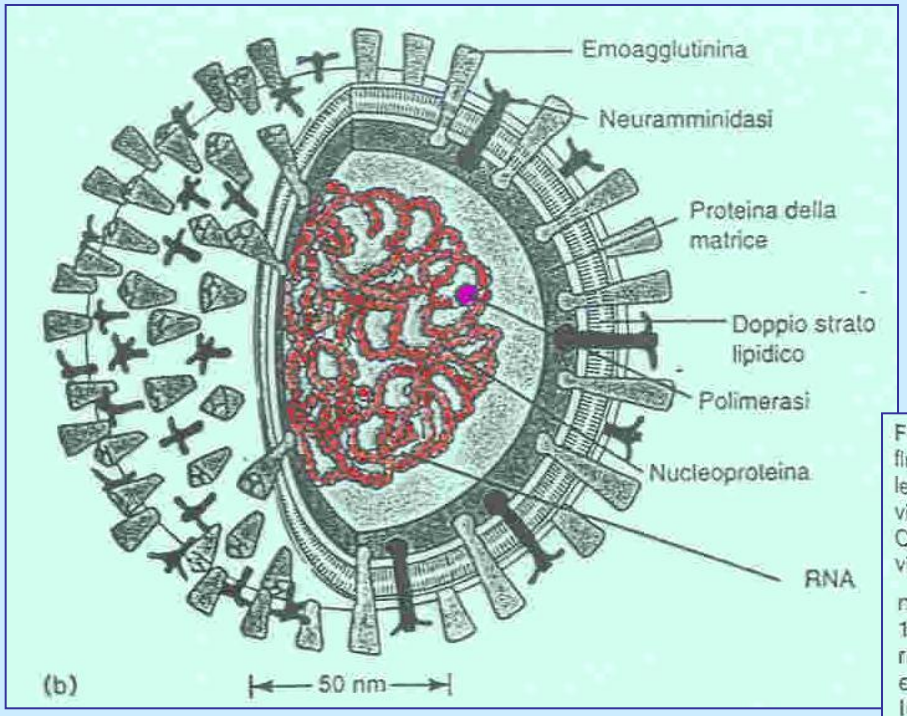


Figure 8.3 Comparison of naked and enveloped virus, two basic types of virus particles.

I VIRUS





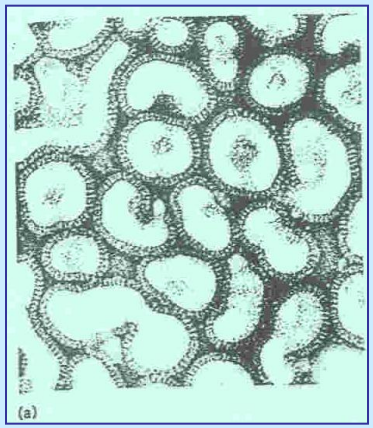
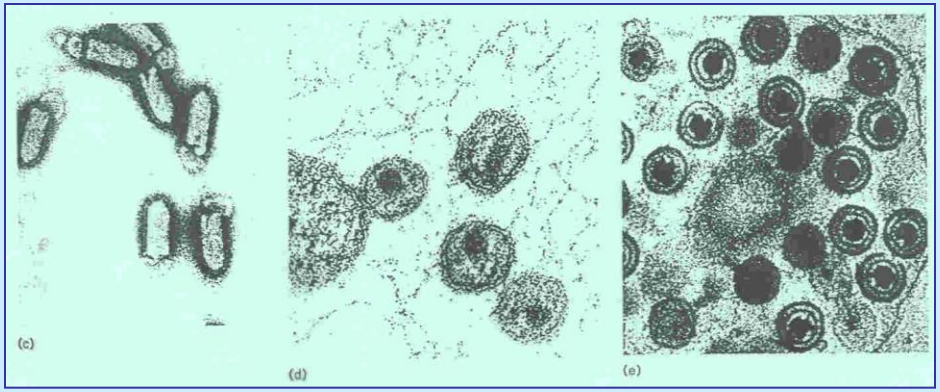
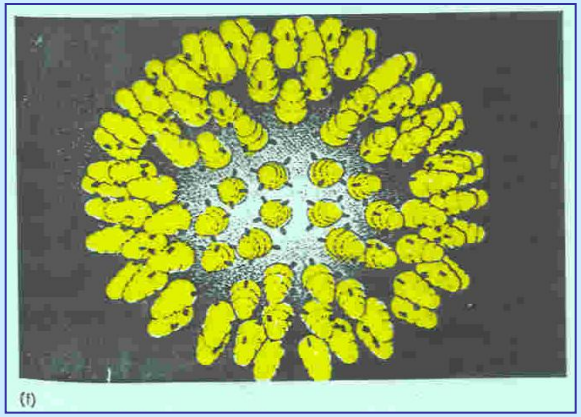
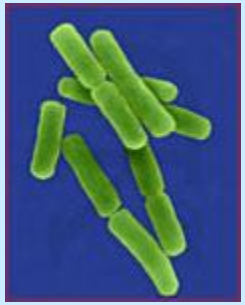


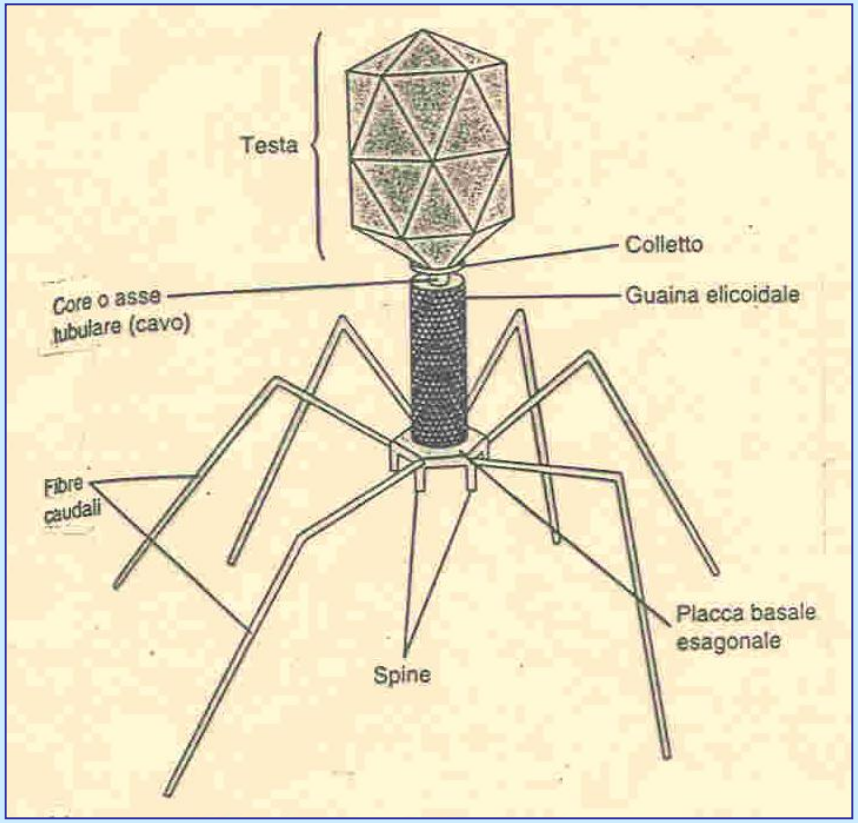
Figura 17.17 Esempi di virus con envelope. (*a*) Virus dell'influenza umano. Da notare la flessibilità dell'envelope e le spicole che si distaccano dalla superficie (× 282000). (*b*) Schema del virione dell'influenza. (*c*) Particelle di Rhabdovirus (× 250000). Questo è il virus della stomatite vescicolare, un virus correlato al virus della rabbia cui somiglia nell'aspetto. (*d*) Virus dell'immunodeficienza umana (HIV) (× 33000). (*e*) Herpesvirus (× 100000). (*f*) Immagine computerizzata del virus di Semliki Forest che causa nell'uomo encefaliti occasionali. (*Fonte b*: D. Voet e J. G. Voet, *Biochemistry*. Copyright 1990 © John Wiley & Sons, Inc., New York.)



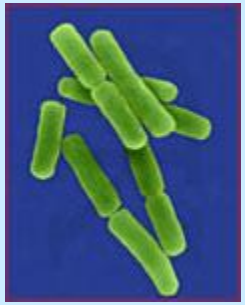


I VIRUS





Batteriofago a struttura complessa della particella virale (fago della serie T-pari: T4)



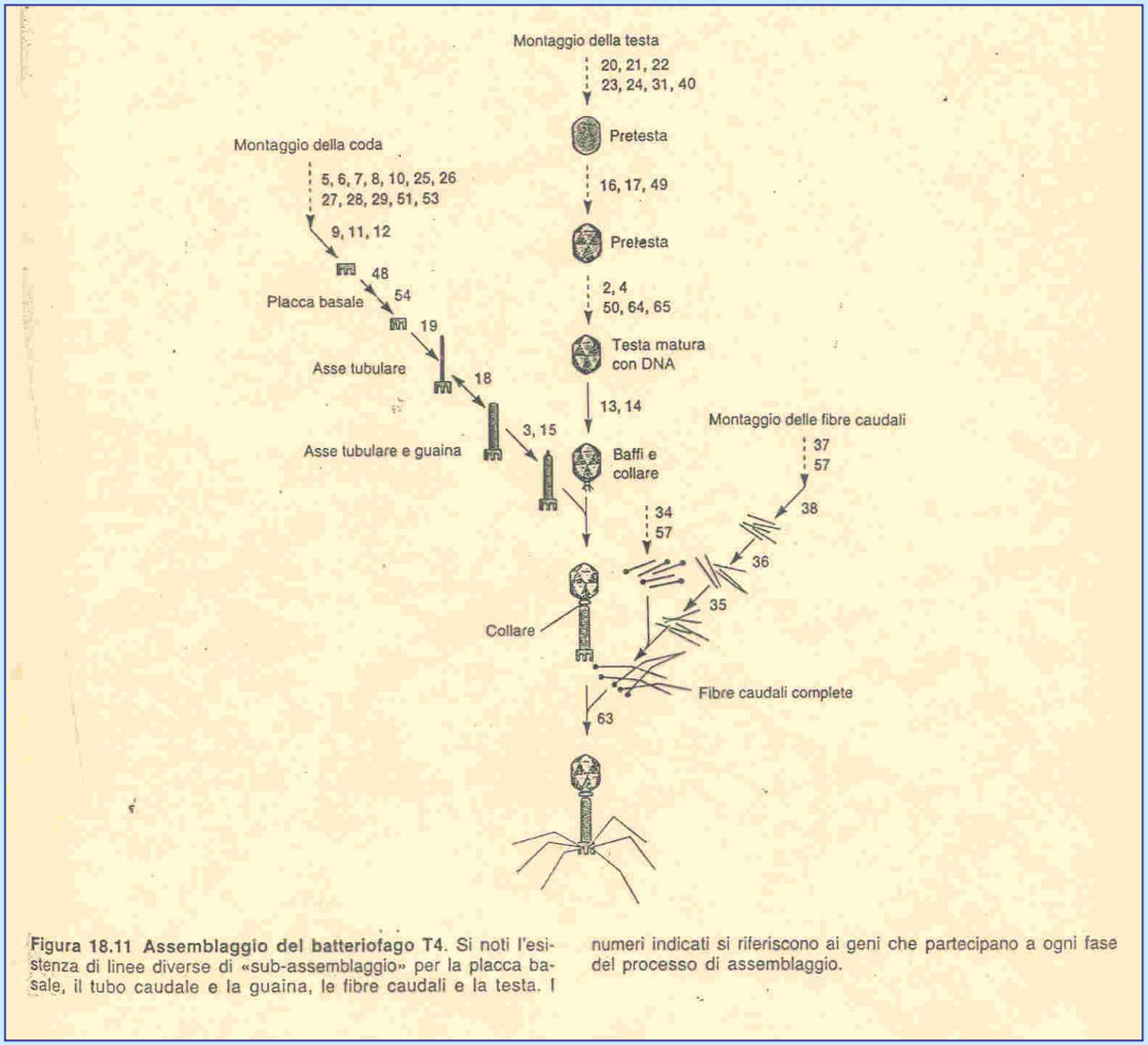
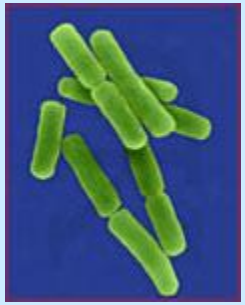
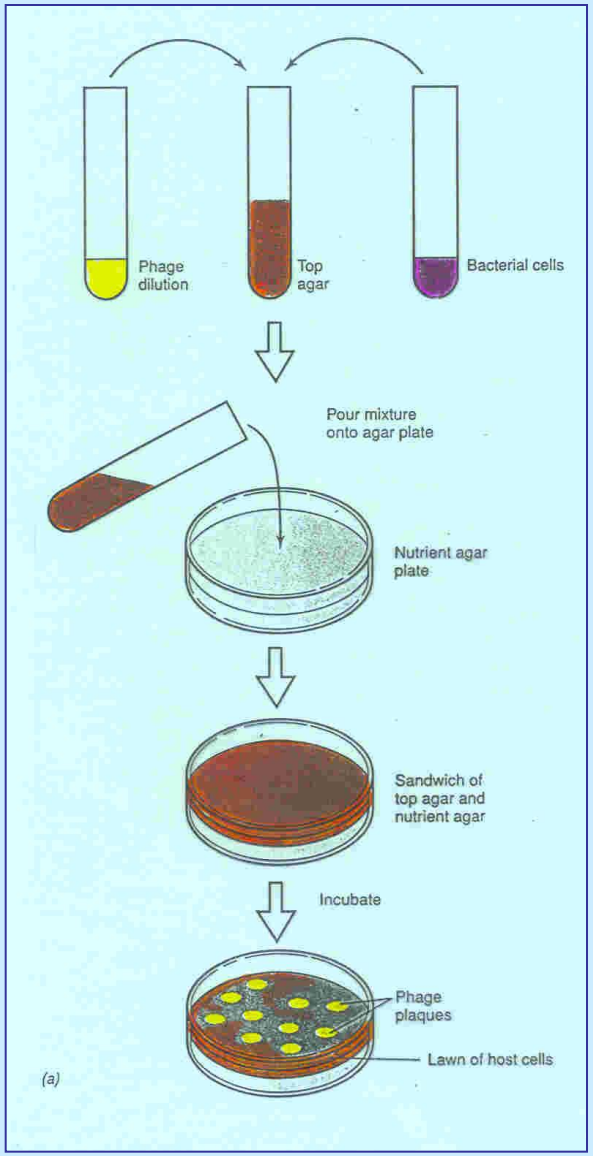


Figura 18.11 Assemblaggio del batteriofago T4. Si noti l'esistenza di linee diverse di «sub-assemblaggio» per la placca basale, il tubo caudale e la guaina, le fibre caudali e la testa. I numeri indicati si riferiscono ai geni che partecipano a ogni fase del processo di assemblaggio.





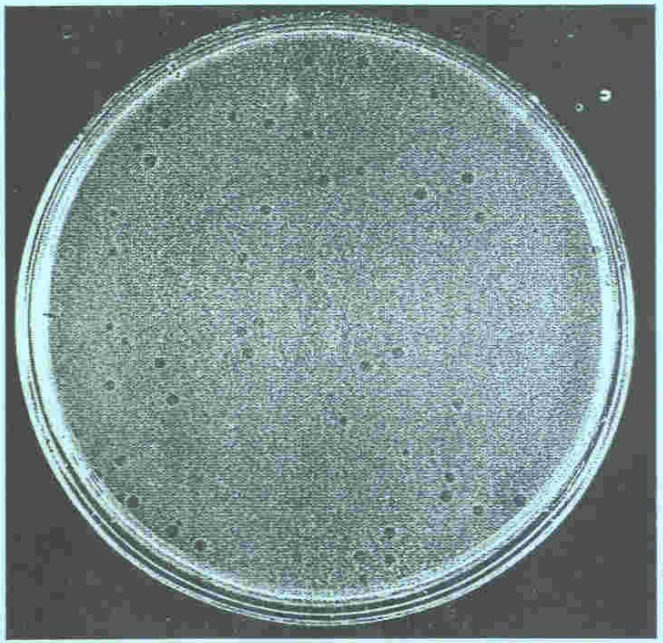
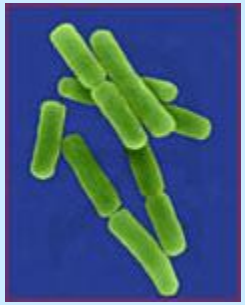


Figure 8.6 Quantification of bacterial virus by plaque assay using the agar overlay technique. (a) A dilution of a suspension containing the virus material is mixed in a small amount of melted agar with the sensitive host bacteria, and the mixture poured on the surface of a nutrient agar plate. The host bacteria, which have been spread uniformly throughout the top agar layer, begin to grow, and after overnight incubation form a *lawn* of confluent growth. Each virus particle that attaches to a cell and reproduces may cause cell lysis, and the virus particles released can spread to adjacent cells in the agar, infect them, be reproduced, and again lead to lysis and release. The size of the plaque formed depends on the virus, the host, and conditions of culture. (b) Photograph of a plate showing plaques formed by bacteriophage on a lawn of sensitive bacteria. The plaques shown are about 1–2 mm in diameter.

I VIRUS



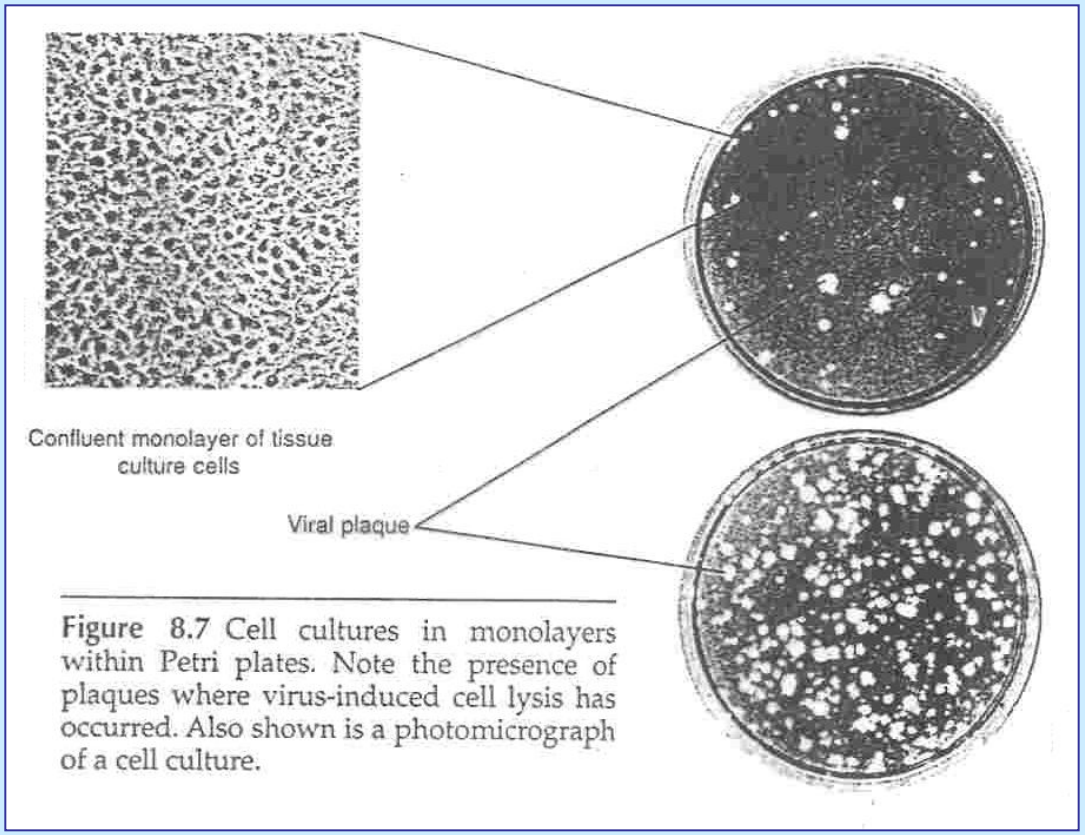
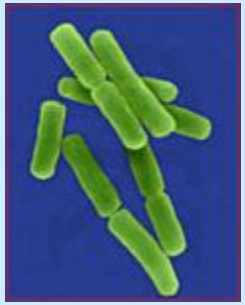


Figure 8.7 Cell cultures in monolayers within Petri plates. Note the presence of plaques where virus-induced cell lysis has occurred. Also shown is a photomicrograph of a cell culture.

I VIRUS



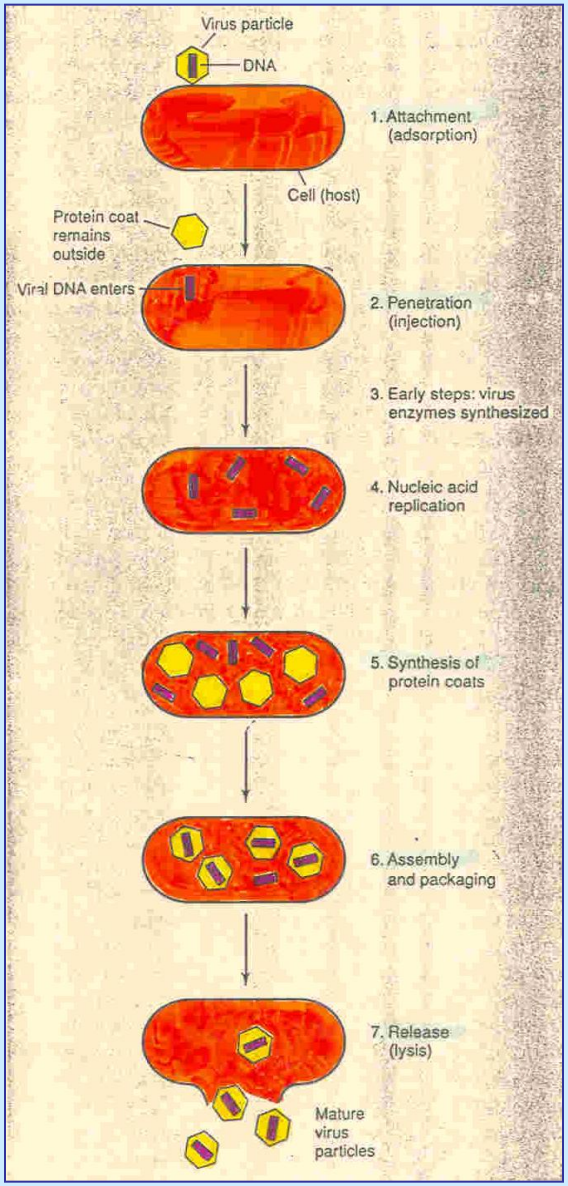
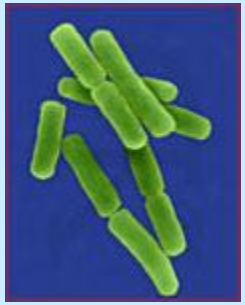


Figure 8.8 The replication cycle of a bacterial virus. The general stages of virus replication are indicated.

I VIRUS



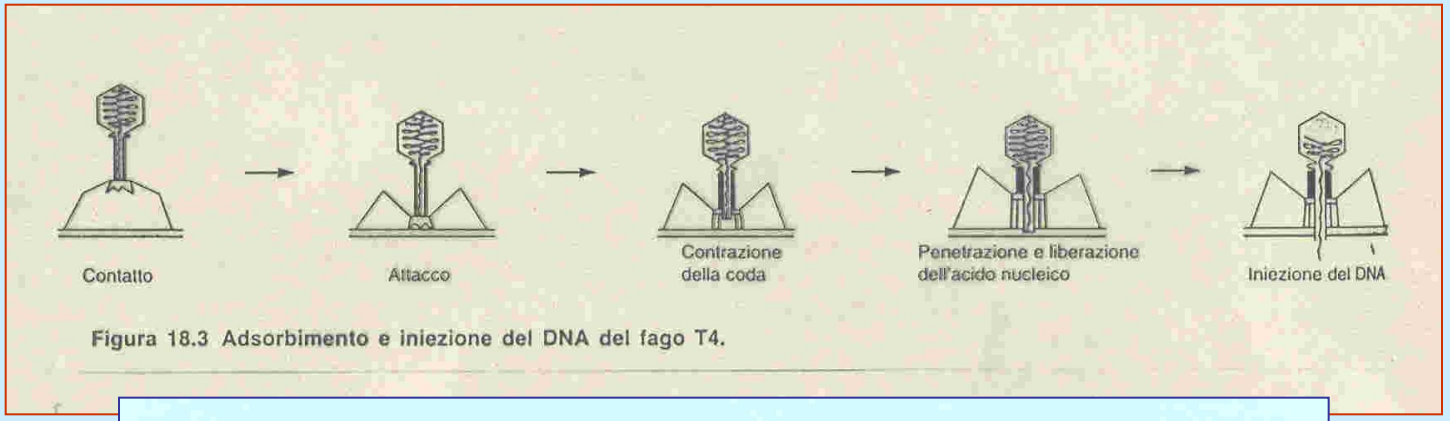



Figura 18.3 Adsorbimento e iniezione del DNA del fago T4.

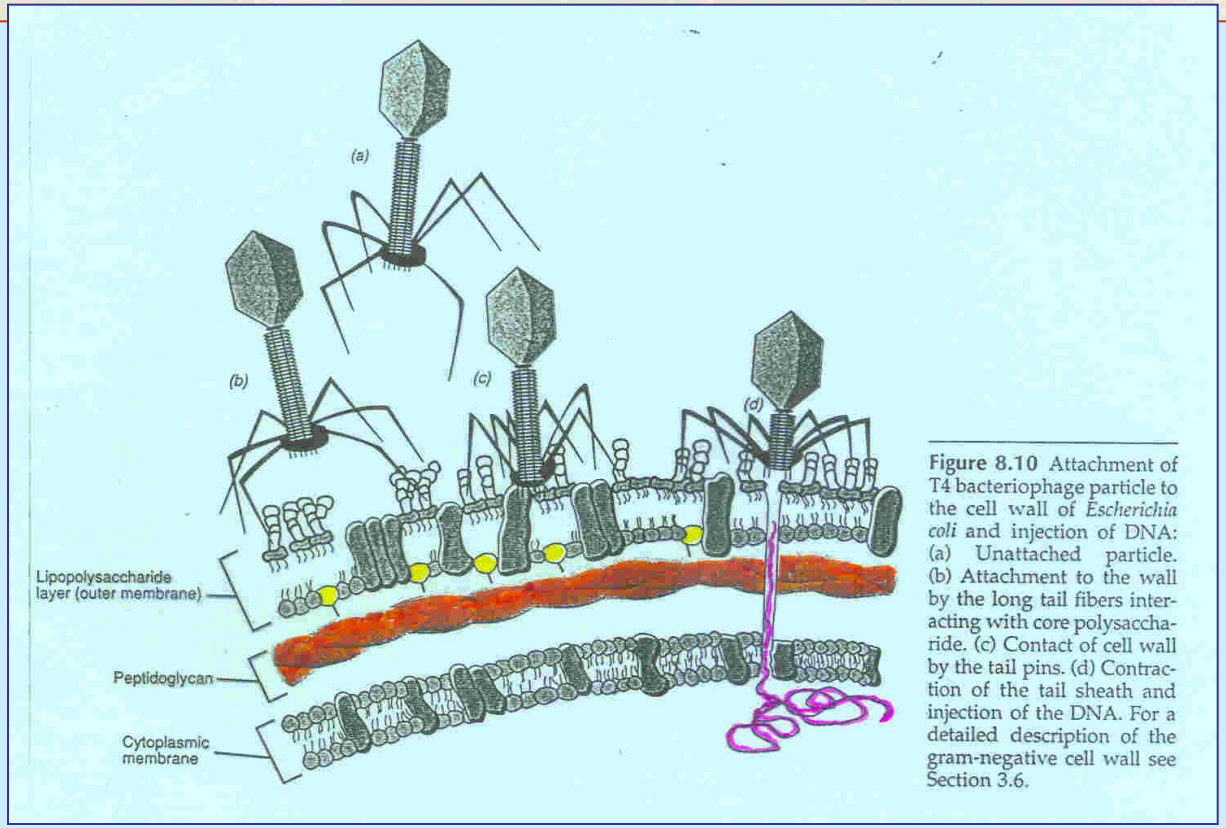
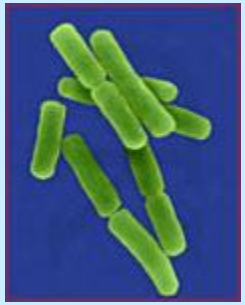


Figure 8.10 Attachment of T4 bacteriophage particle to the cell wall of *Escherichia coli* and injection of DNA: (a) Unattached particle. (b) Attachment to the wall by the long tail fibers interacting with core polysaccharide. (c) Contact of cell wall by the tail pins. (d) Contraction of the tail sheath and injection of the DNA. For a detailed description of the gram-negative cell wall see Section 3.6.

I VIRUS



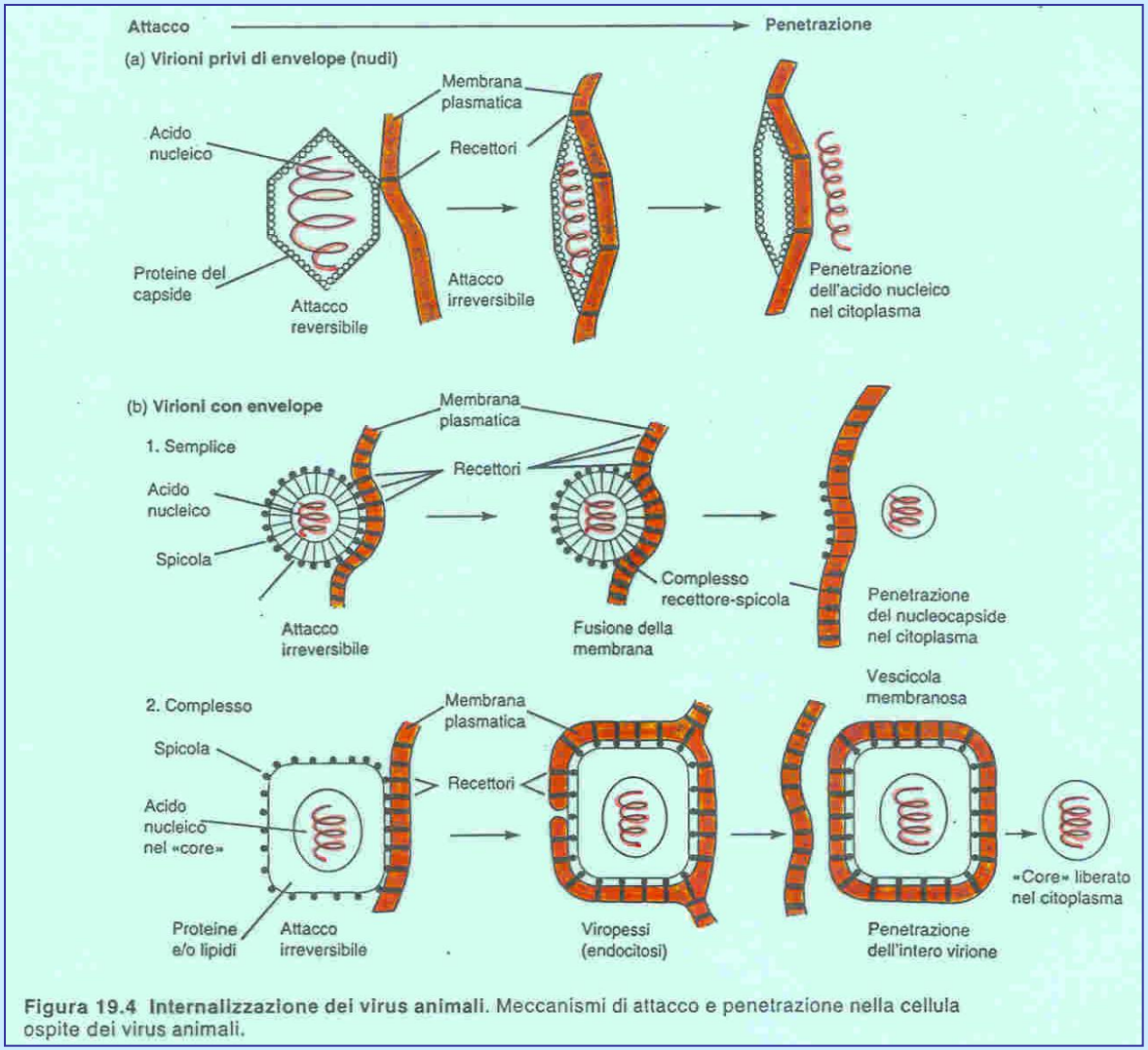
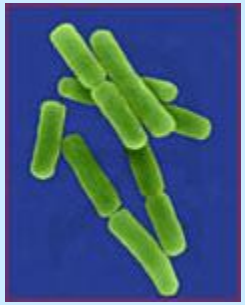


Figura 19.4 Internalizzazione dei virus animali. Meccanismi di attacco e penetrazione nella cellula ospite dei virus animali.

I VIRUS



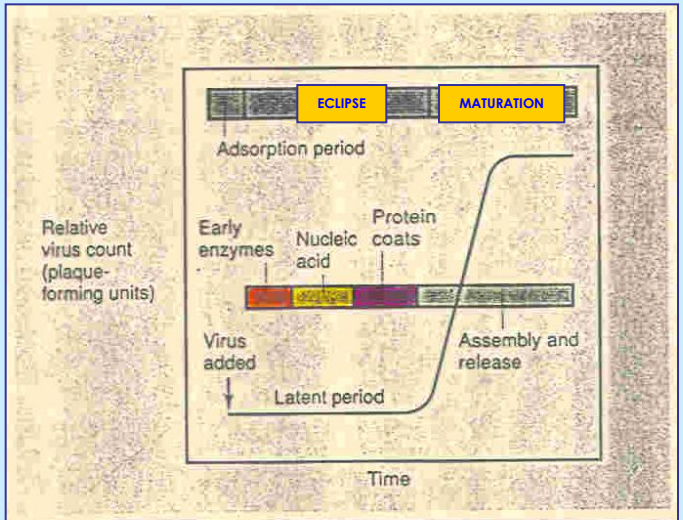
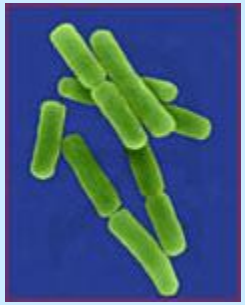
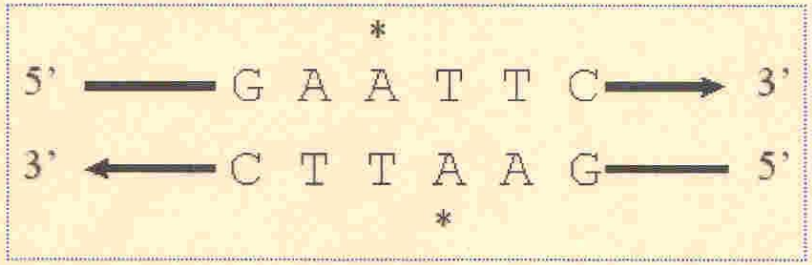


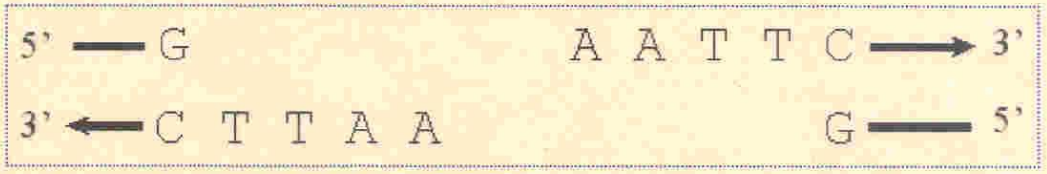
Figure 8.9 The one-step growth curve of virus replication. This graph displays the results of a single round of viral multiplication in a population of cells. Following adsorption, the infectivity of the virus particles disappears, a phenomenon called *eclipse*. This is due to the uncoating of the virus particles. During the *latent period*, replication of viral nucleic acid and protein occurs. The *maturation period* follows, when virus nucleic acid and protein are assembled into mature virus particles. At this time, if the cells are broken up, active virus can be detected. Finally, *release* occurs, either with or without cell lysis. The timing of the one-step growth cycle varies with the virus and host. With many bacterial viruses, the whole cycle may be complete in 30–60 min, whereas with animal viruses 12–24 hr is usually required for a complete cycle. Compare this general picture and color scheme with specific replication events shown for bacteriophage T4 in Figure 8.23.

I VIRUS



ENDONUCLEASI di RESTRIZIONE





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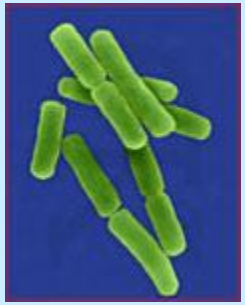


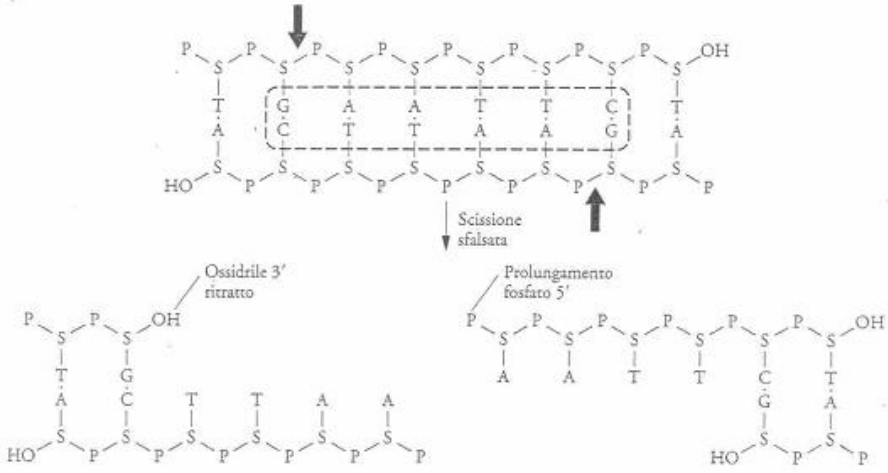



Figura 4.2 Scissione simmetrica e sfalsata di un breve frammento di DNA ad opera dell'endonucleasi di restrizione di tipo II *EcoRI*. Le frecce grandi indicano i siti di scissione dell'ossatura del DNA. S, zucchero deossiribosio; P, gruppo fosfato; OH, gruppo ossidrile; A, adenina; T, timina; C, citosina; G, guanina. La sequenza di riconoscimento di *EcoRI* è stata racchiusa nel rettangolo tratteggiato.

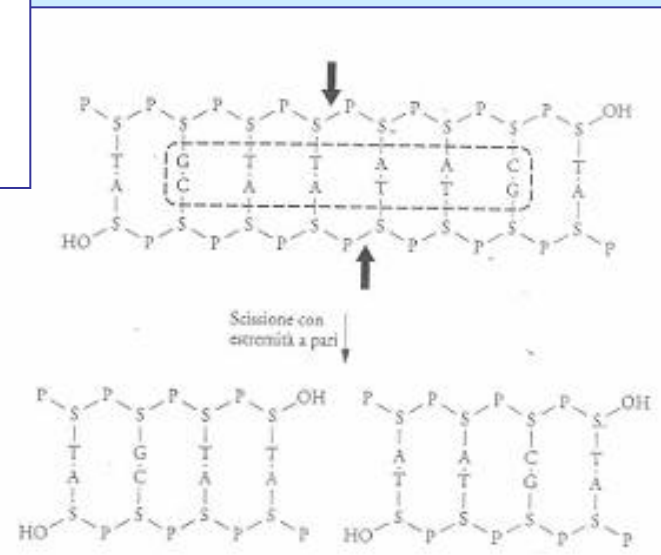
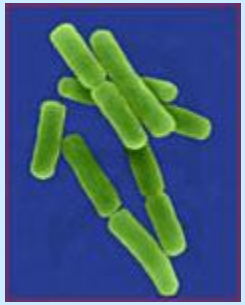


Figura 4.3 La scissione con estremità a pari di un breve frammento di DNA ad opera dell'endonucleasi di restrizione di tipo II *HindII*. Le frecce grandi indicano i siti di scissione nell'ossatura del DNA. Per le abbreviazioni vedi la didascalia di figura 4.2. In evidenza la sequenza di riconoscimento di *HindII*.

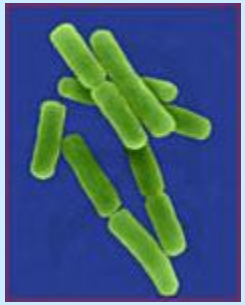
I VIRUS

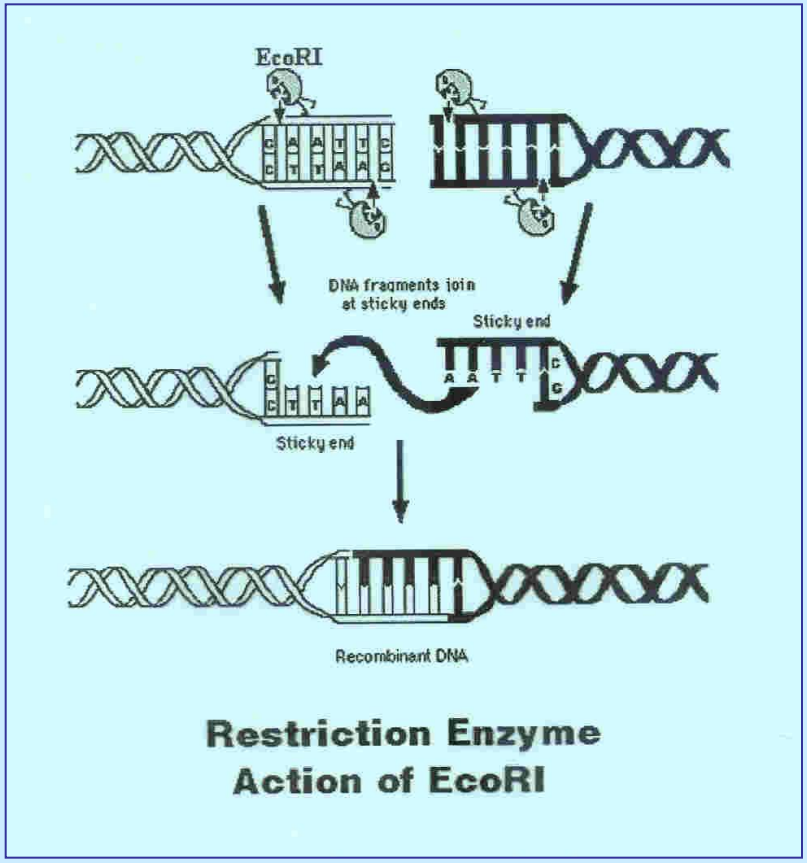


Examples of Restriction Enzymes

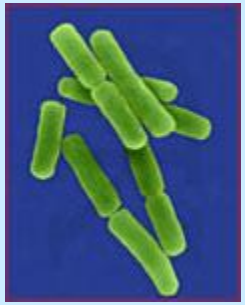
Enzyme	Organism from which derived	Target sequence (cut at *) 5' -->3'
Ava I	Anabaena variabilis	C* C/T C G A/G G
Bam HI	Bacillus amyloliquefaciens	G* G A T C C
Bgl II	Bacillus globigii	A* G A T C T
Eco RI	Escherichia coli RY 13	G* A A T T C
Eco RII	Escherichia coli R245	* C C A/T G G
Hae III	Haemophilus aegyptius	G G * C C
Hha I	Haemophilus haemolyticus	G C G * C
Hind III	Haemophilus inflenzae Rd	A* A G C T T
Hpa I	Haemophilus parainflenzae	G T T * A A C
Kpn I	Klebsiella pneumoniae	G G T A C * C
Mbo I	Moraxella bovis	*G A T C
Mbo I	Moraxella bovis	*G A T C
Pst I	Providencia stuartii	C T G C A * G
Sma I	Serratia marcescens	C C C * G G G
SstI	Streptomyces stanford	G A G C T * C
Sal I	Streptomyces albus G	G * T C G A C
Taq I	Thermophilus aquaticus	T * C G A
Xma I	Xanthamonas malvacearum	C * C C G G G

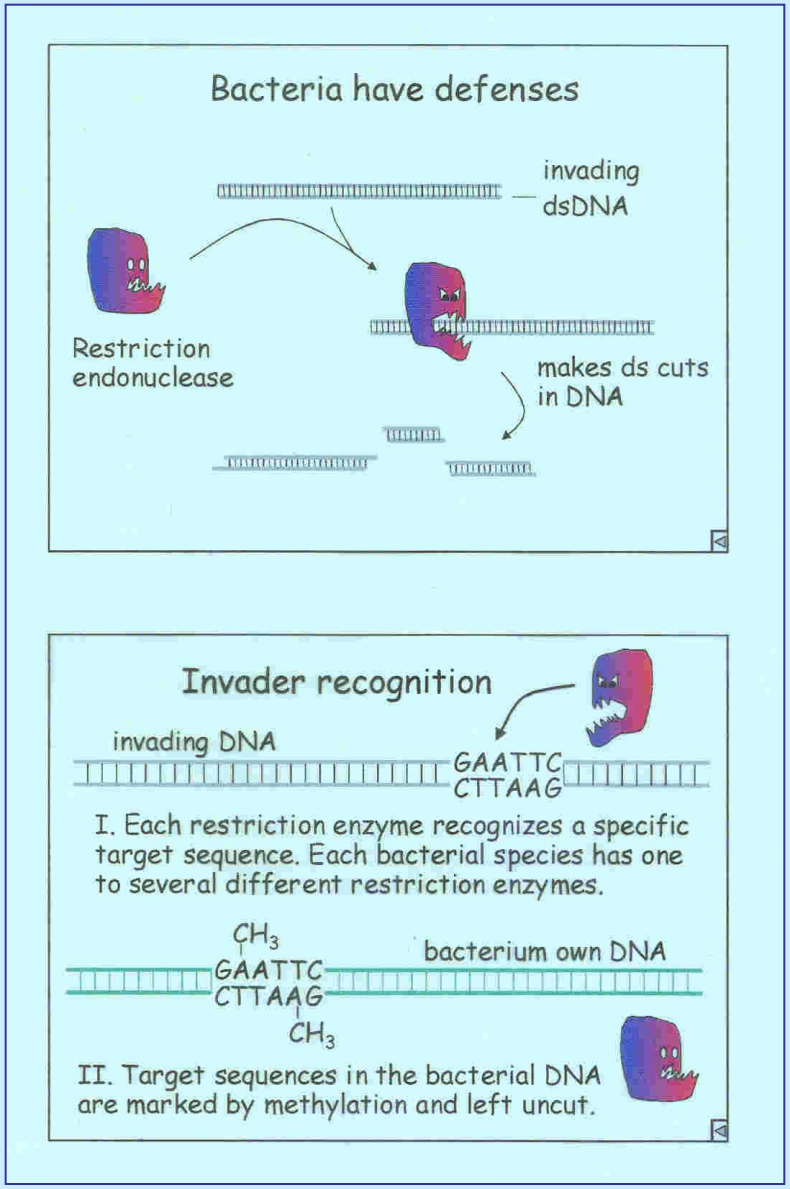
Note: Only one strand of the target DNA is shown, in the interests of clarity. It will be noted that the omitted strand has the same sequence as the one show, but the nucleotides occur in the reverse order. Thus for example The complementary sequence for Sal I (with its point of cleavage indicated as above) is 5' -C A G C T * G-3'. These are hence said to be palindromic sequences, and double-stranded cuts produce short sinlge-stranded cohesive ends.



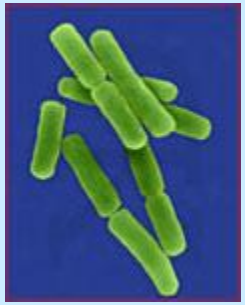

EcoRI
DNA fragments join at sticky ends
Sticky end
Sticky end
Recombinant DNA
Restriction Enzyme Action of EcoRI

I VIRUS

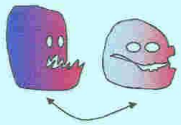



Bacteria have defenses
invading dsDNA
Restriction endonuclease
makes ds cuts in DNA
Invader recognition
invading DNA
GAATTC
CTTAAG
I. Each restriction enzyme recognizes a specific target sequence. Each bacterial species has one to several different restriction enzymes.
CH3
GAATTC
CTTAAG
CH3
bacterium own DNA
II. Target sequences in the bacterial DNA are marked by methylation and left uncut.

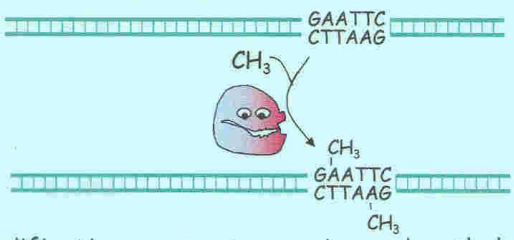
I VIRUS



The modification methylase



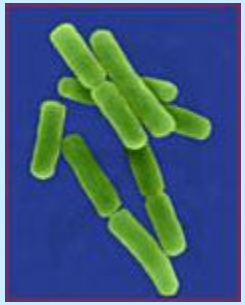
restriction endonucleases are always paired with the corresponding modification methylase

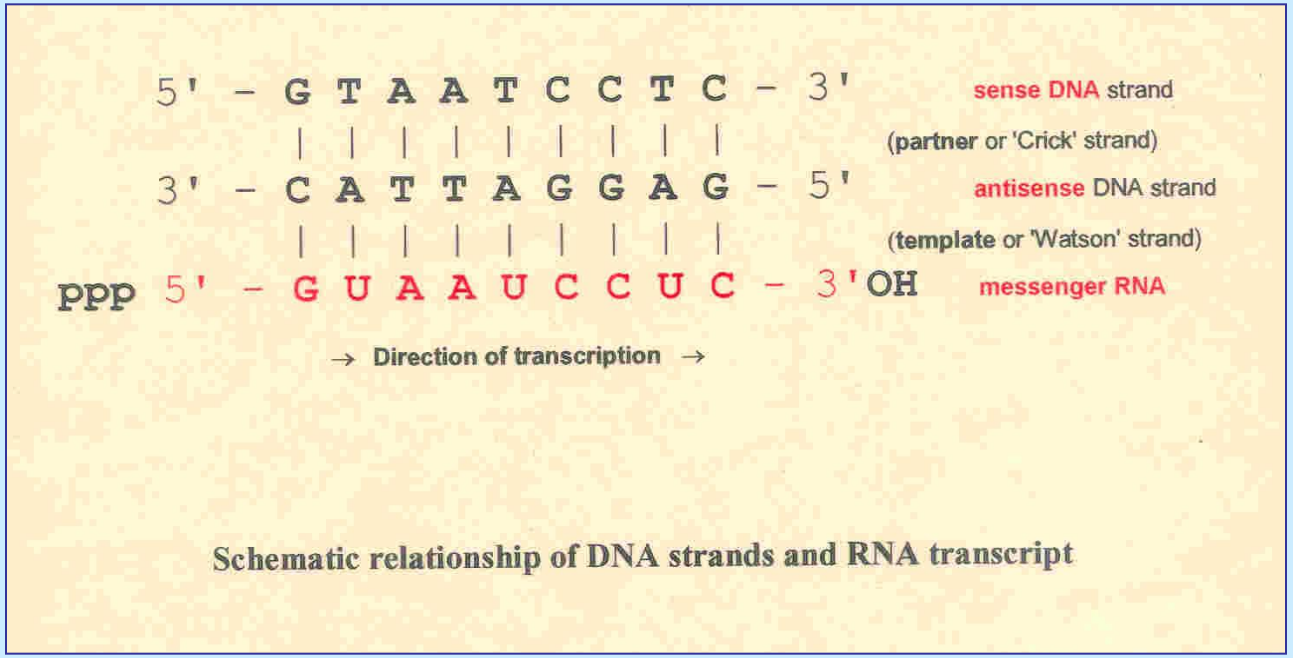


Modification enzymes recognize and methylate the same bacterial DNA sites targeted by the restriction enzyme

Conclusion on restriction-modification systems

- The bacterial DNA is protected by the continuous action of the restriction methylase
- Invading DNA is not marked (= protected) by methylation and is immediately digested by the restriction endonuclease
- Restriction endonucleases can be easily purified. They are useful tools for the analysis and manipulation of DNA



Schematic relationship of DNA strands and RNA transcript

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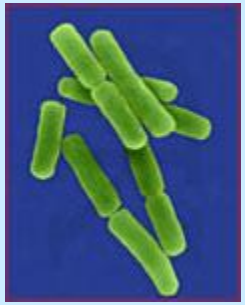

(a)	mRNA	5′ ... GAC UCG AGC ... 3′
(b)	+ strand DNA	5′ ... GAC TCG AGC ... 3′
(c)	− strand DNA	3′ ... CTG AGC TCG ... 5′
(d)	+ strand RNA	5′ ... GAC UCG AGC ... 3′
(e)	− strand RNA	3′ ... CUG AGC UCG ... 5′

Figure 8.11 Comparison of sequences of nucleic acids with different configurations. The strands of nucleic acid in viral genomes are called *plus* (+) if they are in the same configuration as mRNA and *minus* (−) if they are in a configuration opposite that of mRNA. (a) The direction and sequence of an mRNA. (b) The sequence of a + strand of a viral DNA genome that could encode that mRNA (using a − strand DNA intermediate). (c) A − strand of a viral DNA genome that could encode the mRNA. (d, e) The + and − strands of RNA genomes that could encode the mRNA. Note that a single-stranded + strand RNA genome has the same sequence and orientation as the mRNA. The 5′-end of each nucleic acid strand is highlighted in red.

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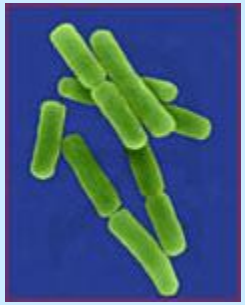

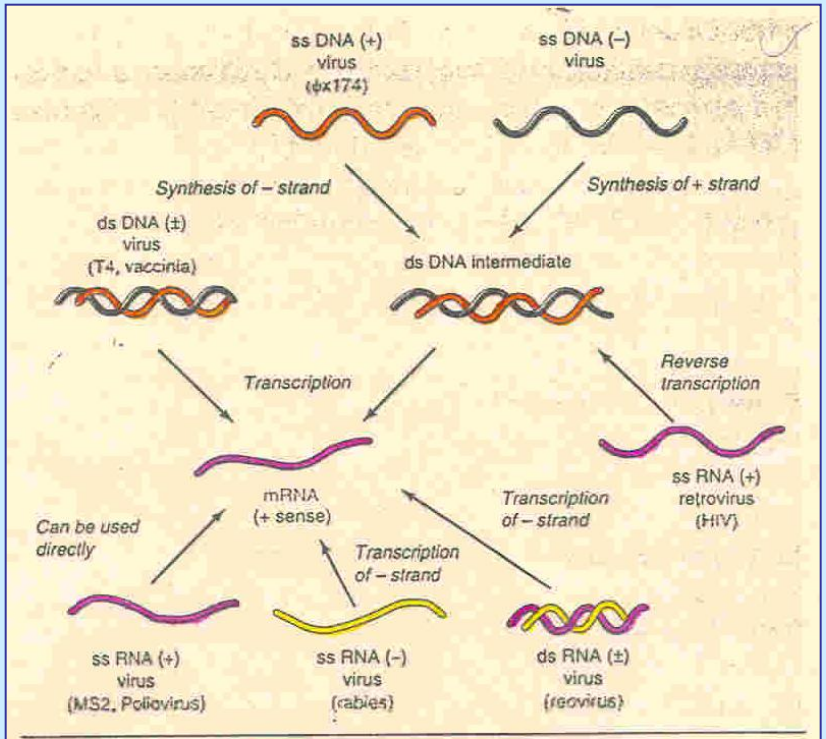

Figure 8.12 Formation of mRNA after infection of cells by viruses of different types. The chemical sense of the mRNA is considered as plus (+). The senses of the various virus nucleic acids are indicated as + if the same as mRNA, as − if opposite, or as ± if double-stranded. Examples are indicated next to the virus nucleic acid. Although examples of viruses containing ss DNA of the − sense are known, none are discussed in the text.

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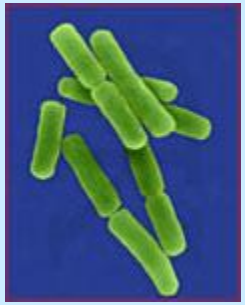


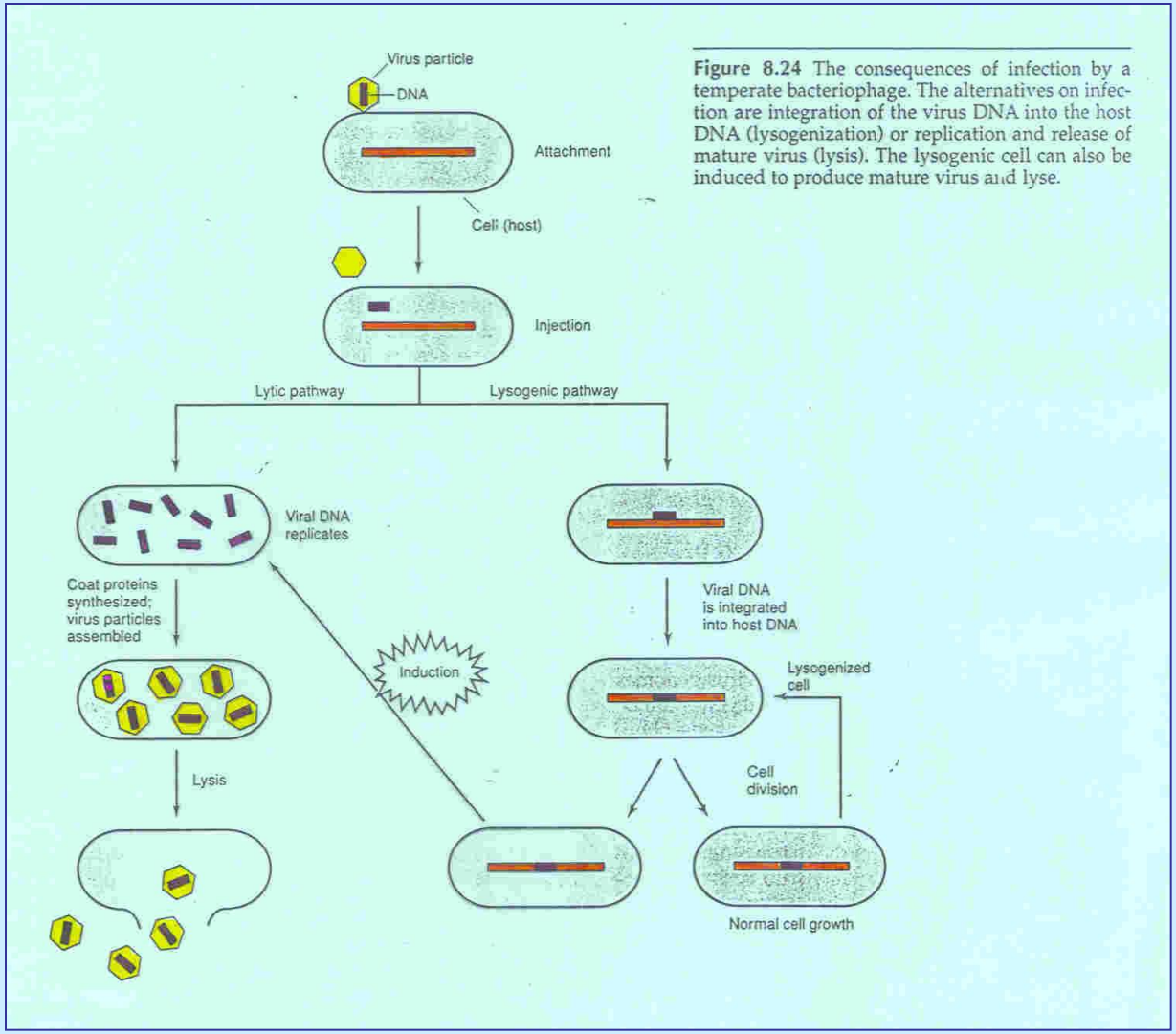
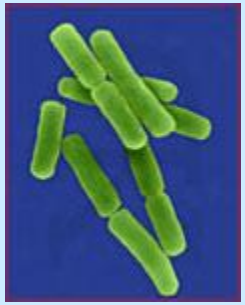


Figure 8.24 The consequences of infection by a temperate bacteriophage. The alternatives on infection are integration of the virus DNA into the host DNA (lysogenization) or replication and release of mature virus (lysis). The lysogenic cell can also be induced to produce mature virus and lyse.



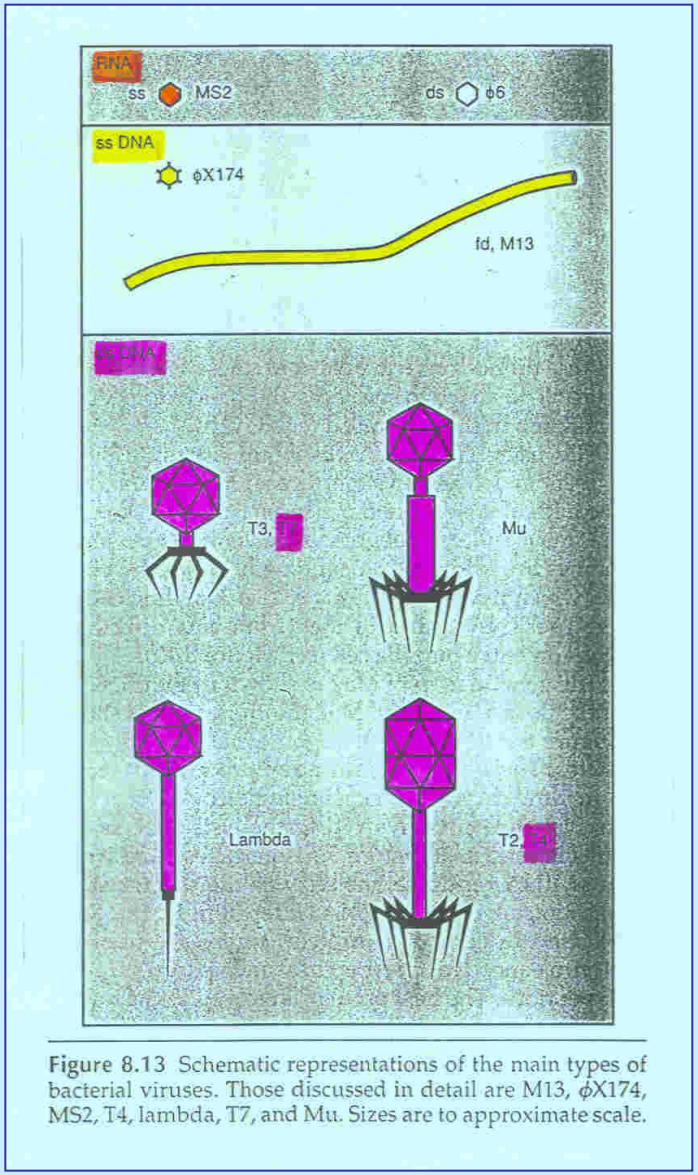


Figure 8.13 Schematic representations of the main types of bacterial viruses. Those discussed in detail are M13, ϕX174, MS2, T4, lambda, T7, and Mu. Sizes are to approximate scale.

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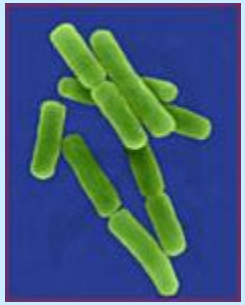

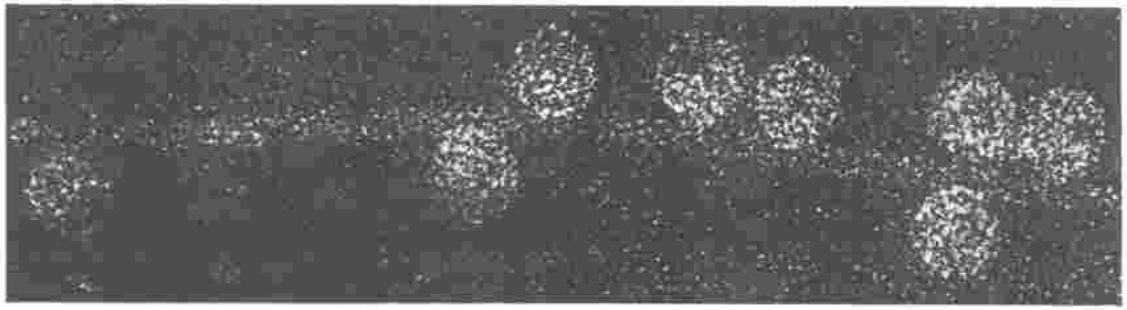

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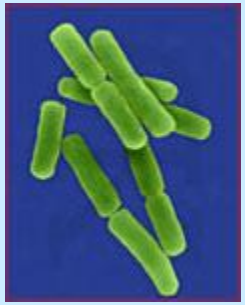
Figure 8.14 Electron micrograph of the pilus of a male bacterial cell of *Escherichia coli* showing virions of a small RNA phage attached to the pilus.



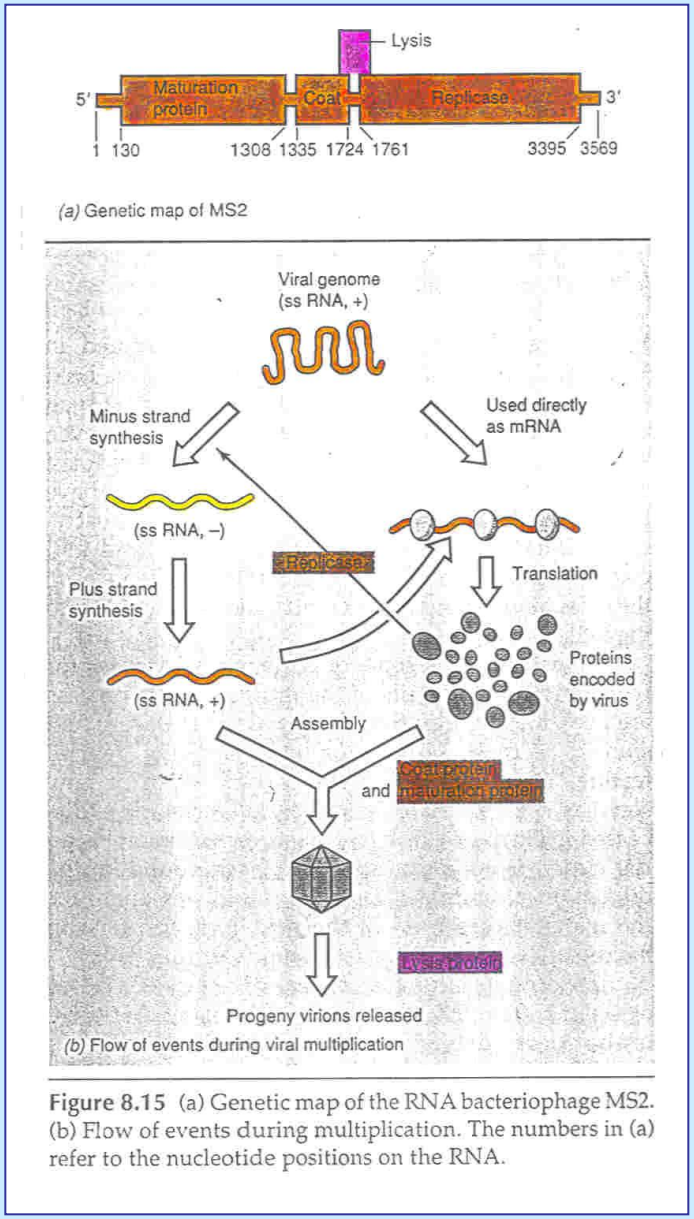


Figure 8.15 (a) Genetic map of the RNA bacteriophage MS2. (b) Flow of events during multiplication. The numbers in (a) refer to the nucleotide positions on the RNA.

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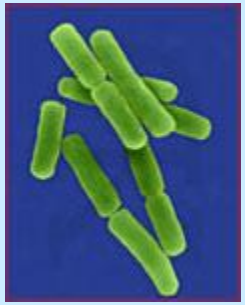


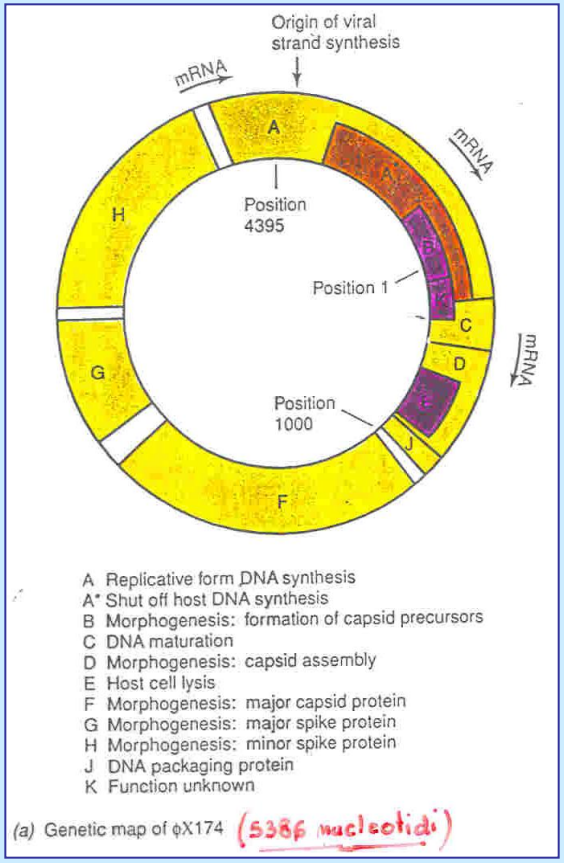

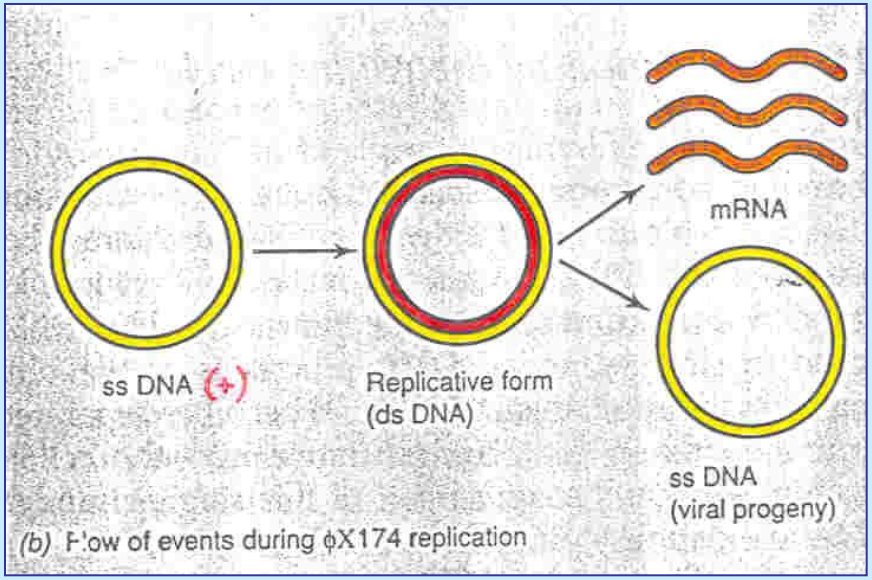

Figure 8.15 Bacteriophage ϕX174, a single-stranded DNA phage. (a) Genetic map. Note the regions of gene overlap (A/B, K/B, K/C, K/A, A/C, and D/E). Intergenic regions are not colored. Protein A* is formed using only part of the coding sequence of gene A by reinitiation of translation (see text). (b) Flow of events in ϕX174 multiplication. The production of progeny ss DNA from replicative form ds DNA involves rolling circle replication and is shown in more detail in Figure 8.17.

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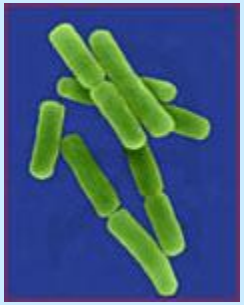

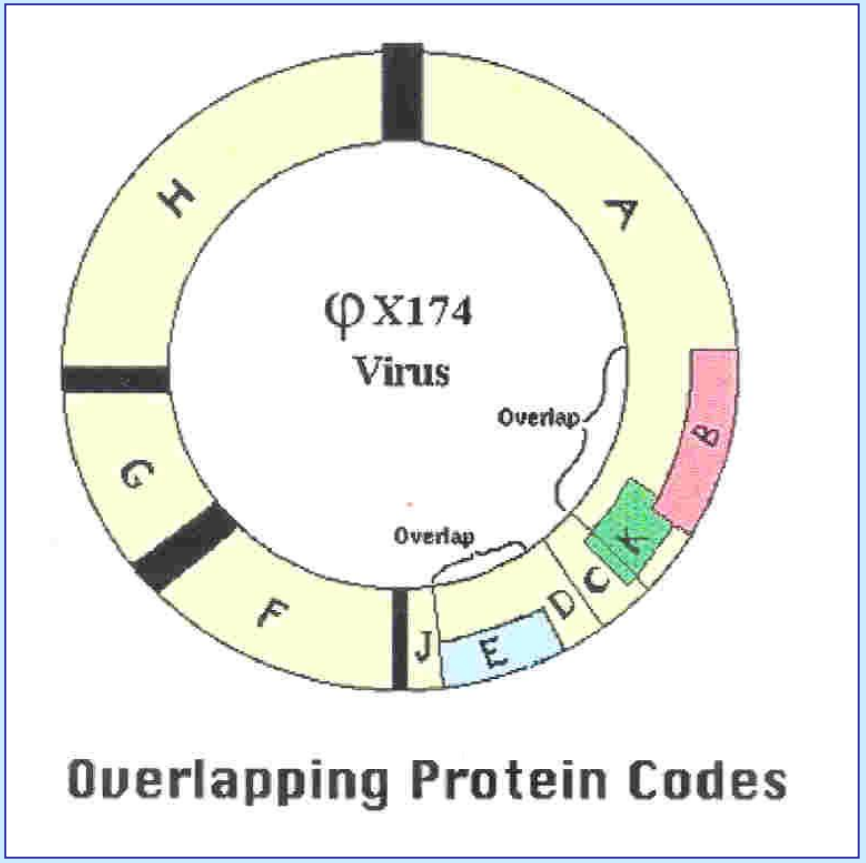

Overlapping Protein Codes

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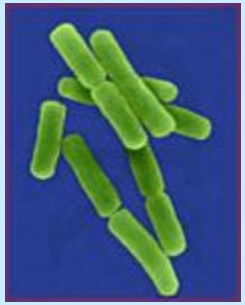


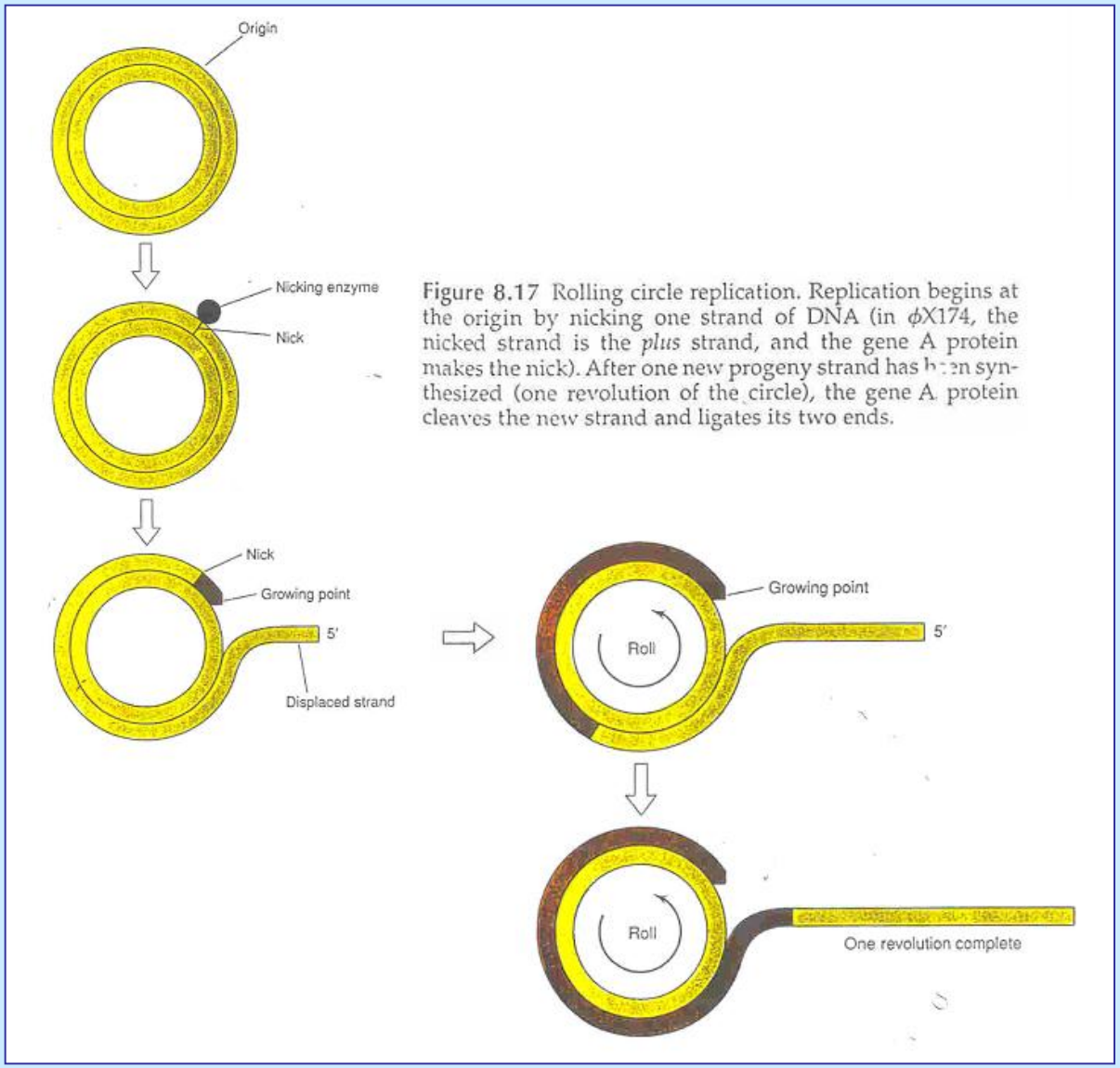
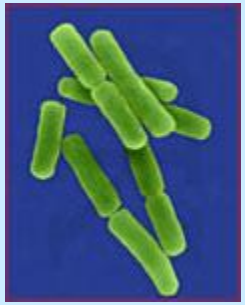


Figure 8.17 Rolling circle replication. Replication begins at the origin by nicking one strand of DNA (in ϕX174, the nicked strand is the *plus* strand, and the gene A protein makes the nick). After one new progeny strand has been synthesized (one revolution of the circle), the gene A protein cleaves the new strand and ligates its two ends.



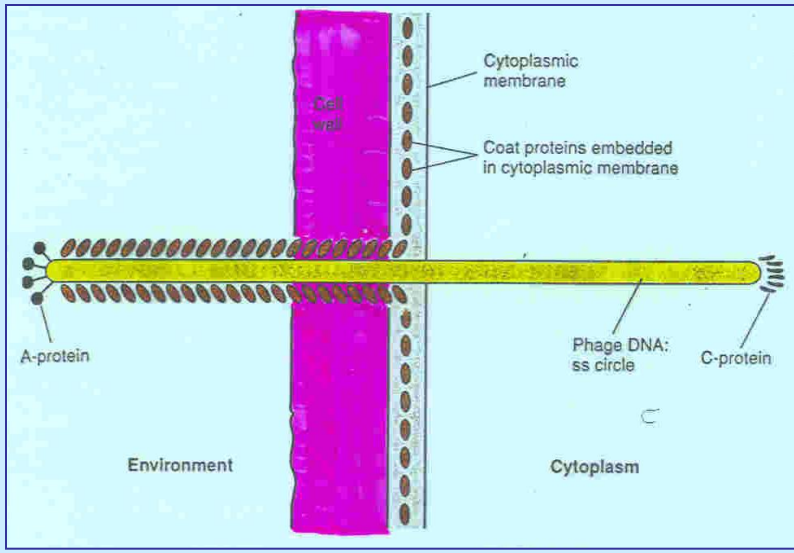


Figure 8.18 Illustration of the manner in which the virion of a filamentous single-stranded phage (such as M13 or fd) leaves an infected cell without lysis. The A-protein passes first through the membrane at a site on the membrane where coat protein molecules have first become embedded. The intracellular circular DNA is coated with dimers of another phage protein, which is displaced by coat protein as the DNA passes through the intact cytoplasmic membrane.

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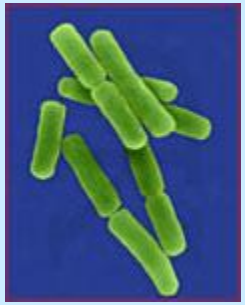

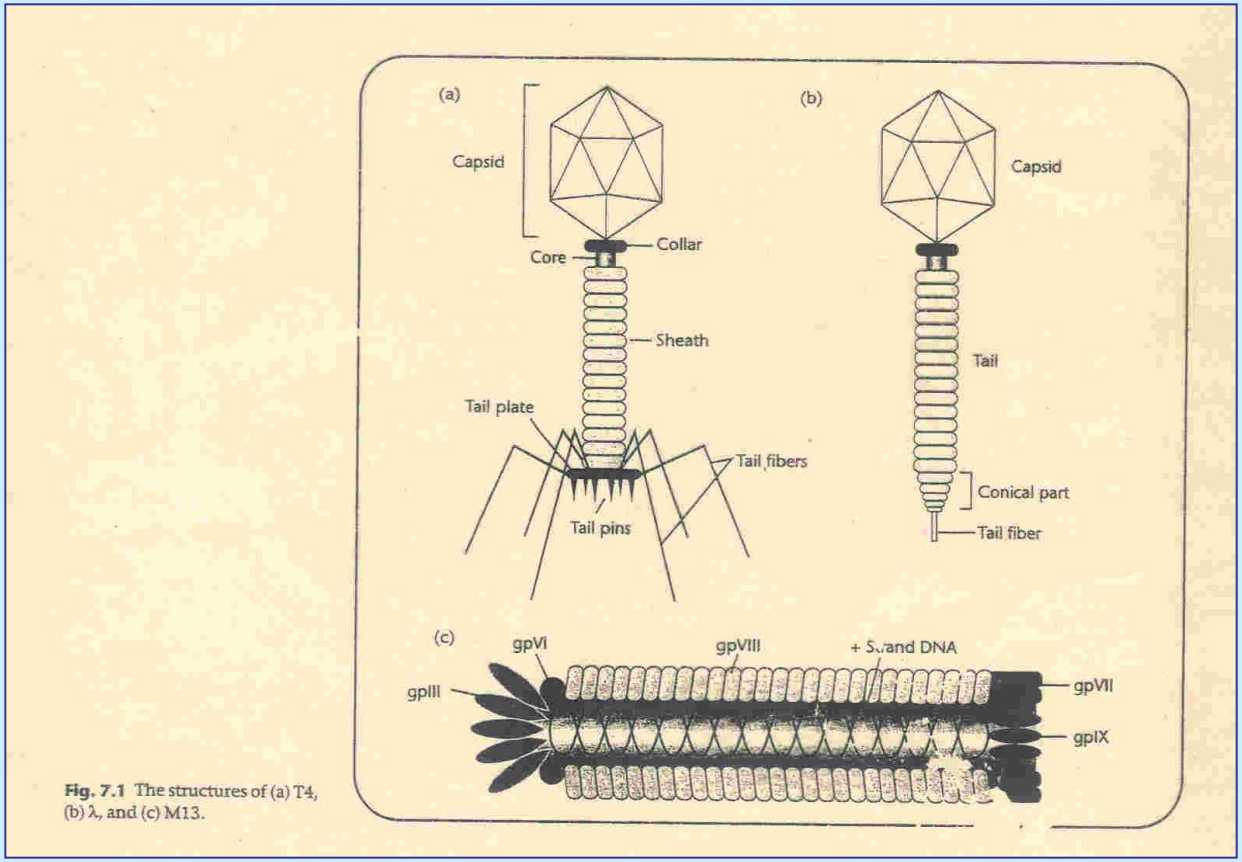

Fig. 7.1 The structures of (a) T4, (b) λ, and (c) M13.

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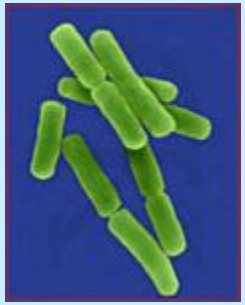


Fig. 7.12 The conversion of the M13 plus strand to a double-stranded DNA molecule. The plus strand enters the cell (a and b) with gpIII attached. It is immediately coated with host SSB (c). RNA polymerase synthesizes a short primer (d) and DNA polymerase synthesizes the minus strand.

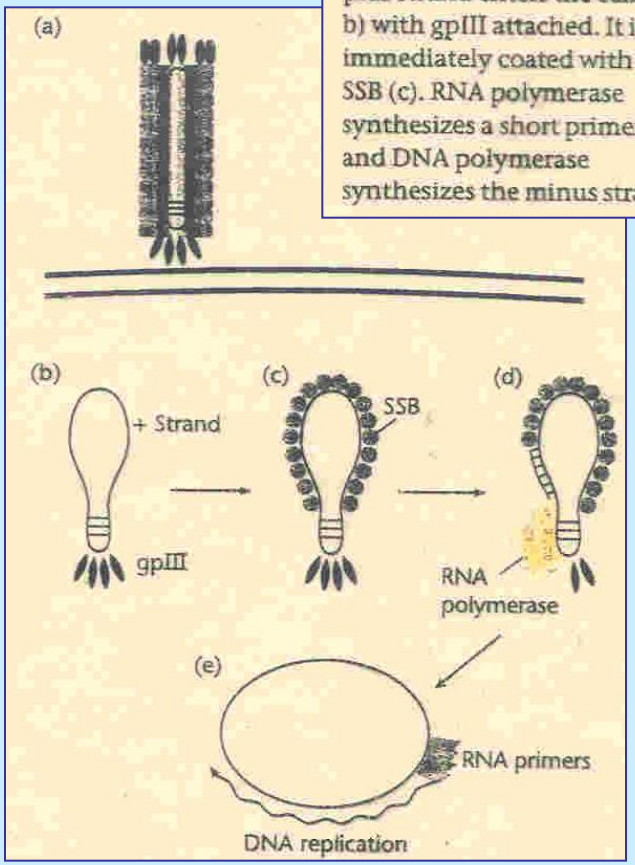
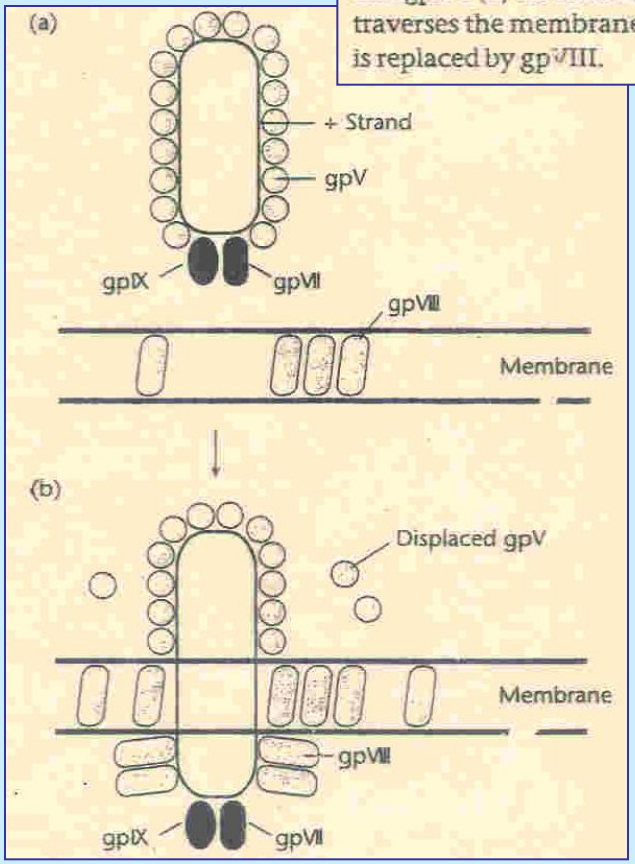


Fig. 7.13 M13 is released from the cell without lysing the bacterium. (a) The plus strand, coated with gpV interacts with the membrane through gpVII and gpIX. (b) As the DNA traverses the membrane, the gpV is replaced by gpVIII.



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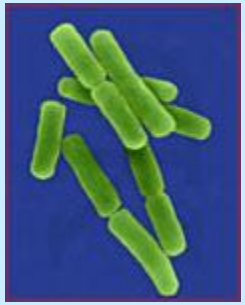

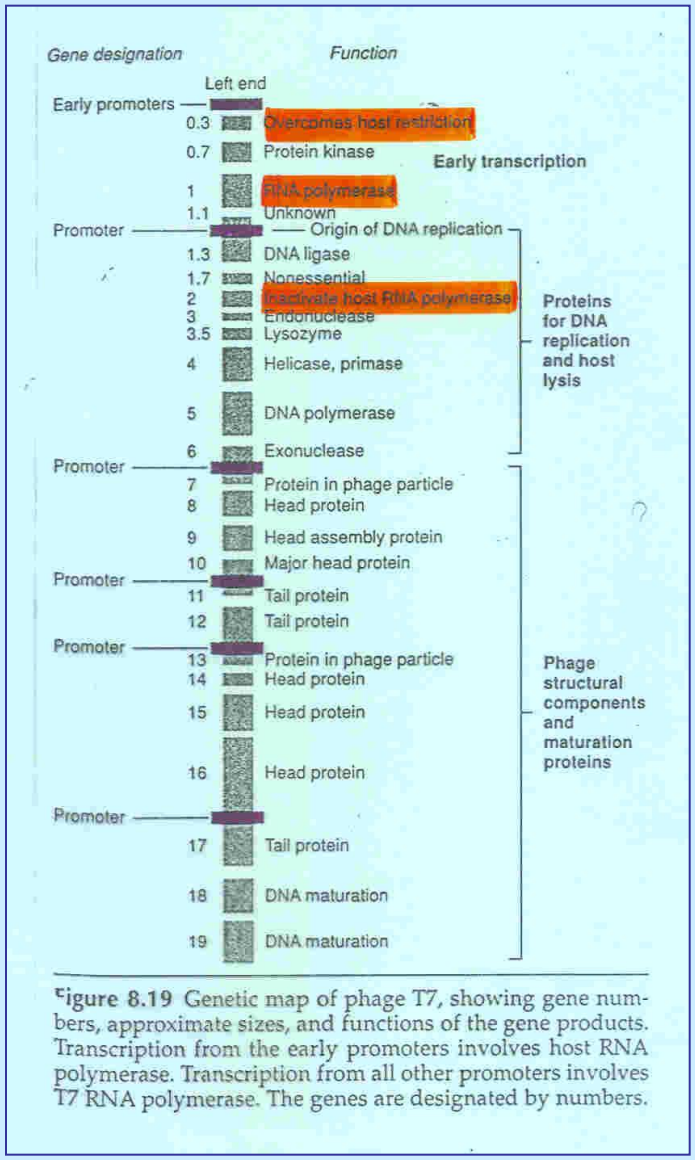

Figure 8.19 Genetic map of phage T7, showing gene numbers, approximate sizes, and functions of the gene products. Transcription from the early promoters involves host RNA polymerase. Transcription from all other promoters involves T7 RNA polymerase. The genes are designated by numbers.

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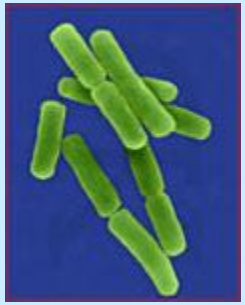


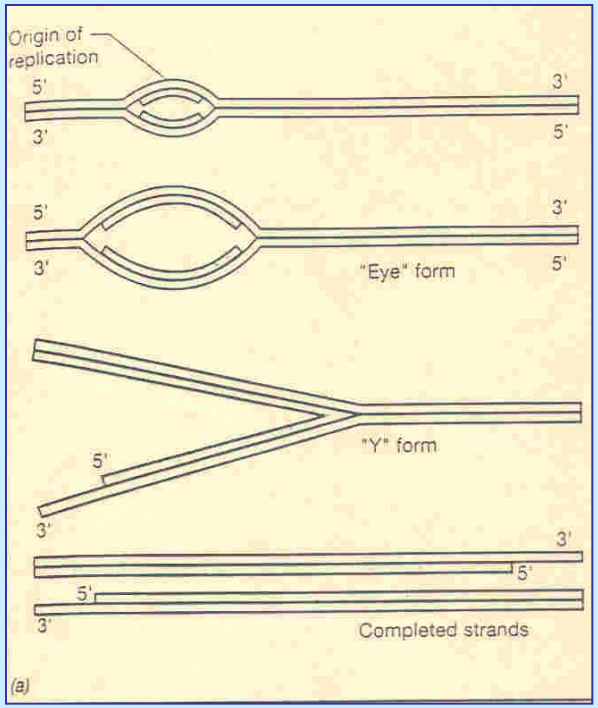

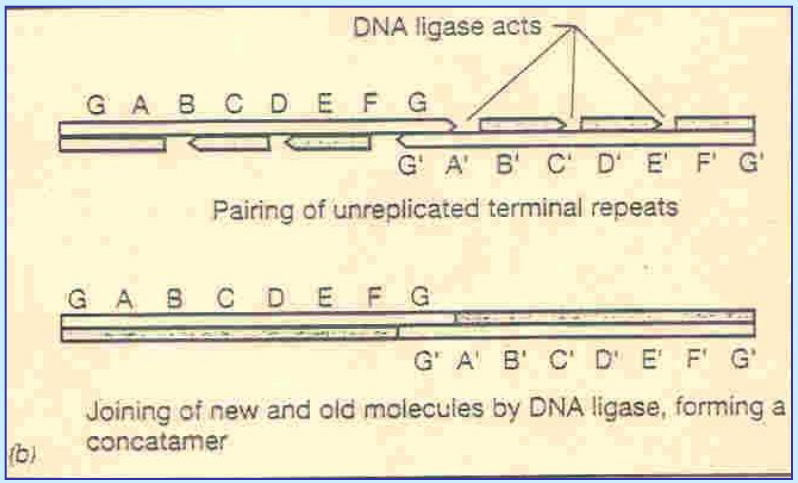

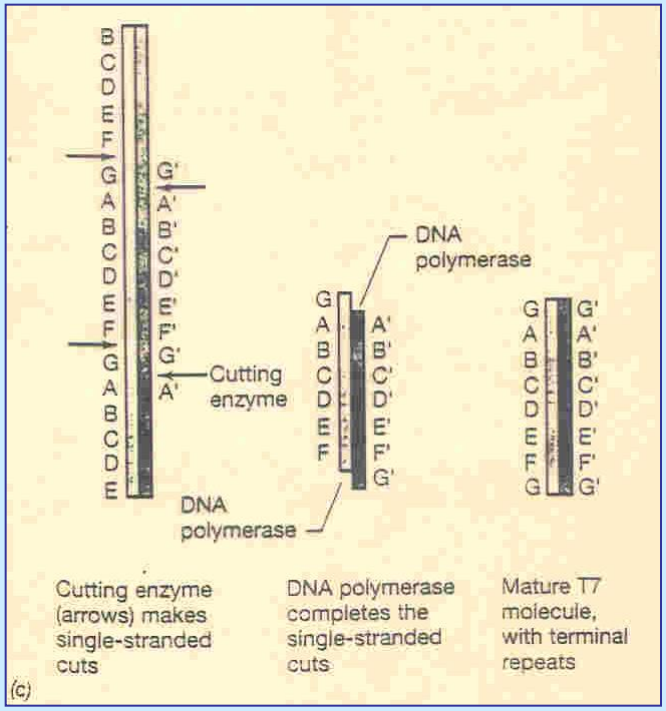

FIGURE 8.20 Replication of the linear, double-stranded DNA genome of bacteriophage T7. (a) Bidirectional replication of DNA giving rise to intermediate "eye" and "Y" forms. (b) Formation of concatamers by joining DNA molecules at the unreplicated terminal ends. The designation of the genes is arbitrary. (c) Production of mature viral DNA molecules from T7 concatamers by action of cutting enzyme, an endonuclease. Left: The enzyme makes single-stranded cuts of specific sequences (arrows); center: DNA polymerase completes the single-stranded ends; right: the mature T7 molecule with terminal repeats.

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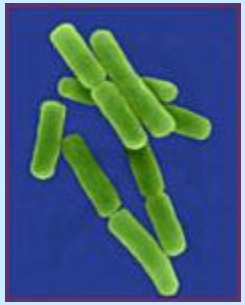

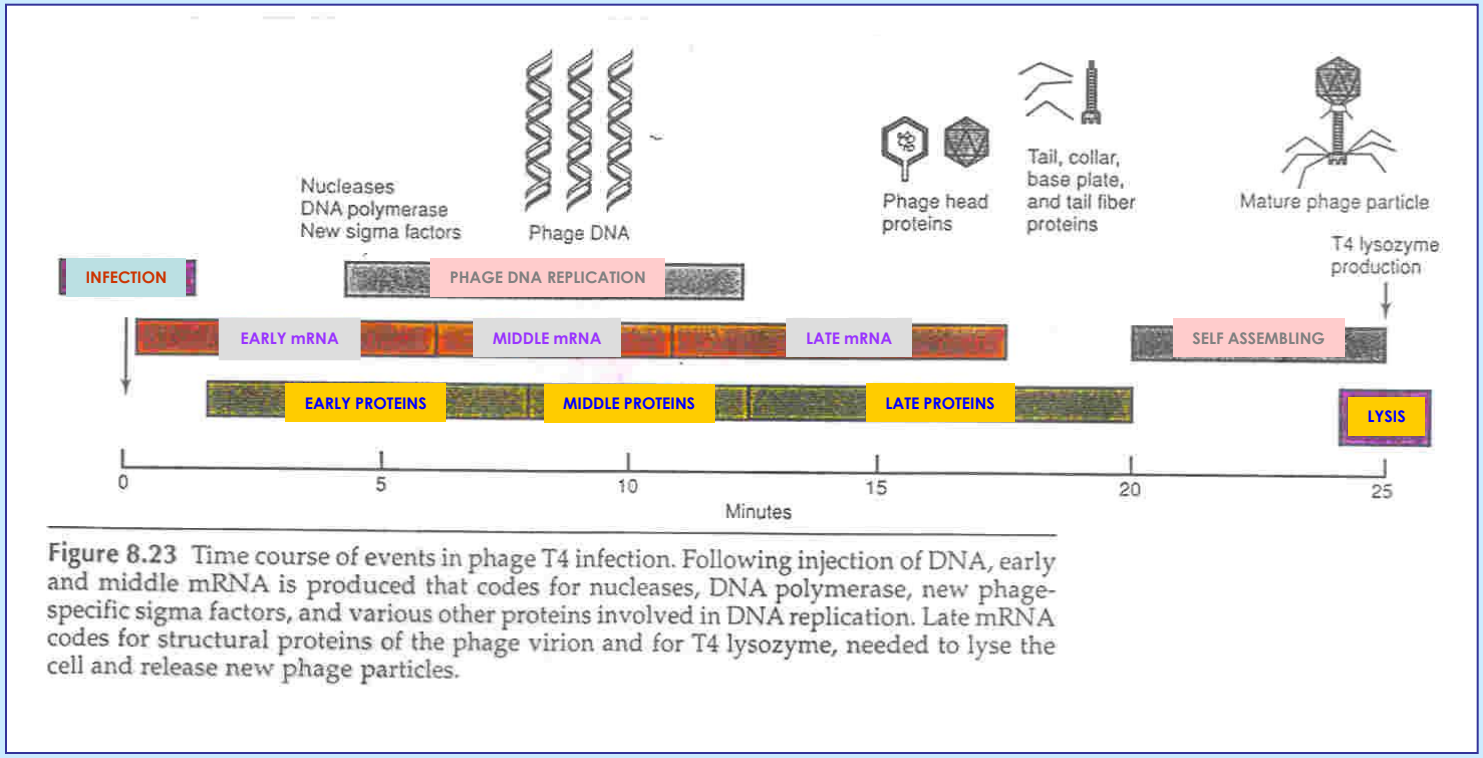

Figure 8.23 Time course of events in phage T4 infection. Following injection of DNA, early and middle mRNA is produced that codes for nucleases, DNA polymerase, new phage-specific sigma factors, and various other proteins involved in DNA replication. Late mRNA codes for structural proteins of the phage virion and for T4 lysozyme, needed to lyse the cell and release new phage particles.

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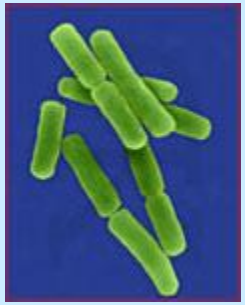


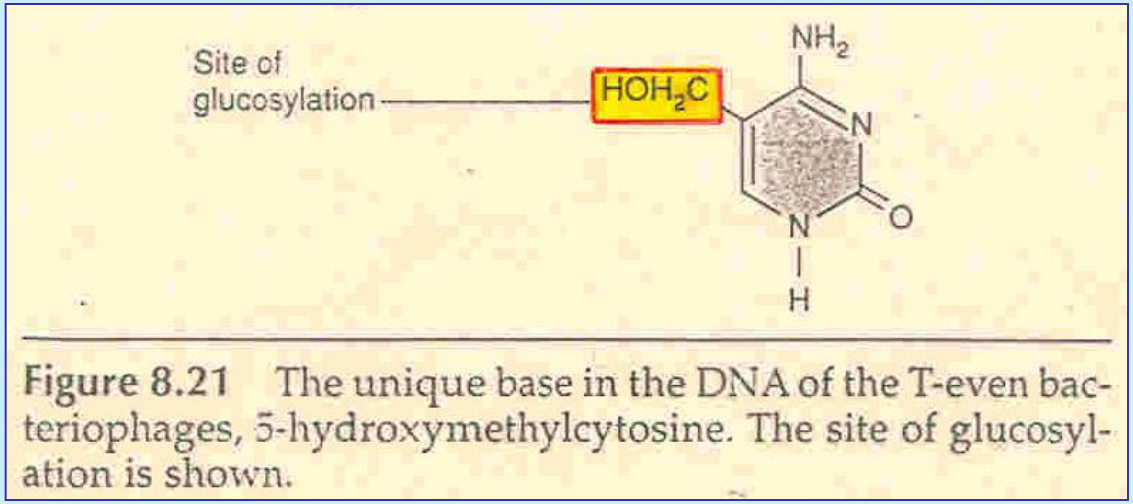

Figure 8.21 The unique base in the DNA of the T-even bacteriophages, 5-hydroxymethylcytosine. The site of glucosylation is shown.

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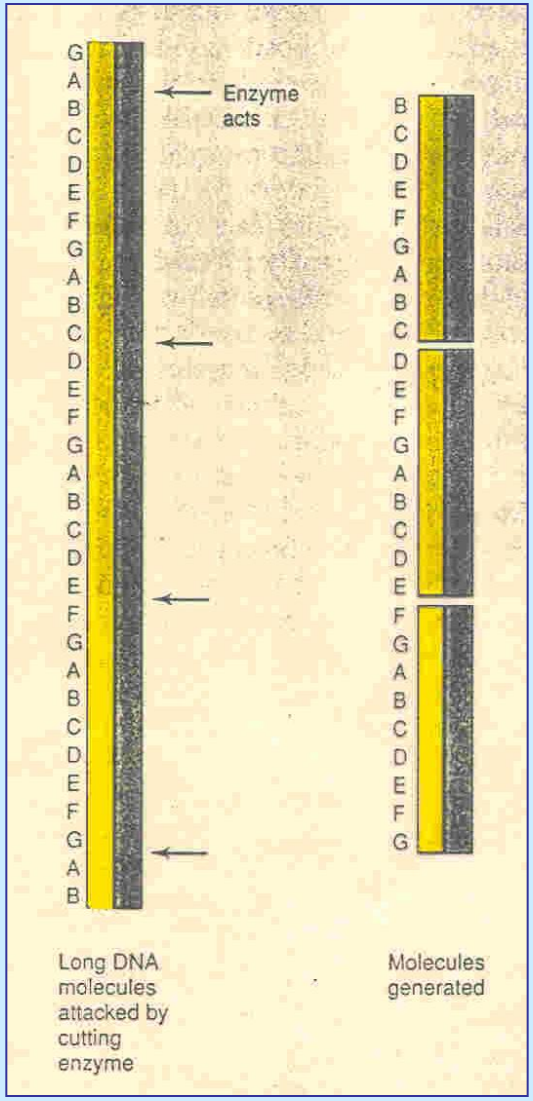
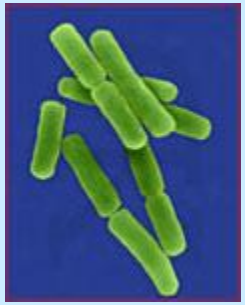


Figure 8.22 Generation in T4 of virus length DNA molecules with permuted sequences by a cutting enzyme, which cuts off constant lengths of DNA irrespective of the sequence. Left: arrows, sites of enzyme attack; right: molecules generated.



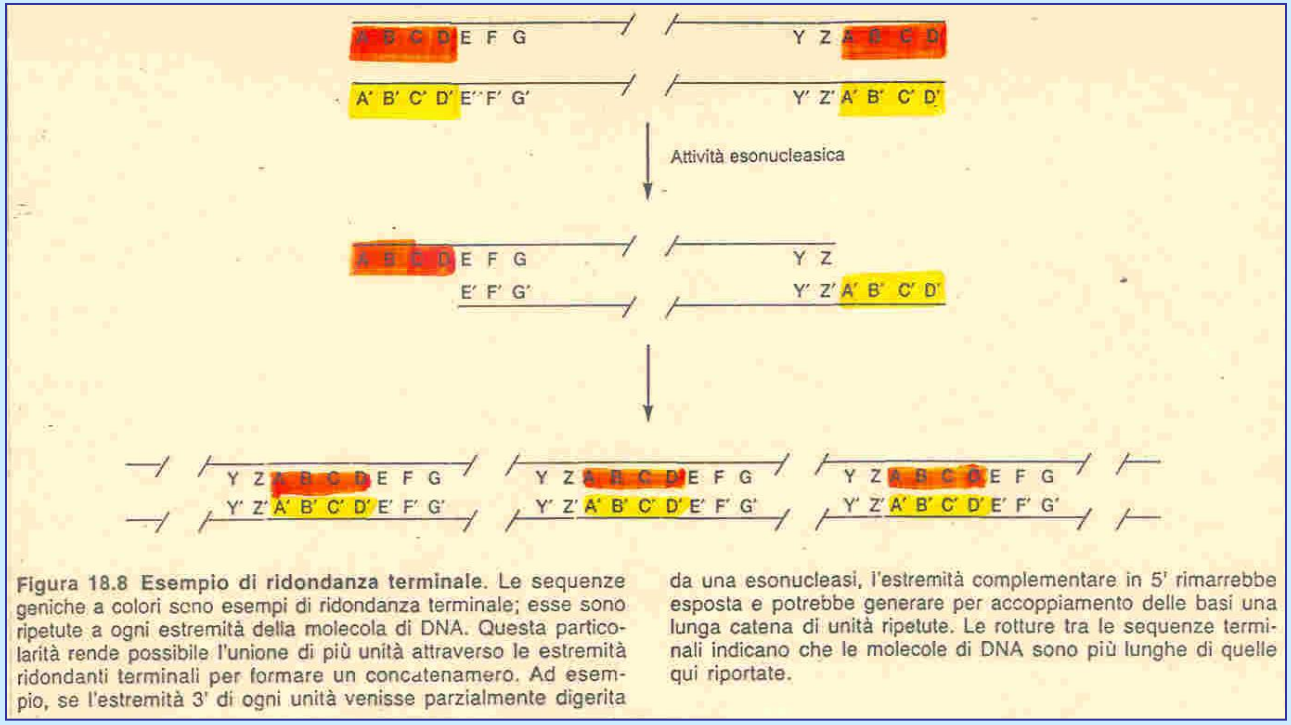
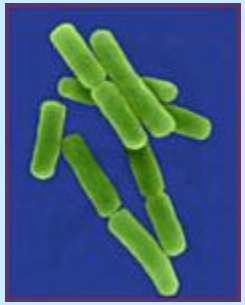


Figura 18.8 Esempio di ridondanza terminale. Le sequenze geniche a colori sono esempi di ridondanza terminale; esse sono ripetute a ogni estremità della molecola di DNA. Questa particolarità rende possibile l'unione di più unità attraverso le estremità ridondanti terminali per formare un concatenamero. Ad esempio, se l'estremità 3' di ogni unità venisse parzialmente digerita da una esonucleasi, l'estremità complementare in 5' rimarrebbe esposta e potrebbe generare per accoppiamento delle basi una lunga catena di unità ripetute. Le rotture tra le sequenze terminali indicano che le molecole di DNA sono più lunghe di quelle qui riportate.



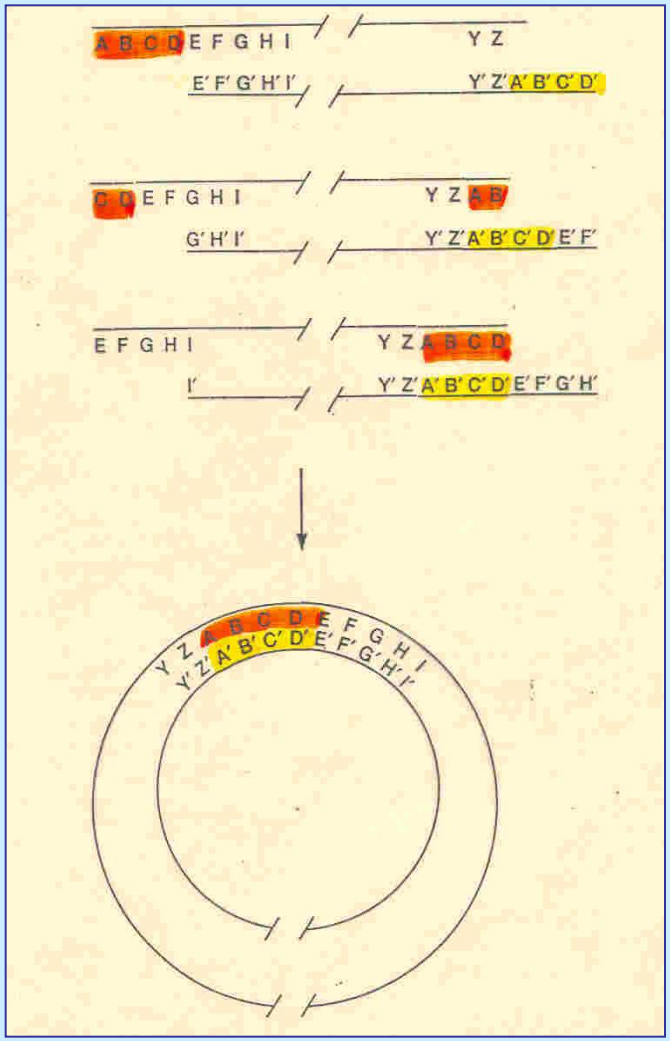
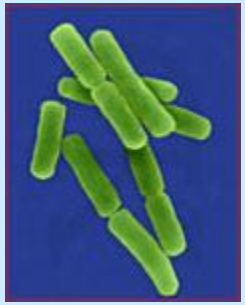


Figura 18.9 Genomi permutati circolarmente estratti da un concatenamero. Il concatenamero formato in figura 18.8 può essere tagliato a qualsiasi livello in pezzi di uguale lunghezza, che contengono un corredo genico completo, anche se alle estremità sono presenti geni differenti. Se ogni pezzo ha estremità coesive a singolo filamento come in figura 18.8, esso si avvolgerà a formare un cerchio il cui ordine genico sarà identico a quello presente nei cerchi prodotti da pezzi diversi.



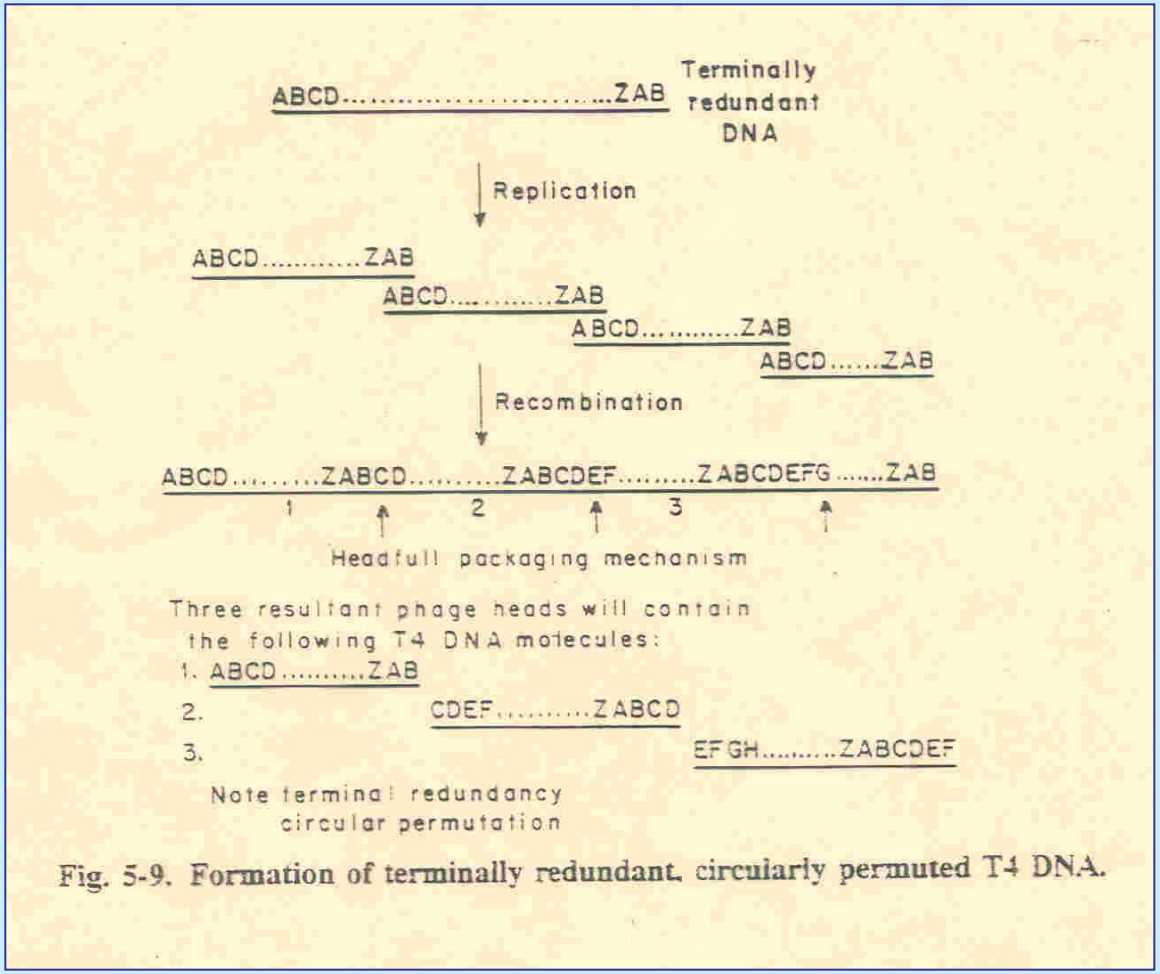
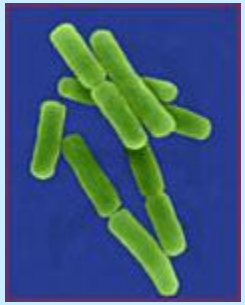
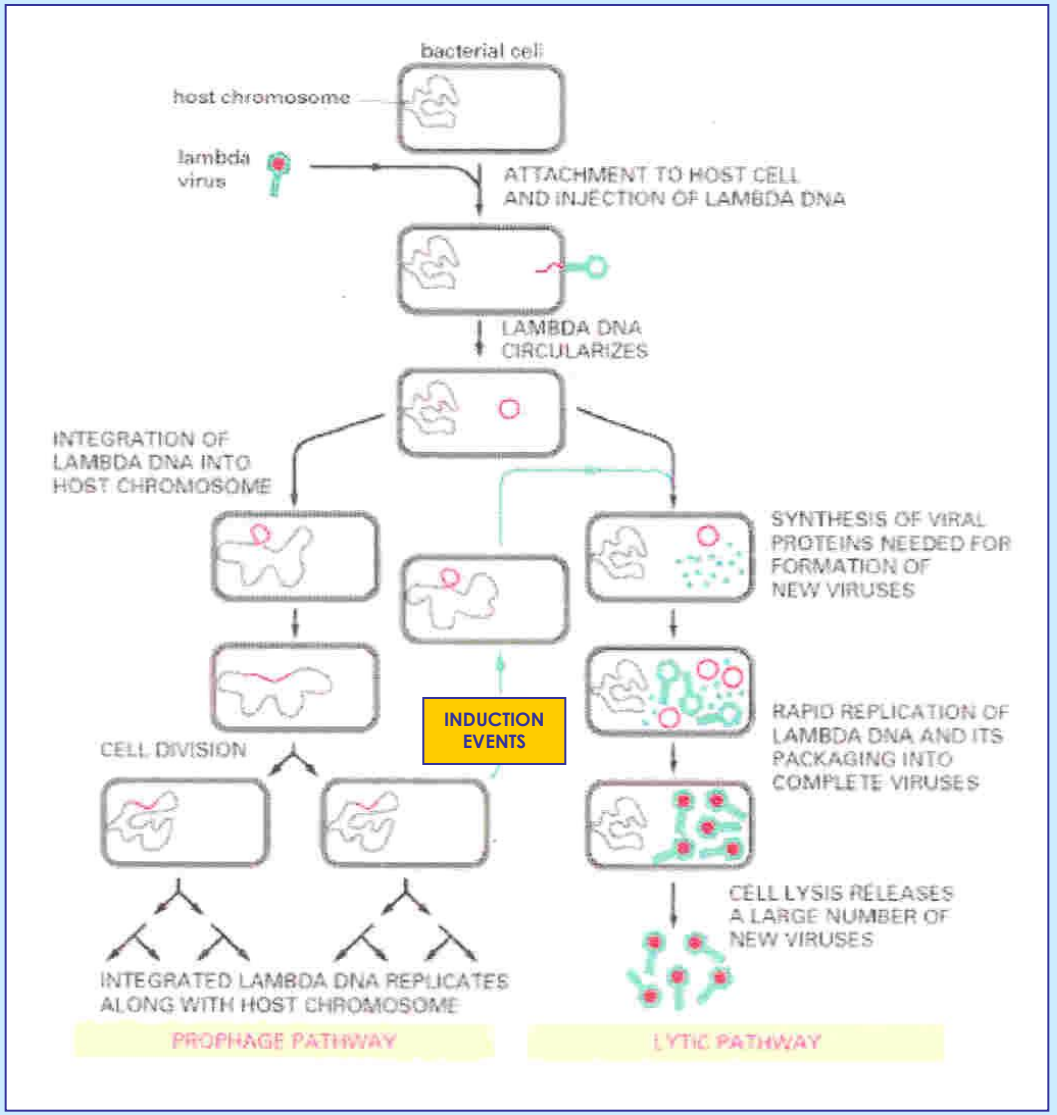
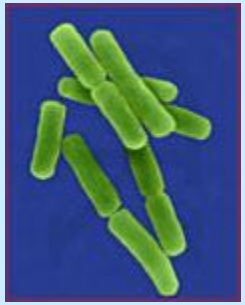


Fig. 5-9. Formation of terminally redundant, circularly permuted T4 DNA.




bacterial cell
host chromosome
lambda virus
ATTACHMENT TO HOST CELL AND INJECTION OF LAMBDA DNA
LAMBDA DNA CIRCULARIZES
INTEGRATION OF LAMBDA DNA INTO HOST CHROMOSOME
SYNTHESIS OF VIRAL PROTEINS NEEDED FOR FORMATION OF NEW VIRUSES
INDUCTION EVENTS
CELL DIVISION
RAPID REPLICATION OF LAMBDA DNA AND ITS PACKAGING INTO COMPLETE VIRUSES
CELL LYSIS RELEASES A LARGE NUMBER OF NEW VIRUSES
INTEGRATED LAMBDA DNA REPLICATES ALONG WITH HOST CHROMOSOME
PROPHAGE PATHWAY
LYTIC PATHWAY

I VIRUS



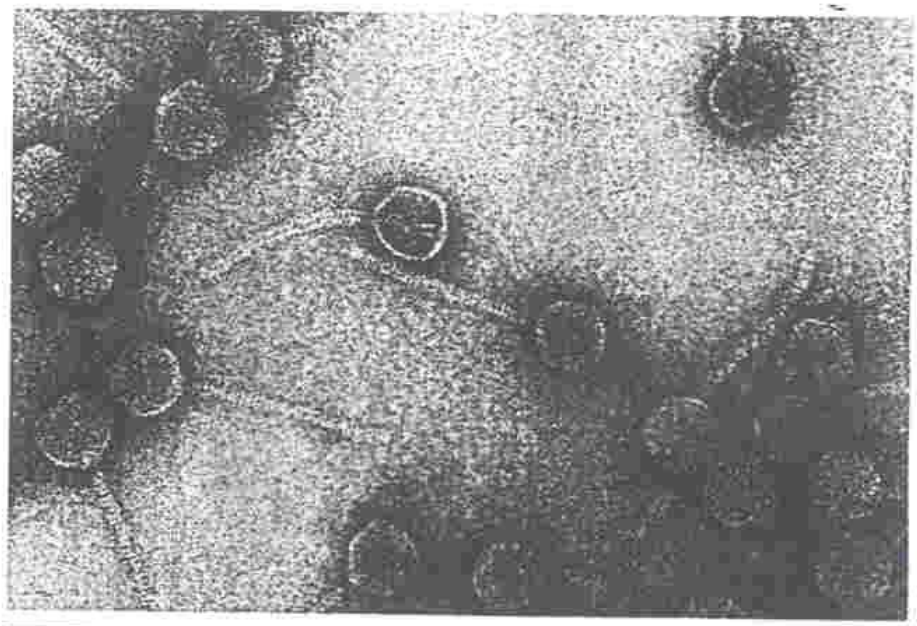
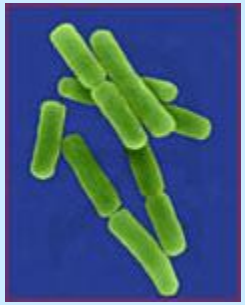


Figure 8.25 Electron micrograph by negative staining of bacteriophage lambda particles. The head of each particle is about 65 nm in diameter.

I VIRUS



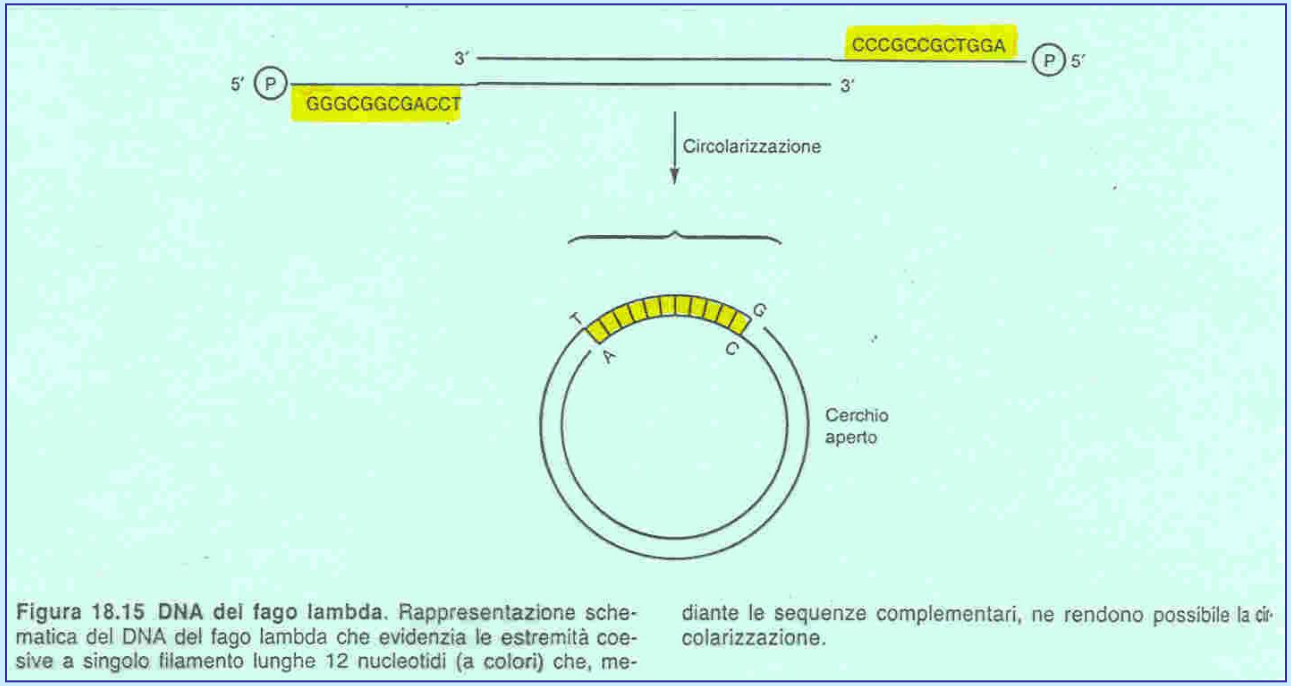
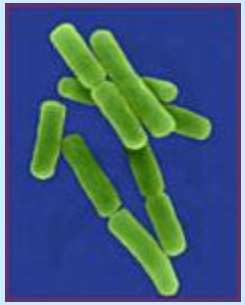


Figura 18.15 DNA del fago lambda. Rappresentazione schematica del DNA del fago lambda che evidenzia le estremità coesive a singolo filamento lunghe 12 nucleotidi (a colori) che, mediante le sequenze complementari, ne rendono possibile la circolarizzazione.

I VIRUS



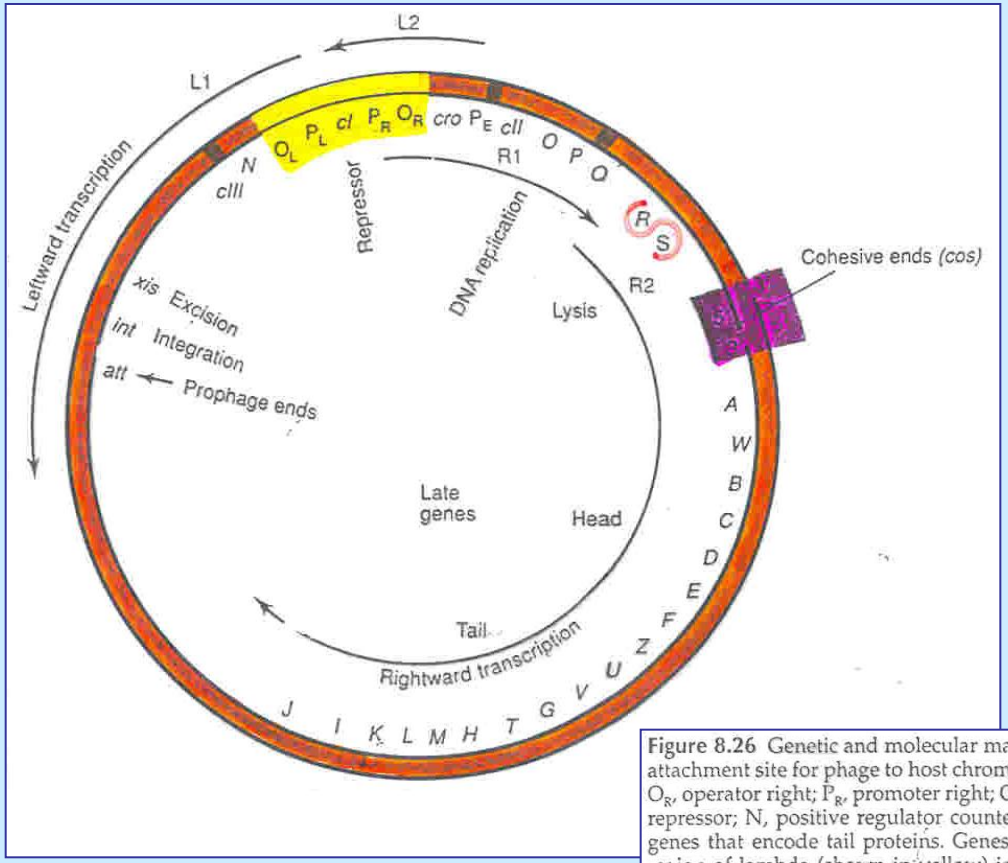
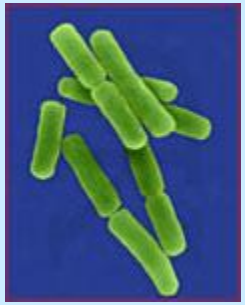



Figure 8.26 Genetic and molecular map of lambda. The genes are designated by letters; *att*, attachment site for phage to host chromosome. Genes of special interest: *cI*, repressor protein; O_R, operator right; P_R, promoter right; O_L, operator left; P_L, promoter left; *cro*, gene for second repressor; N, positive regulator counteracting rho-dependent termination. J through U are genes that encode tail proteins. Genes Z through A encode head proteins. The regulatory region of lambda (shown in yellow) is positioned at the top of this circular map. It is also known as the immunity region and contains the *cI* gene. The site created when the cohesive ends of the lambda genome join is called *cos* (shown in blue). Early transcription in lambda is primarily leftward (counterclockwise) from P_L and rightward (clockwise) from P_R. The main leftward transcript is labeled L1, and the main early rightward transcript is labeled R1. The three transcript[illegible] terminators affected by the N-protein are shown as gray boxes on the DNA. The late rightward transcript, which encodes head and tail proteins and proteins for lytic function, is labeled R2 and begins at a promoter near the Q gene. The transcript labeled L2 is the positively regulated transcript from P_E that encodes the repressor protein.

I VIRUS



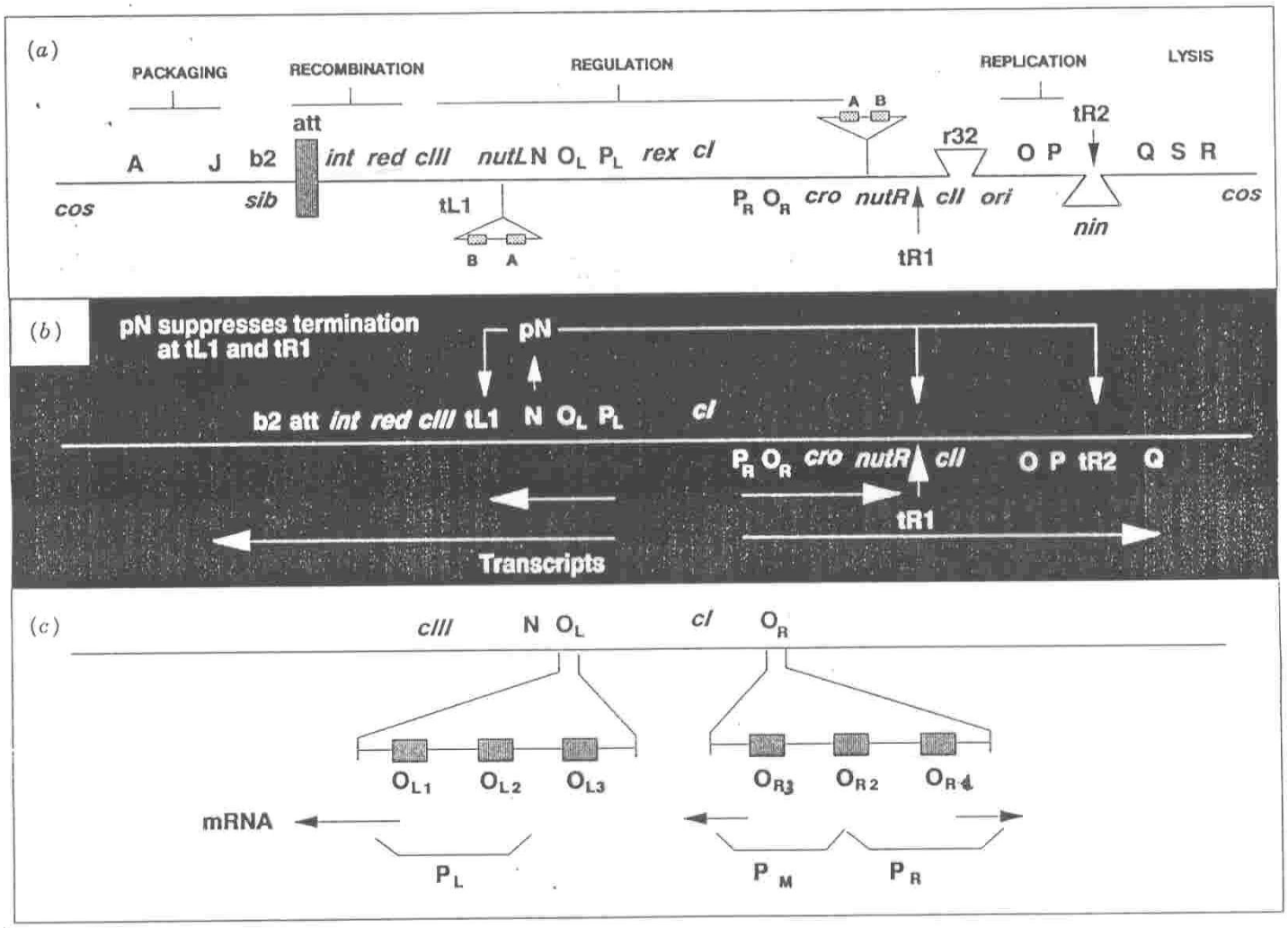
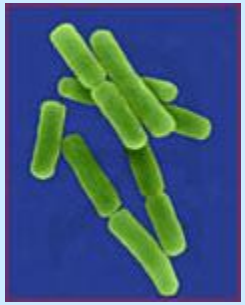


Fig. 5-13. Lambda phage. (A) Genetic organization. (B) Transcriptional organization. (C) Structure of the leftward and rightward promoter/operator regions.

I VIRUS



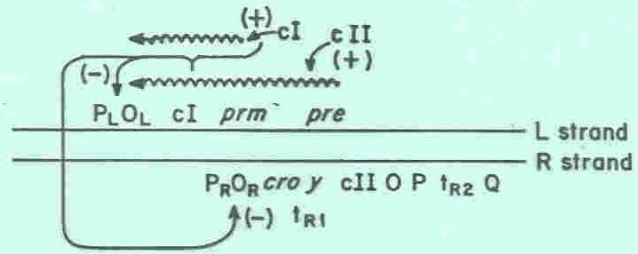
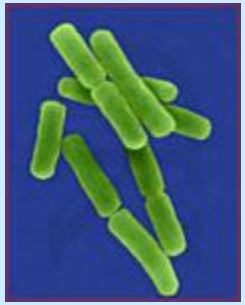
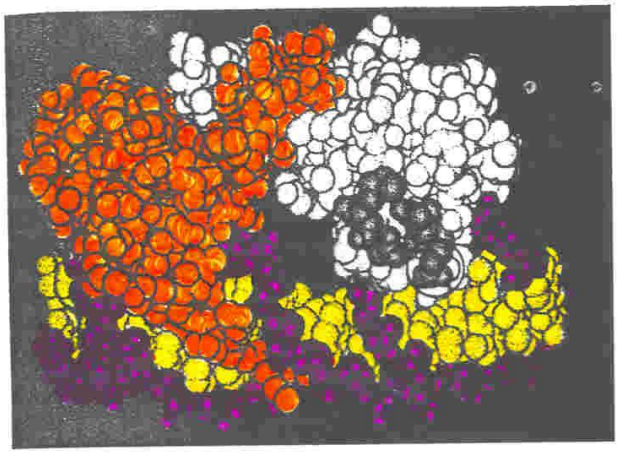


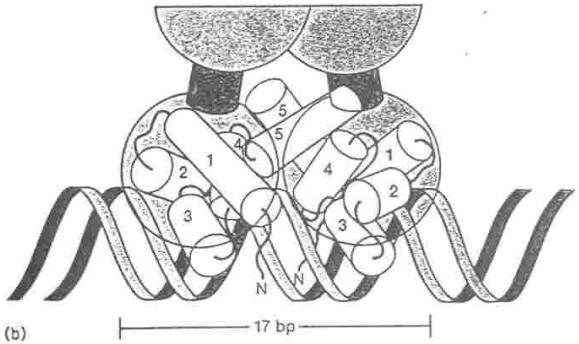
Fig. 5-15. Establishment of lysogeny by λ phage. The CII protein stimulates the transcription of *cI* from *pre*, the promoter for repressor establishment. Once CI protein is produced it will stimulate its own transcription from *prm*, the promoter for repressor maintenance. Note that *prm* overlaps O_R (Fig. 5-13C) such that when CI binds to O_{R1} and O_{R2} transcription is inhibited from P_R but stimulated from *prm*.

I VIRUS





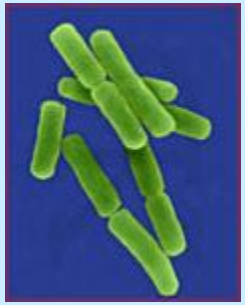
(a)



(b)

Figura 18.17 Legame del repressore lambda. (*a*) Immagine elaborata al computer del legame del repressore lambda all'operatore lambda. Il dimero del repressore (marrone e verde) è legato al DNA (blu e bianco). Le braccia flessibili del dimero circondano il solco principale della doppia elica. (*b*) Segmento di 17 pb dell'operatore. L'α-elica 3 (i segmenti di α-elica sono numerati partendo dall'estremità N-terminale della catena) è la parte più a contatto con la sporgenza dell'operatore. (*Fonte b*: D. Voet e J. G. Voet, *Biochemistry*. Copyright © 1990 John Wiley & Sons, Inc., New York. Riproduzione autorizzata.)

I VIRUS



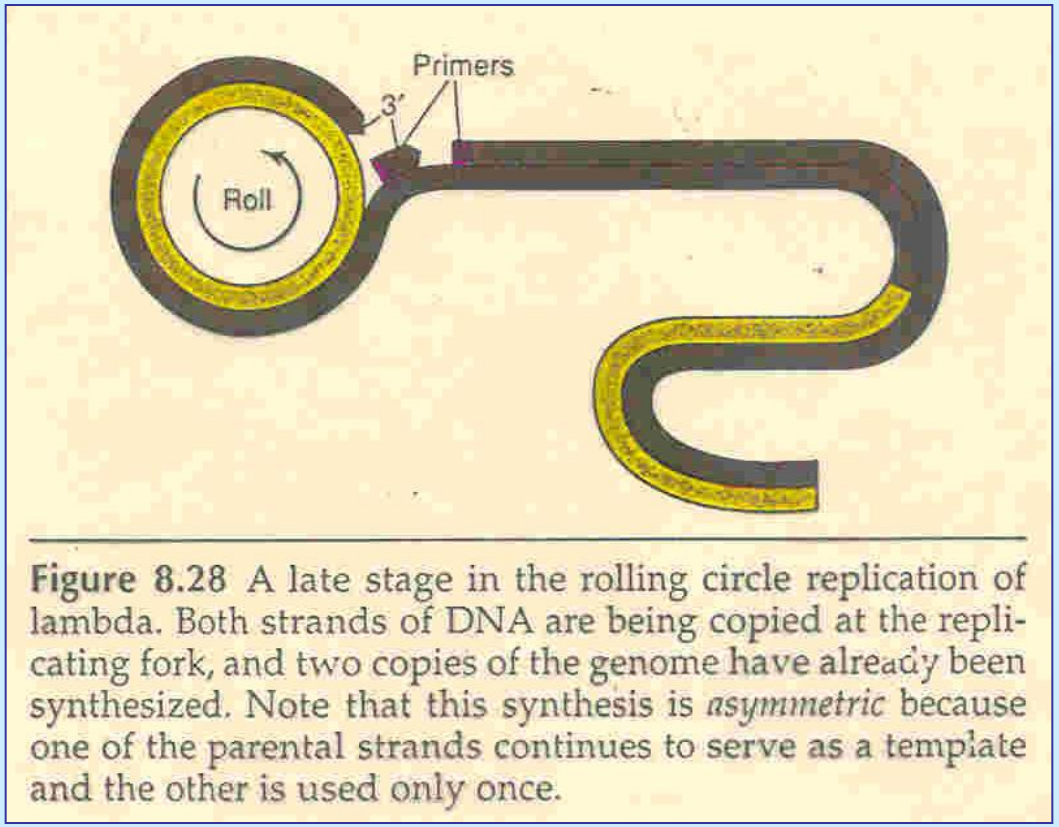
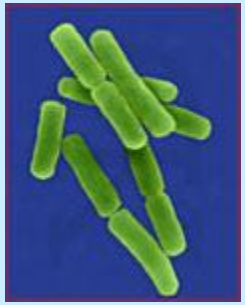



Figure 8.28 A late stage in the rolling circle replication of lambda. Both strands of DNA are being copied at the replicating fork, and two copies of the genome have already been synthesized. Note that this synthesis is *asymmetric* because one of the parental strands continues to serve as a template and the other is used only once.



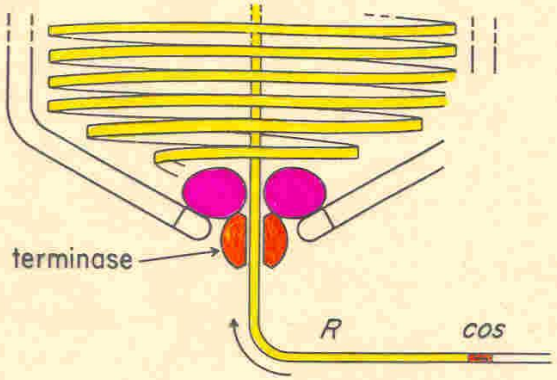
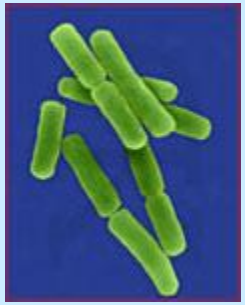



Fig. 5-20. Scanning for the terminal *cos* site, showing part of a nearly filled head with terminase positioned at the connector. The DNA strand being packaged is "scanned" by terminase until the cohesive end symmetry segment of the terminal *cos* site is recognized and cut. Other aspects of packaging, such as the terminase-connector interaction, are purely hypothetical. From Feiss, M. and A. Becker. In *Lambda II*. R. W. Hendrix, J. W. Roberts, F. W. Stahl, and R. A. Weisberg (Eds.). 1983. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

I VIRUS



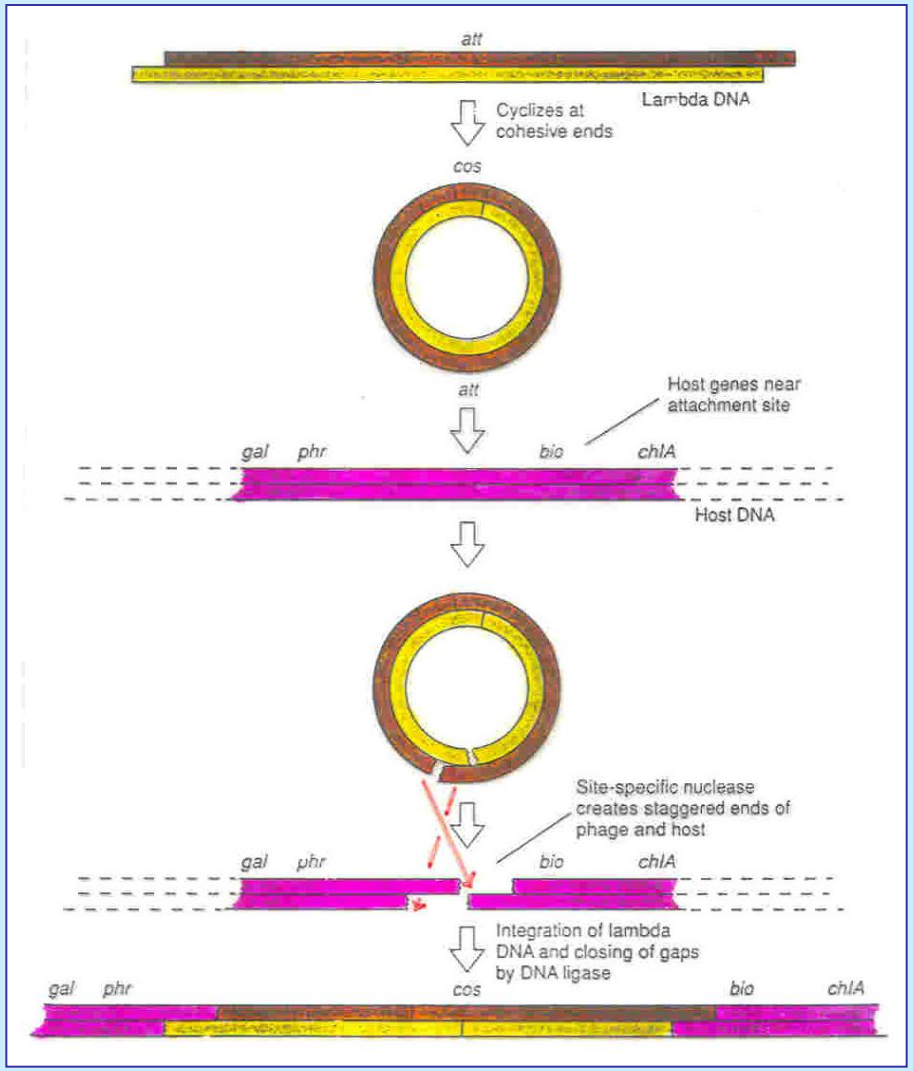
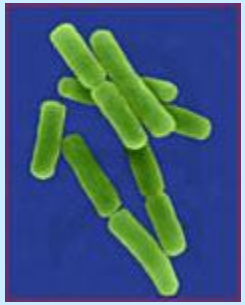
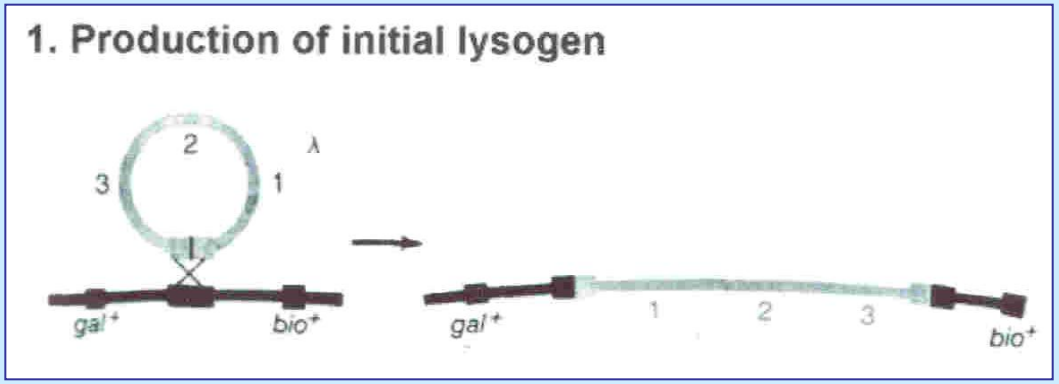
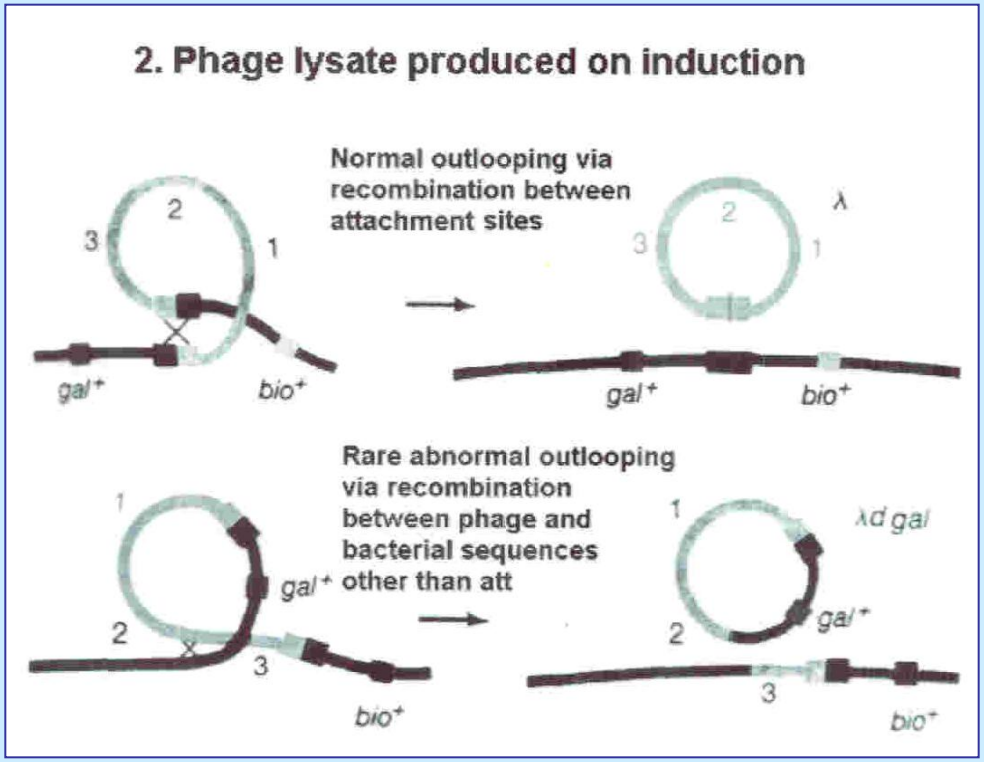


Figure 8.29 Integration of lambda DNA into the host. See the genetic map, Figure 8.26, for details of the gene order. Integration always occurs at a specific site on the host DNA, involving a specific attachment site (*att*) on the phage. Some of the host genes near the attachment site are given. A site-specific enzyme (integrase) is involved, and specific pairing of the complementary ends results in integration of phage DNA.

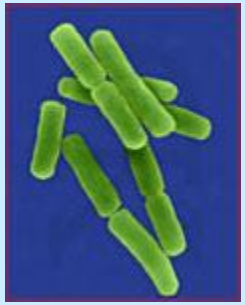
I VIRUS





1. Production of initial lysogen
2
λ
3
1
gal+
bio+
gal+
1
2
3
bio+


2. Phage lysate produced on induction
Normal outlooping via recombination between attachment sites
2
λ
3
1
gal+
bio+
gal+
bio+
Rare abnormal outlooping via recombination between phage and bacterial sequences other than att
1
gal+
2
3
bio+
1
λd gal
gal+
2
3
bio+

I VIRUS



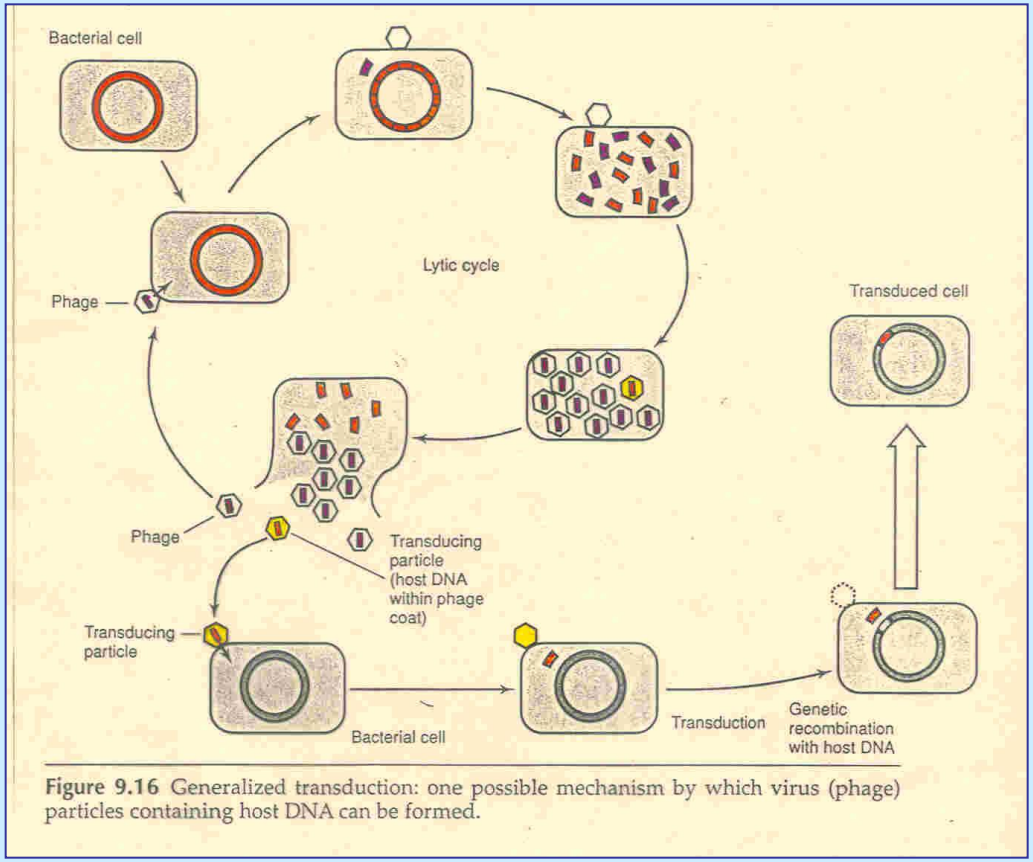
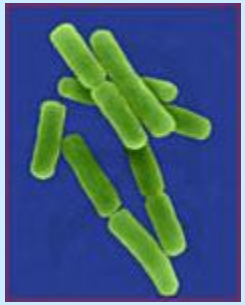


Figure 9.16 Generalized transduction: one possible mechanism by which virus (phage) particles containing host DNA can be formed.

I VIRUS



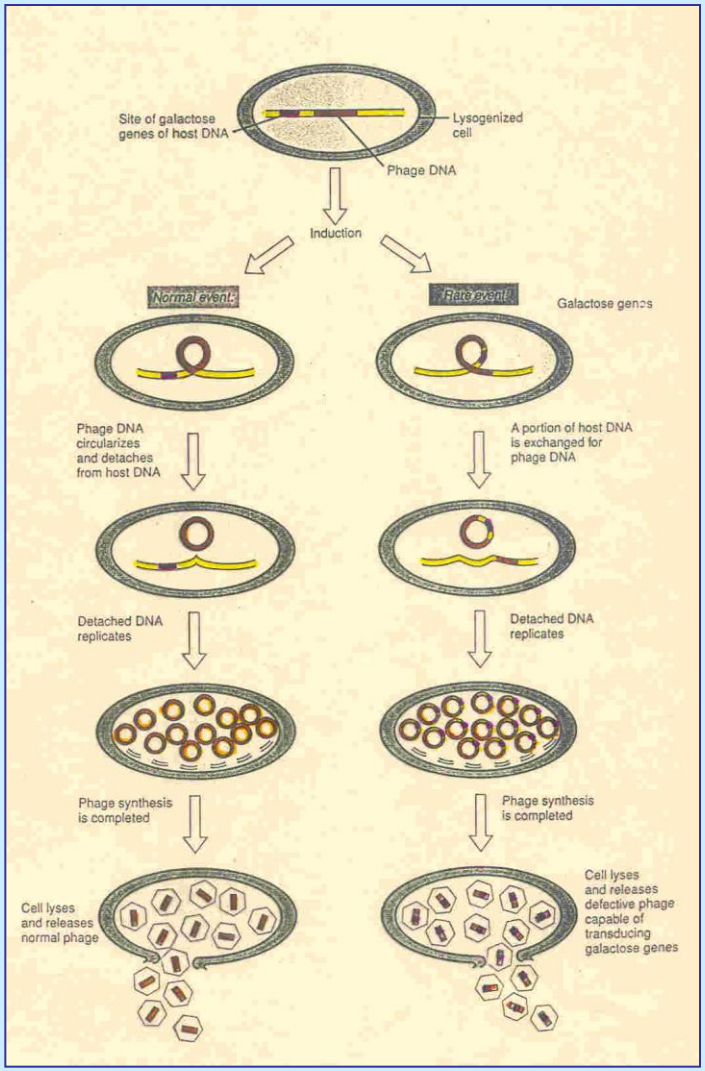
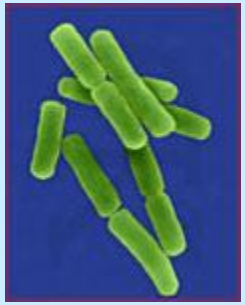


Figure 9.17 Normal lytic events and the production of particles transducing the galactose genes in an *Escherichia coli* cell containing a lambda prophage.



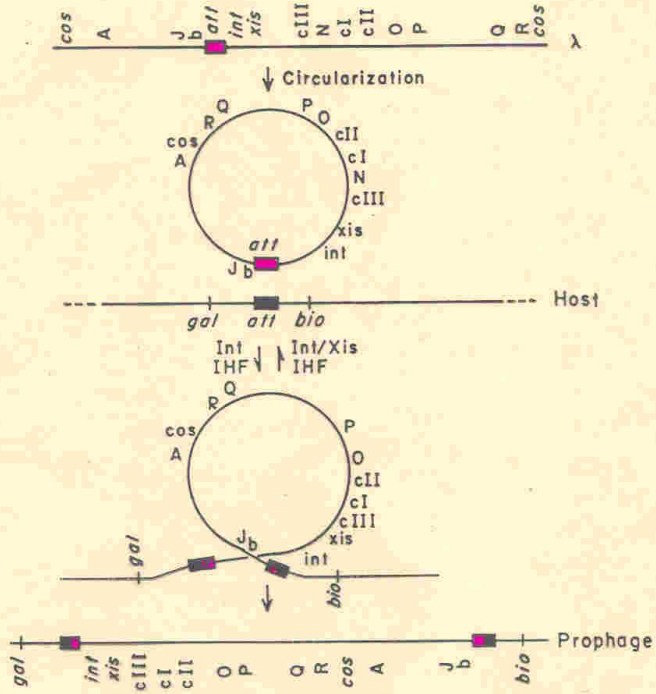
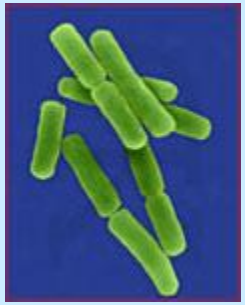


Fig. 5-16 Integration and excision of the λ genome (see text for details). Note the permutation of gene order as a result of integration. (i.e., *b* and *int* are proximal in the vegetative form but distal in the prophage).



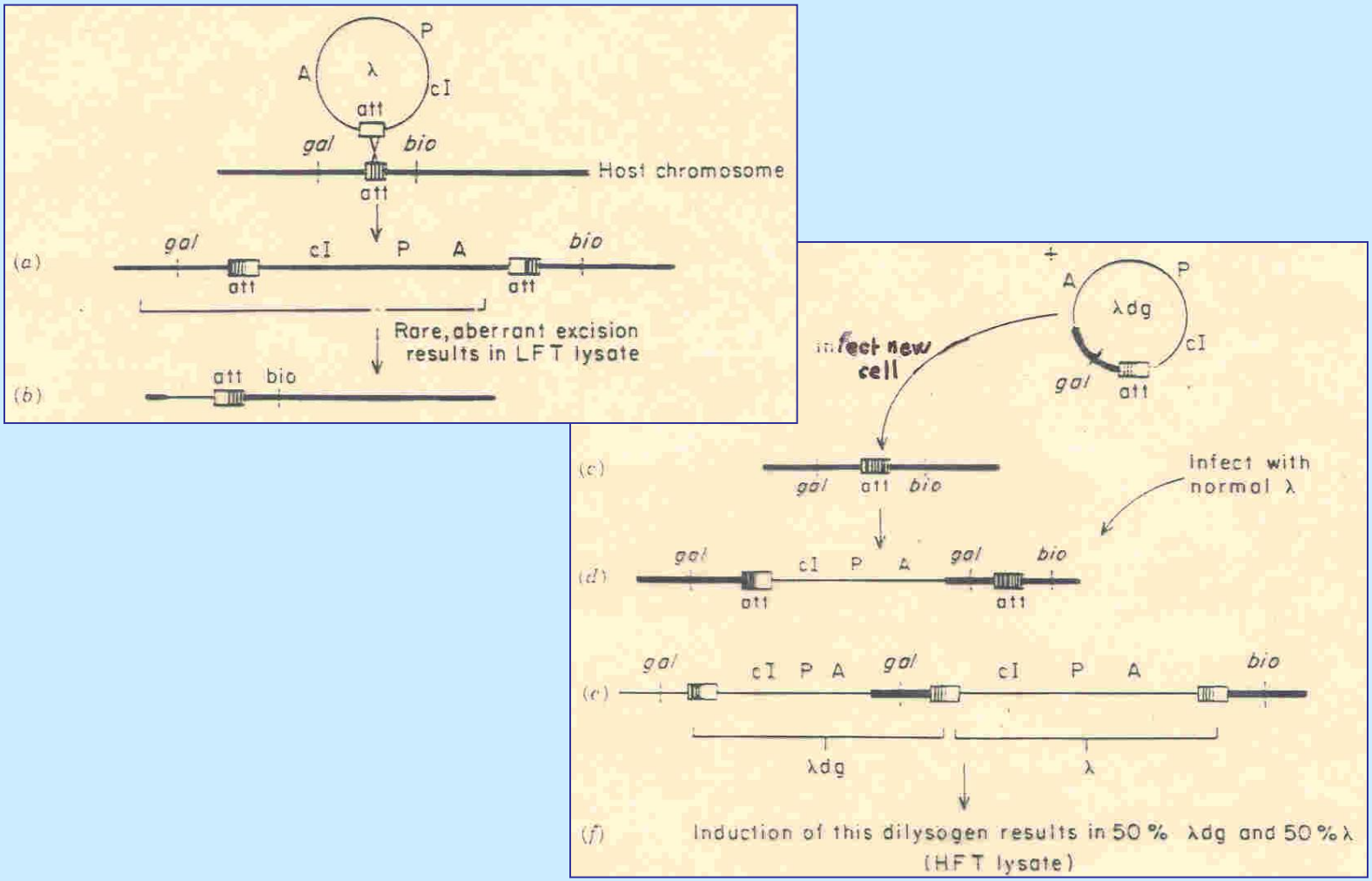
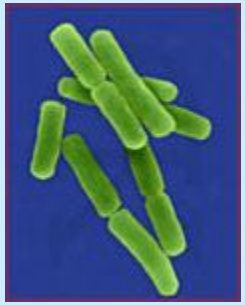



Fig. 3-12. Specialized transduction with λ phage showing the production of low-frequency transducing (LFT) and high-frequency transducing (HFT) lysates.



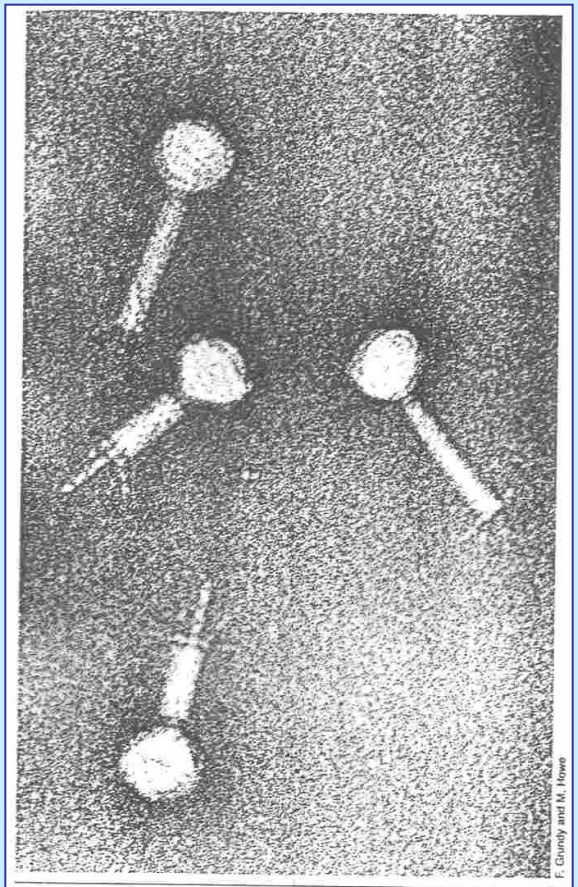
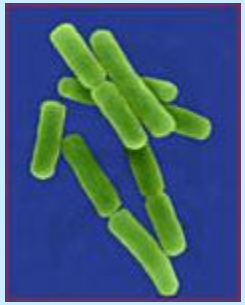
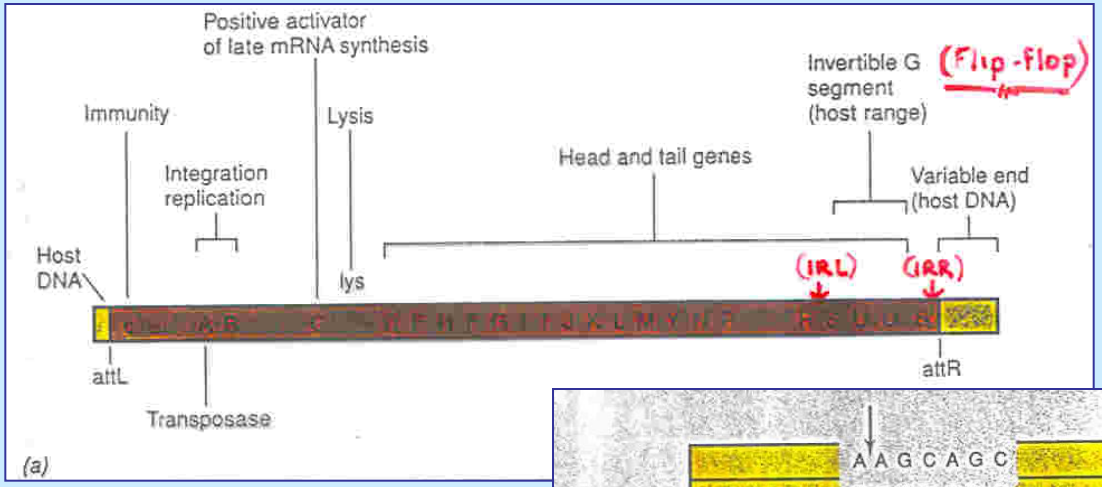
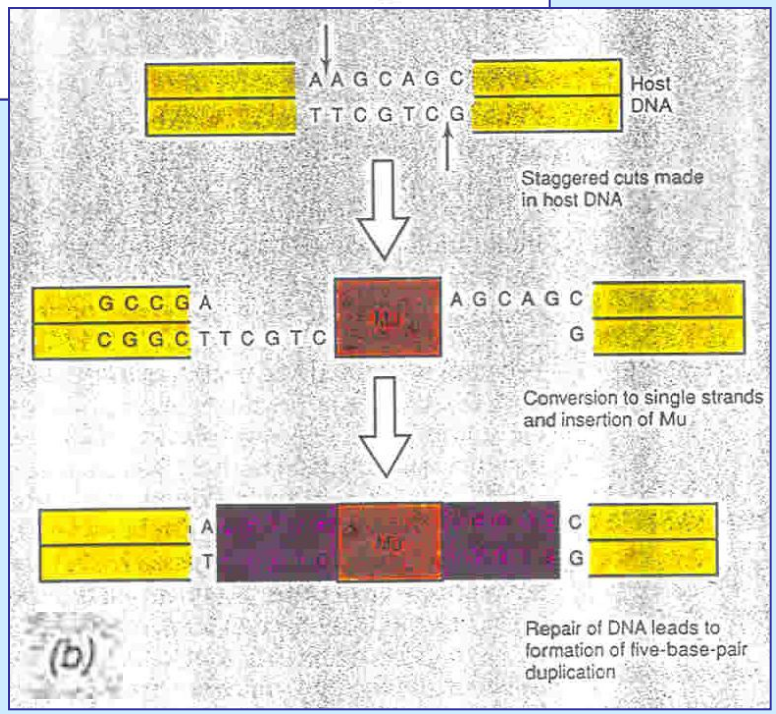


Figure 8.30 Electron micrograph of virions of bacteriophage Mu, the mutator phage.

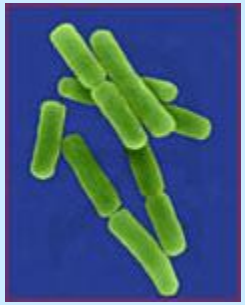
I VIRUS




Positive activator of late mRNA synthesis
Immunity
Lysis
Integration replication
Head and tail genes
Invertible G segment (host range)
(Flip-flop)
Variable end (host DNA)
Host DNA
lys
(IRL)
(IRR)
attL
attR
Transposase
(a)


A A G C A G C
T T C G T C G
Host DNA
Staggered cuts made in host DNA
G C C G A
C G G C T T C G T C
Mu
A G C A G C
G
Conversion to single strands and insertion of Mu
A
T
C
G
(b)
Repair of DNA leads to formation of five-base-pair duplication

I VIRUS



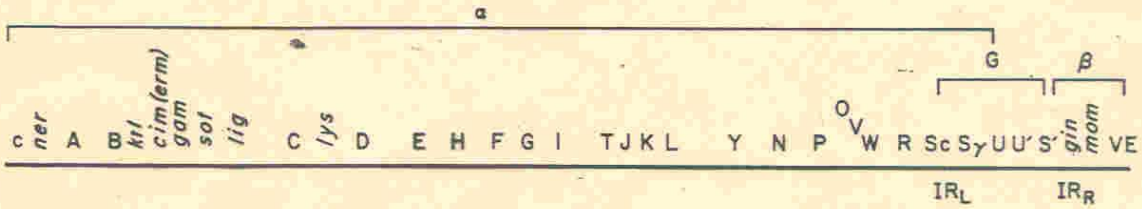
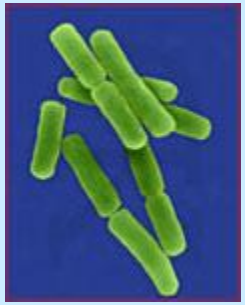


Fig. 5-21. Map of Mu DNA. The DNA is about 37 kb long. Lengths are shown in kilobases (not drawn to scale). Mature Mu DNA in phage particles is flanked by heterogeneous host sequences, which result from packaging of Mu from different sites. α, G, and β are different segments of the Mu genome. The α segment contains the early genes and the genes for head and tail morphogenesis. The early region contains the repressor gene *c* and a negative regulator (*ner*). In addition to transposase gene *A*, the main replicator gene *B*, and a gene for amplified replication of Mu (*arm*), other genes in this region are involved in DNA metabolism. Gene *C* appears to control the late functions. The G segment contains genes for the Mu host range and is flanked by 34 bp inverted repeats. The *gin* protein, whose gene is located to the right of G, acts on the 34 bp inverted repeats to invert the G segment; hence G is known at the flip–flop segment. The rightmost gene in Mu encodes the DNA modification function (*mom*). From Toussaint, A. and A. Resibois. 1983. Phage Mu: Transposition as a lifestyle. In *Mobile genetic elements*. J. A. Shapiro (Ed.). Academic, New York, pp. 105–158.



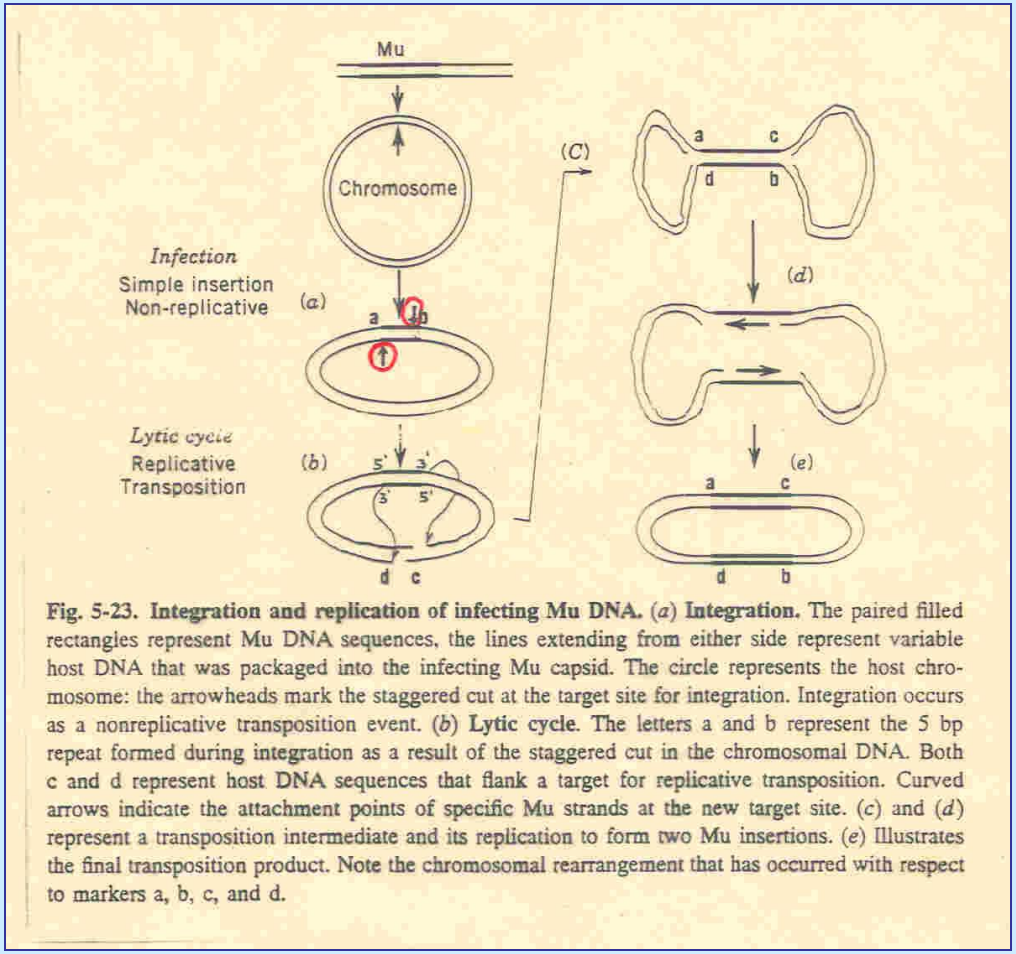
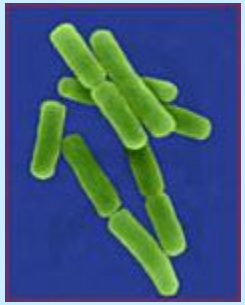
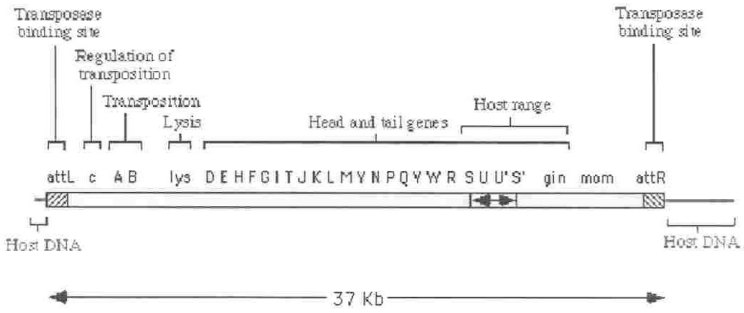


Fig. 5-23. Integration and replication of infecting Mu DNA. (*a*) **Integration.** The paired filled rectangles represent Mu DNA sequences, the lines extending from either side represent variable host DNA that was packaged into the infecting Mu capsid. The circle represents the host chromosome: the arrowheads mark the staggered cut at the target site for integration. Integration occurs as a nonreplicative transposition event. (*b*) **Lytic cycle.** The letters a and b represent the 5 bp repeat formed during integration as a result of the staggered cut in the chromosomal DNA. Both c and d represent host DNA sequences that flank a target for replicative transposition. Curved arrows indicate the attachment points of specific Mu strands at the new target site. (*c*) and (*d*) represent a transposition intermediate and its replication to form two Mu insertions. (*e*) Illustrates the final transposition product. Note the chromosomal rearrangement that has occurred with respect to markers a, b, c, and d.



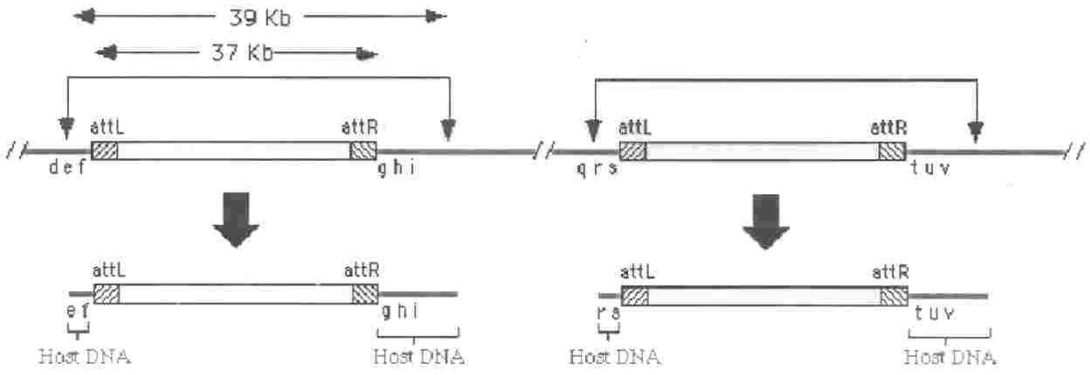
I VIRUS

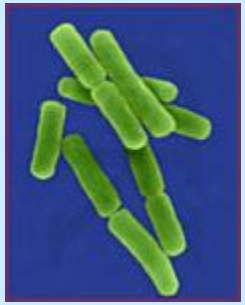
Phage Mu is a phage that reproduces by transposition. A simplified cartoon of the Mu genetic map is shown below.

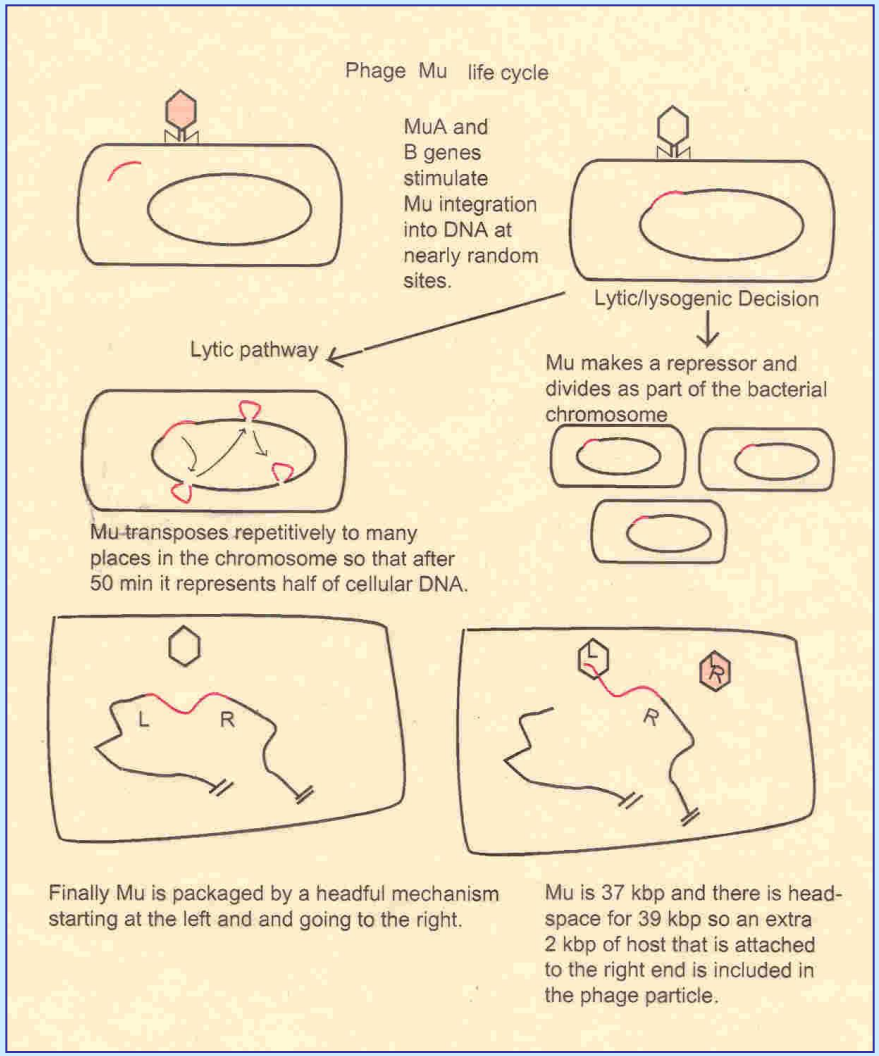


Note the following features of Mu phage:

- The *A* and *B* gene products encode transposase -- the A protein is required for all transposition events, but the B protein is only required for replicative transposition events.
- Expression of the transposase genes is repressed by the *c* gene product.
- Transposition requires the two ends of Mu, labeled attL and attR (sometimes called MuL and MuR).
- When Mu DNA is packaged into a phage head it includes about 50-150 bp of host DNA at the left end and a variable amount of host DNA on the right end. For wild-type Mu the amount of host DNA on the right end is about 2 Kb but, because of the headful packaging mechanism shown below, the length of host DNA on this end increases if part of the Mu genome is deleted. Each Mu is packaged from a different site in the host genome, so the host DNA on the ends of Mu is unique in every different phage head.






Phage Mu life cycle
MuA and B genes stimulate Mu integration into DNA at nearly random sites.
Lytic/lysogenic Decision
Lytic pathway
Mu makes a repressor and divides as part of the bacterial chromosome
Mu transposes repetitively to many places in the chromosome so that after 50 min it represents half of cellular DNA.
L
R
L
R
Finally Mu is packaged by a headful mechanism starting at the left and and going to the right.
Mu is 37 kbp and there is head-space for 39 kbp so an extra 2 kbp of host that is attached to the right end is included in the phage particle.

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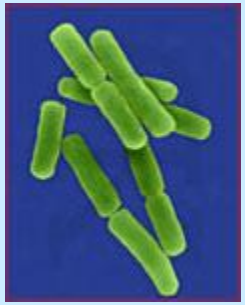

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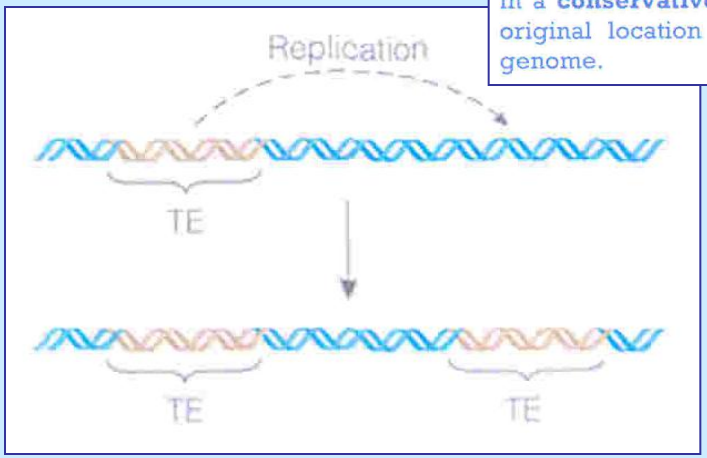
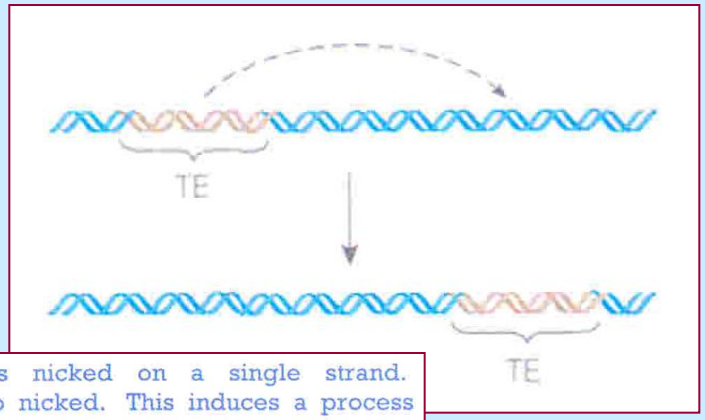
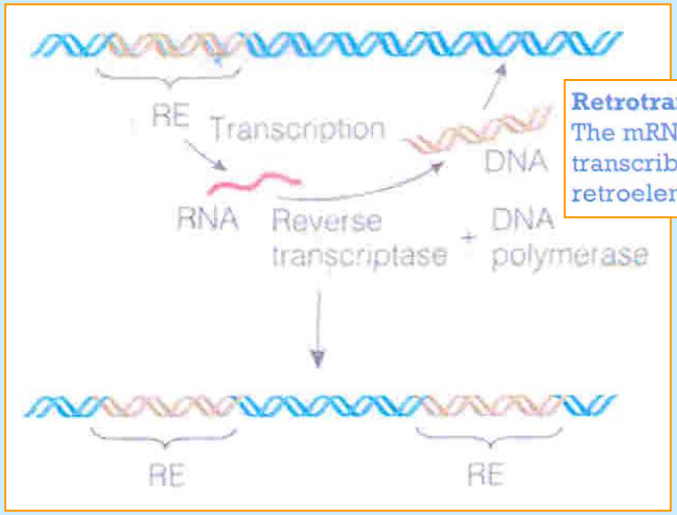
In a **conservative** (or simple) **transposition** event, the TE is excised from its original location by transposase and transferred to a new position in the genome.

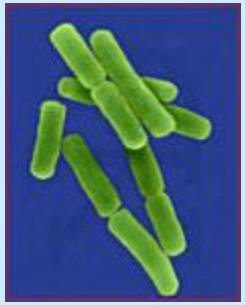




In **replicative transposition**, the TE is nicked on a single strand. Simultaneously, the target DNA site is also nicked. This induces a process similar to recombination, in which one strand of the TE inserts itself into the target DNA site. Gap repair synthesis fills in the missing bases, resulting in a TE in both the original and target DNA.



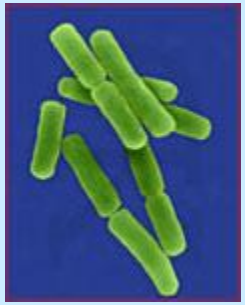
Retrotransposition - Retroelements transpose by way of an RNA intermediary. The mRNA product of retroelement transcription is used as a template to reverse transcribe the mRNA into DNA. This newly made DNA, which is a copy of the retroelement, can insert itself in a new location in the menome.

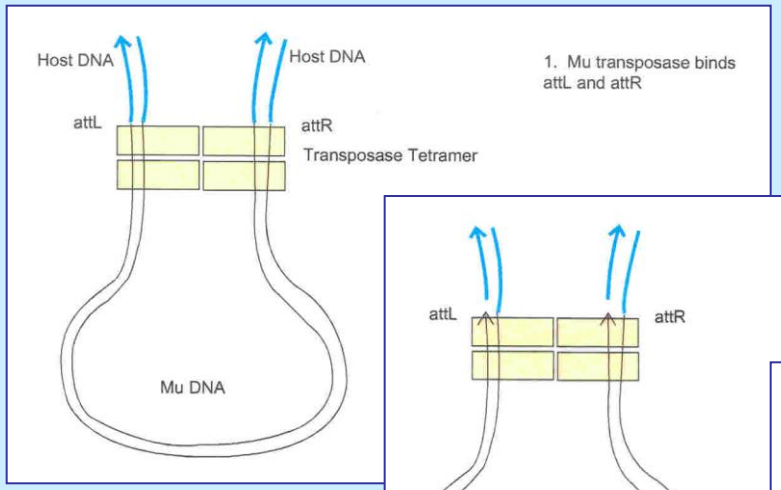


Mu Transposition Is a process that can be described as occurring in 5 discrete steps. The basic elements of this model were predicted by Jim Shapiro, discoverer of bacterial transposons (Shapiro, J. A. (1969). Mutations caused by the insertion of genetic material into the galactose operon of *E. coli*, J Mol Biol *40*, 93-105) in a model published in 1979 (Shapiro, J. A. (1979). Molecular model for the transposition and replication of bacteriophage Mu and other transposable elements, Proc Natl Acad Sci USA *76*, 1933-1937.)

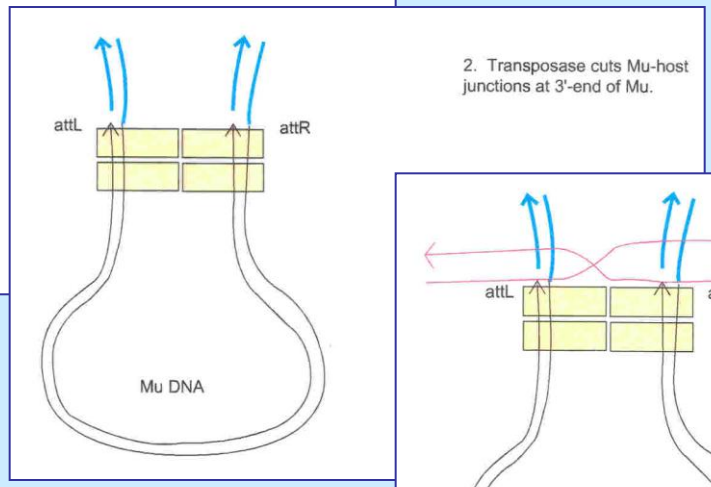
The 5 steps in the modern version are:

1) Transposase binds to attL and attR and bring them together in a synapse. In this structure, the Mu transposase (gene A product) forms a stable tetramer.
2) Transposase cuts each Mu-host junction uniquely at the 3'-OH that separates Mu from the host DNA. This cleaved intermediate is known as a "transposasome".
3) The Mu B protein acts a a target site selector and brings the target to the cleaved ends of the transposasome.
4) The 3'-OH ends of Mu attack the target and carry out a transesterification reaction. Chemistry is similar to that of DNA ligase in the step after formation of adenylylated enzyme intermediate and in DNA polymerization by polymerases.
5) A replisome is formed at the Mu left end and this machine replicates from attL to attR with semi-discontinuous synthesis, just like the E. coli chromosome.

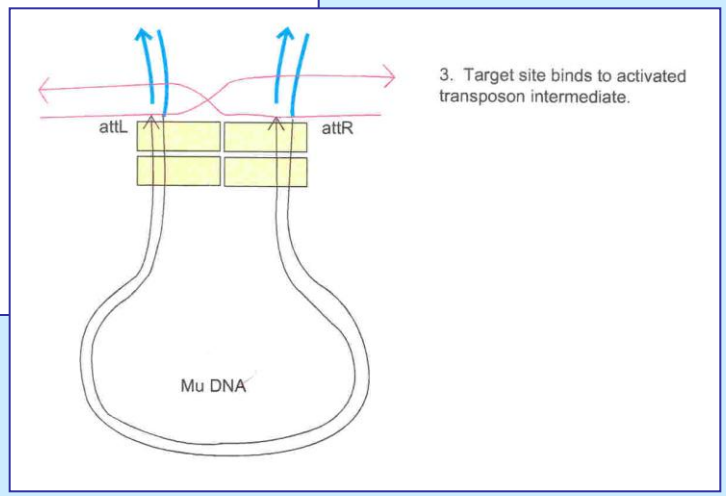




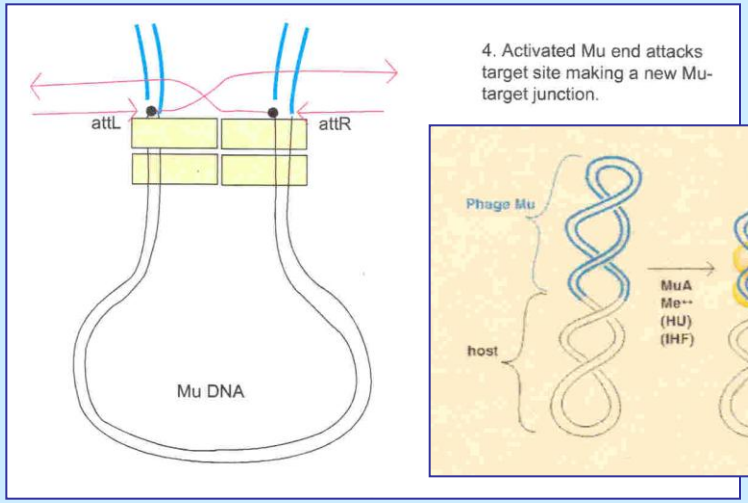
1. Mu transposase binds attL and attR



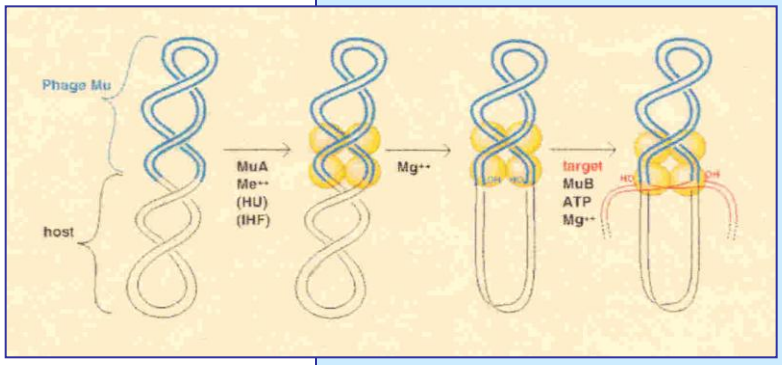
2. Transposase cuts Mu-host junctions at 3'-end of Mu.



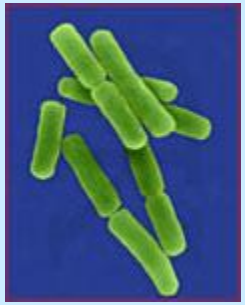
3. Target site binds to activated transposon intermediate.



4. Activated Mu end attacks target site making a new Mu-target junction.



I VIRUS



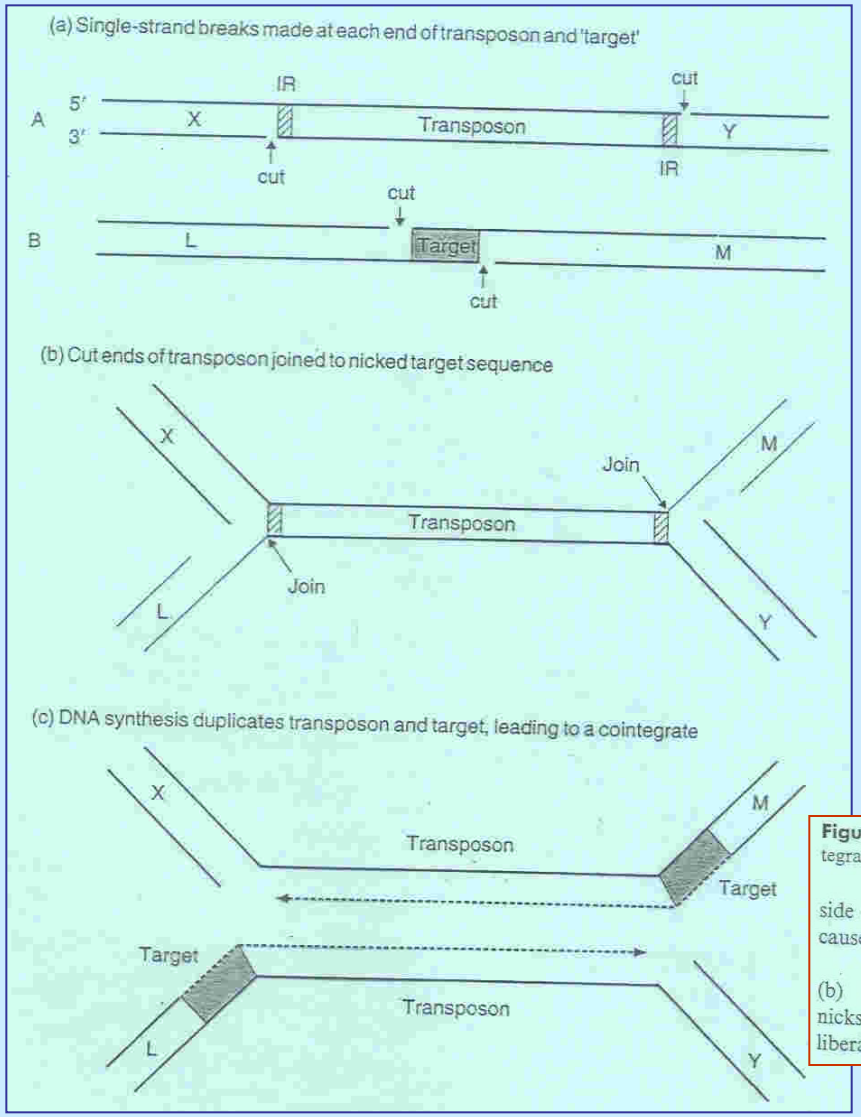


Figure 7.9 Model of the mechanism of replicative transposition: formation of a cointegrate (see the text for details)

side of a short target sequence. The staggered nature of the nicks is the ultimate cause of the duplication of the target sequence.

(b) The free ends of the transposon are joined to the free ends generated by the nicks in the recipient plasmid. At no stage in this process is the transposon itself liberated as an independent molecule.

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