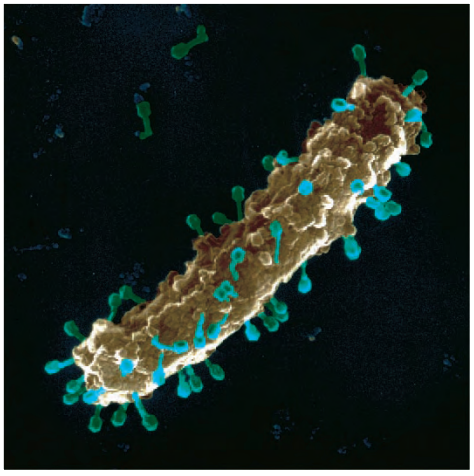
7

LINKAGE AND MAPPING IN PROKARYOTES AND BACTERIAL VIRUSES

STUDY OBJECTIVES

1. To define bacteria and bacterial viruses and learn about methods of studying them 149
2. To study life cycles and sexual processes in bacteria and bacteriophages 154, 163
3. To make use of the sexual processes of bacteria and their viruses to map their chromosomes 155, 166

STUDY OUTLINE



Scanning electron micrograph (color enhanced) of an *Escherichia coli* bacterium with adsorbed T-family bacteriophages (36,000x). (© Oliver Meckes/MPI-Tubingen/Photo Researchers.)

All organisms and viruses have genes located sequentially in their genetic material; and almost all can undergo recombination between homologous (equivalent) pieces of genetic material. Because recombination can occur, it is possible to map, by analytical methods, the locations and sequence of genes along the chromosomes of all organisms and almost all viruses. In this chapter, the viruses we look at are those that attack bacteria. Through work with bacteria and viruses, we have entered the modern era of molecular genetics, the subject of the next section of this book.

Bacteria (including the cyanobacteria, the blue-green algae) are prokaryotes. The prokaryotes also include the **archaea,** or archaebacteria, a kingdom recognized in 1980. These highly specialized organisms (previously classified as bacteria), along with the bacteria and eukaryotes, make up the three domains of life on Earth.

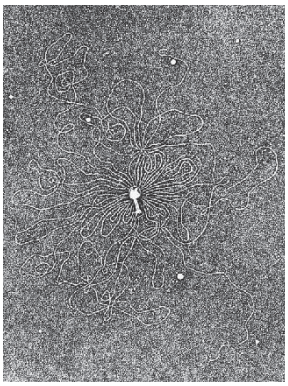
The true bacteria can be classified according to shape: a spherical bacterium is called a **coccus;** a rod-shaped bacterium is called a **bacillus;** and a spiral bacterium is called a **spirillum.** Prokaryotes do not undergo mitosis or meiosis but simply divide in two after their chromosome (usually only one), most often a circle of DNA, has replicated (see chapter 9). Bacterial viruses do not even divide; they are mass-produced within a host cell.

BACTERIA AND BACTERIAL VIRUSES IN GENETIC RESEARCH

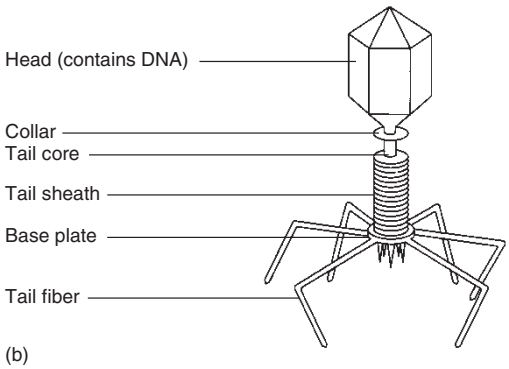
Several properties of bacteria and viruses make them especially suitable for genetic research. First, bacteria and their viruses generally have a short generation time. Some viruses increase three-hundredfold in about a half hour; an *Escherichia coli* cell divides every twenty minutes. In contrast, generation time is fourteen days in fruit flies, a year in corn, and twenty years or so in human beings. (*E. coli,* the common intestinal bacterium, was discovered by Theodor Escherich in 1885.)

Another reason bacteria and bacterial viruses are so well-suited for genetic research is because they have much less genetic material than eukaryotes do, and the organization of this material is much simpler. The term *prokaryote* arises from the lack of a true nucleus (*pro* means before and *karyon* means kernel or nucleus); they have no nuclear membranes (see fig. 3.2) and usually have only a single, relatively "naked" chromosome, so they are haploid. Bacteria may, however, contain small, auxiliary circles of DNA, called **plasmids.** Bacterial viruses are even simpler. Although animal and plant viruses, discussed in more detail later in the book (chapters 13 and 16), can be more complicated, the viruses we are interested in studying in this chapter—the bacterial viruses, **bacteriophages,** or just **phages** (Greek: eating)—are exclusively genetic material surrounded by a protein coat.

Bacteriophages are usually classified first by the type of genetic material (nucleic acid) they have (DNA or RNA, single- or double-stranded), then by structural features of their protein surfaces **(capsids)** such as type or symmetry and number of discrete protein subunits **(capsomeres)** in the capsid, and general size. Most bacteriophages are complex, like T2 (fig. 7.1), or made up of a headlike capsule like T2 without the tail appendages, or filamentous. Most contain double-stranded DNA. Bacteriophages are obligate parasites; outside of a host, they are inert molecules. Once their genetic material penetrates a host cell, they can take over the metabolism of that cell and construct multiple copies of themselves. We will discuss details of this and alternative infection



(a)



(b)

Figure 7.1 Phage T2 and its chromosome. (*a*) The chromosome, which is about 50 μm long, has burst from the head. (*b*) The intact phage. The phage attaches to a bacterium using its tail fibers and base plate and then injects its genetic material into the host cell. ([*a*] A. K. Kleinschmidt, et al., "Darstellung und Langen messungen des gesamten Deoxyribose-nucleinsaure Inhaltes von T2-Bacteriophagen" *Biochemica et Biophysica Acta,* 61:857–64, 1962. Reproduced by permission of Elsevier Science Publishers.)

pathways later in the chapter. The smallest bacteriophages (e.g., R17) have RNA as their genetic material and contain just three genes, one each for a coat protein, an attachment protein, and an enzyme to replicate their RNA. The larger bacteriophages (T2, T4) have DNA as their genetic material and contain up to 130 genes.

A third reason for the use of bacteria and viruses in genetic study is their ease of handling. A researcher can handle millions of bacteria in a single culture with a minimal amount of work compared with the effort required to grow the same number of eukaryotic organisms such as fruit flies or corn. (Some eukaryotes, such as yeast or *Neurospora,* can, of course, be handled using prokaryotic techniques, as we saw in chapter 6.) Let us look at an expansion of the techniques, introduced in chapter 6, that geneticists use in bacterial and viral studies.

TECHNIQUES OF CULTIVATION

All organisms need an energy source, a carbon source, nitrogen, sulfur, phosphorus, several metallic ions, and water. Those that require an organic form of carbon are termed **heterotrophs.** Those that can utilize carbon as carbon dioxide are termed **autotrophs.** All bacteria obtain their energy either by photosynthesis or chemical oxidation. Bacteria are usually grown in or on a chemically defined **synthetic medium,** either in liquid in flasks or test tubes, or on petri plates using an agar base to supply rigidity. When one cell is placed on the medium in the plate, it will begin to divide. After incubation, often overnight, a colony, or clone, will exist where previously was only one cell. Overlapping colonies form a confluent growth (fig. 7.2). A culture medium that has only the minimal necessities required by the bacterial species is referred to as *minimal medium* (table 7.1).

Alternatively, bacteria can grow on a medium that supplies, in addition to their minimal requirements, the more complex substances that the bacteria normally synthesize, including amino acids, vitamins, and so on. A medium of this kind allows the growth of strains of bacteria, called **auxotrophs,** that have particular nutritional requirements. (The parent, or wild-type, strain is referred to as a **prototroph.**) For example, a strain that has an enzyme defect in the pathway that produces the amino acid histidine will not grow on a minimal medium because it has no way of obtaining histidine; it is a histidine-requiring auxotroph. If, however, histidine were provided in the medium, the organisms could grow. This type of mutant is called a **conditional-lethal mutant.** The organism would normally die, but under appropriate conditions, such as the addition of histidine, the organism can survive.

This histidine-requiring auxotrophic mutant can grow only on an **enriched** or **complete medium,** whereas the parent prototroph could grow on a minimal medium. Media are often enriched by adding complex mixtures of organic substances such as blood, beef extract, yeast extract, or peptone, a digestion product. Many media, however, are made up of a minimal medium with the addition of only one other substance, such as an amino acid or a vitamin. These are called **selective media;** we will discuss their uses later in the chapter. In addition to minimal, complete, and selective media, other media exist for specific purposes such as aiding in counting colonies, helping maintain cells in a nongrowth phase, and so on.

Table 7.1 **Minimal Synthetic Medium for Growing *E. coli,* a Heterotroph**

Component	Quantity
$NH_4H_2PO_4$	1 g
Glucose	5 g
NaCl	5 g
$MgSO_4 \cdot 7H_2O$	0.2 g
K_2HPO_4	1 g
H_2O	1,000 ml

Source: Data from M. Rogosa, et al., *Journal of Bacteriology,* 54:13, 1947.

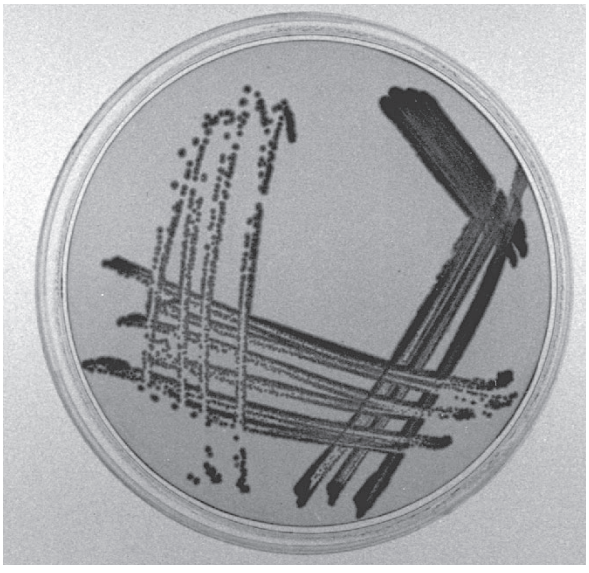


Figure 7.2 Confluent growth of bacterial colonies on a petri plate. Bacteria were streaked on the petri plate with an inoculation loop—a metal wire with a looped end—covered with bacteria. Streaks began at the upper right and continued around clockwise. With a heavy inoculation on the loop, bacterial growth is confluent. Eventually, only a few bacteria are left; they form single colonies at the upper left. (Photo by Robert Tamarin.)

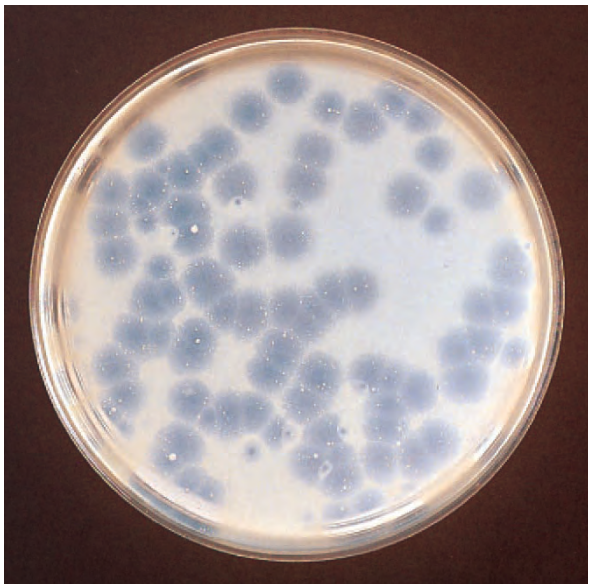


Figure 7.3 Viral plaques (phage T1) on a bacterial lawn of *E. coli.* (© Bruce Iverson, BSc.)

The experimental cultivation of viruses is somewhat different. Since viruses are obligate parasites, they can grow only in living cells. Thus, for the cultivation of phages, petri plates of appropriate media are inoculated with enough bacteria to form a continuous cover, or **bacterial lawn.** This bacterial culture serves as a medium for the growth of viruses added to the plate. Since the virus attack usually results in rupture, or **lysis,** of the bacterial cell, addition of the virus usually produces clear spots, known as **plaques,** on the petri plates (fig. 7.3). Large quantities of viruses can be grown in flasks of bacteria.

BACTERIAL PHENOTYPES

Bacterial phenotypes fall into three general classes: colony morphology, nutritional requirements, and drug or infection resistance.

Colony Morphology

The first of these classes, colony morphology, relates simply to the form, color, and size of the colony that grows from a single cell. A bacterial cell growing on a petri plate in an incubator at 37° C divides as frequently as once every twenty minutes. Each cell gives rise to a colony, or clone, at its original position. In a relatively short amount of time (e.g., overnight), the colonies will consist of enough cells to be seen with the unaided eye. The different morphologies observed among the colonies are usually under genetic control (fig. 7.4).

Nutritional Requirements

The second basis for classifying bacteria—by their nutritional requirements—reflects the failure of one or more enzymes in the bacteria's biosynthetic pathways. If an auxotroph has a requirement for the amino acid cysteine that the parent strain (prototroph) does not have, then that auxotroph most likely has a nonfunctional enzyme in the pathway for the synthesis of cysteine. Figure 7.5 shows five steps in cysteine synthesis; a different enzyme controls each step. All enzymes are proteins, and the information in one or more genes determines the sequences in the strings of amino acids that make up those proteins (chapter 11). A normal or wild-type allele

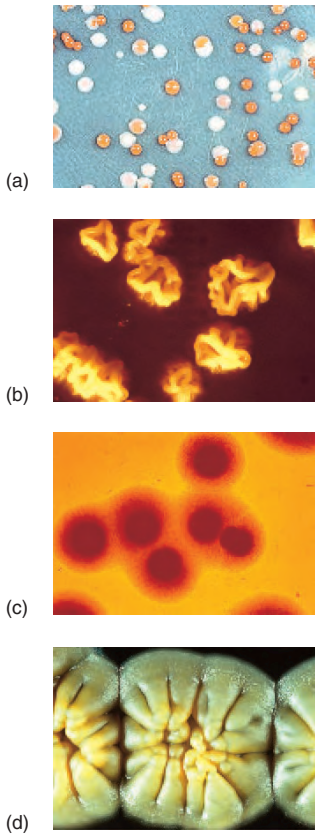


Figure 7.4 Various bacterial colony forms on agar petri plates. (*a*) Red and white colonies of *Serratia marcescens.* (*b*) Irregular raised folds of *Streptomyces griseus*. (*c*) Round colonies with concentrated centers and diffuse edges of *Mycoplasma.* (*d*) Irregularly folded raised colonies of *Streptomyces antibioticus.* ([*a*] © Dr. E. Buttone/Peter Arnold, Inc., [*b*] © C. Case/Visuals Unlimited, [*c*] © Michael G. Gabridge/Visuals Unlimited, [*d*] © Cabisco/Visuals Unlimited.)

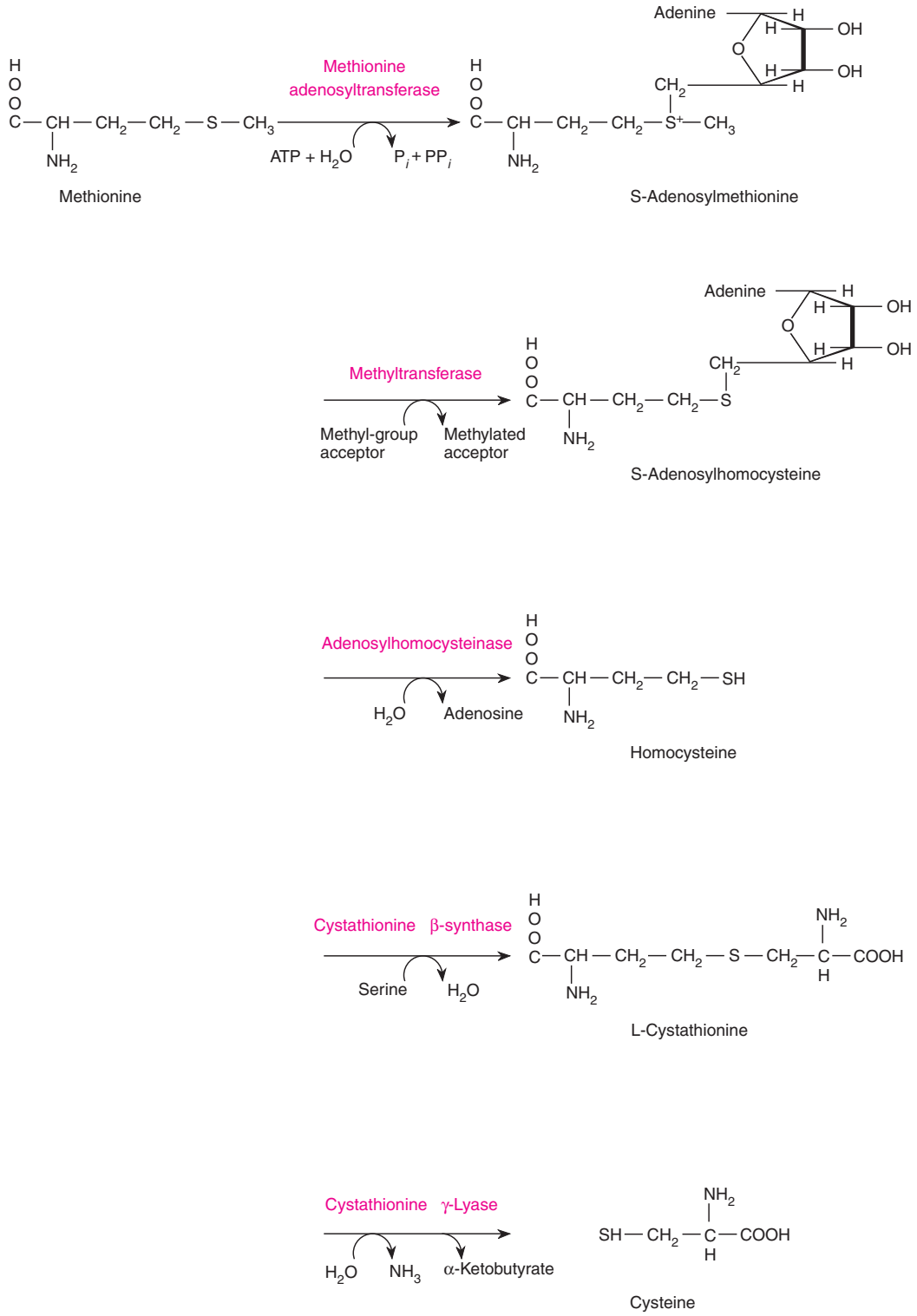
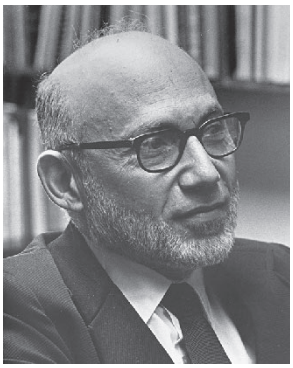


Figure 7.5 Five-step conversion of methionine to cysteine. Each step is controlled by a different enzyme (*red*).



Joshua Lederberg (1925–).
(Courtesy of Dr. Joshua Lederberg.)

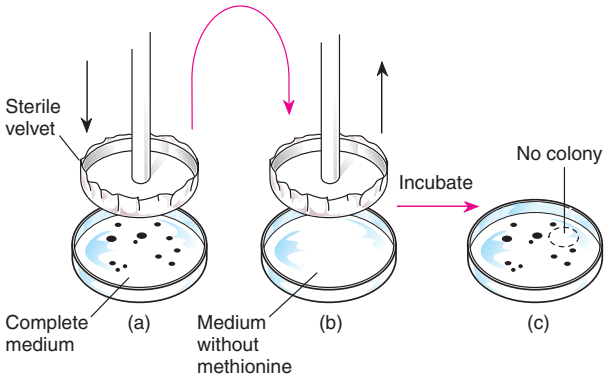


Figure 7.6 Replica-plating technique. (*a*) A pattern of colonies from a plate of complete medium is transferred (*b*) to a second plate of medium that lacks methionine. (*c*) In the locations where colonies fail to grow on the second plate, we can infer that the original colony was a methionine-requiring auxotroph.

produces a normal, functional enzyme. The alternative allele may produce a nonfunctional enzyme. Recall the one-gene-one-enzyme hypothesis from chapter 2.

A technique known as **replica-plating,** devised by Joshua Lederberg, is a rapid **screening technique** that makes it possible to determine quickly whether a given strain of bacteria is auxotrophic for a particular metabolite. In this technique, a petri plate of complete medium is inoculated with bacteria. The resulting growth will have a certain configuration of colonies. This plate of colonies is pressed onto a piece of sterilized velvet. Then any number of petri plates, each containing a medium that lacks some specific metabolite, can be pressed onto this velvet to pick up inocula in the same pattern as the growth on the original plate (fig. 7.6). If a colony grows on the complete medium but does not grow on a plate with a medium missing a metabolite, the inference is that the colony is made of auxotrophic cells that require the absent metabolite. Samples of this bacterial strain can be obtained from the colony growing on complete medium for further study. The nutritional requirement of this strain is its phenotype. The methionine-requiring auxotroph of figure 7.6 would be designated as Met^- (methionine-minus or Met-minus).

In terms of energy sources, the plus or minus notation has a different meaning. For example, a strain of bacteria that can utilize the sugar galactose as an energy source would be Gal^+. If it could not utilize galactose, it would be called Gal^-. The latter strain will not grow if galactose is its sole carbon source. It will grow if a sugar other than galactose is present. Note that a Met^- strain needs methionine to grow, whereas a Gal^- strain needs a carbon source *other* than galactose; it cannot use galactose.

Resistance and Sensitivity

The third common classification of phenotypes in bacteria involves resistance and sensitivity to drugs, phages, and other environmental insults. For example, penicillin, an antibiotic that prevents the final stage of cell-wall construction in bacteria, will kill growing bacterial cells. Nevertheless, we frequently find a number of cells that do grow in the presence of penicillin. These colonies are resistant to the drug, and this resistance is under simple genetic control. The phenotype is penicillin resistant (Pen^r) as compared with penicillin sensitive (Pen^s), the normal condition, or wild-type. Numerous antibiotics are used in bacterial studies (table 7.2).

Table 7.2 Some Antibiotics and Their Antibacterial Mechanisms

Antibiotic	Microbial Origin	Mode of Action
Penicillin G	*Penicillium chrysogenum*	Blocks cell-wall synthesis
Tetracycline	*Streptomyces aureofaciens*	Blocks protein synthesis
Streptomycin	*Streptomyces griseus*	Interferes with protein synthesis
Terramycin	*Streptomyces rimosus*	Blocks protein synthesis
Erythromycin	*Streptomyces erythraeus*	Blocks protein synthesis
Bacitracin	*Bacillus subtilis*	Blocks cell-wall synthesis

Drug sensitivity provides another screening technique for isolating nutritional mutations. For example, if we were looking for mutants that lacked the ability to synthesize a particular amino acid (e.g., methionine), we could grow large quantities of bacteria (prototrophs) and then place them on a medium that lacked methionine but contained penicillin. Here, any growing cells would be killed. But methionine auxotrophs would not grow, and, therefore, they would not be killed. The penicillin could then be washed out and the cells reinoculated onto a complete medium. The only colonies that form should be composed of methionine auxotrophs (Met^-).

Screening for resistance to phages is similar to screening for drug resistance. When bacteria are placed in a medium containing phages, only those bacteria that are resistant to the phages will grow and produce colonies. They can thus be easily isolated and studied.

VIRAL PHENOTYPES

Bacteriophage phenotypes fall generally into two categories: plaque morphology and growth characteristics on different bacterial strains. For example, T2, an *E. coli* phage (see fig. 7.1), produces small plaques with fuzzy edges (genotype r^+). Rapid-lysis mutants (genotype *r*) produce large, smooth-edged plaques (fig. 7.7). Similarly, T4, another *E. coli* phage, has rapid-lysis mutants that produce large, smooth-edged plaques on *E. coli* B but will not grow at all on *E. coli* K12, a different strain. Here, rapid-lysis mutants illustrate both the colony morphology phenotypes and the growth-restriction phenotypes of phages.

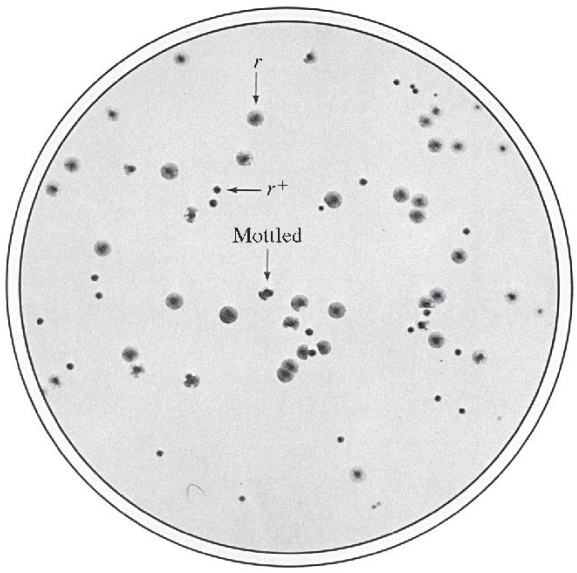


Figure 7.7 Normal (r^+) and rapid-lysis (*r*) mutants of phage T2. Mottled plaques occur when *r* and r^+ phages grow together. (From *Molecular Biology of Bacterial Viruses* by Gunther S. Stent. © 1963, 1978 by W. H. Freeman and Company. Used with permission.)

SEXUAL PROCESSES IN BACTERIA AND BACTERIOPHAGES

Although bacteria and viruses are ideal subjects for biochemical analysis, they would not be useful for genetic study if they did not have sexual processes. If we define a sexual process as combining genetic material from two individuals, then the life cycles of bacteria and viruses include sexual processes. Although they do not undergo sexual reproduction by the fusion of haploid gametes, bacteria and viruses do undergo processes that incorporate genetic material from one cell or virus into another cell or virus, forming recombinants. Actually, bacteria have three different methods to gain access to foreign genetic material: **transformation, conjugation,** and **transduction** (fig. 7.8).

Phages can exchange genetic material when a bacterium is infected by more than one virus particle **(virion).** During the process of viral infection, the genetic material of different phages can exchange parts (or recombine; see fig. 7.8). We will examine the exchange processes in bacteria and then in bacteriophages, and then proceed to the use of these methods for mapping bacterial and viral chromosomes. (*Chromosome* refers to the structural entity in the cell or virus made up of the genetic material. In eukaryotes, it is double-stranded DNA complexed with proteins [chapter 15]. Staining of this eukaryotic organelle led to the term *chromosome,* which means "colored body." In prokaryotes, the chromosome is a circle [usually] of double-stranded DNA. In viruses, it is virtually any combination of linear or circular, single- or double-stranded RNA or DNA. Sometimes the term **genophore** is used for the prokaryotic and viral genetic material, limiting the word *chromosome* to the eukaryotic version. We will use the term *chromosome* for the intact genetic material of any organism or virus.)

Transformation

Transformation was first observed in 1928 by F. Griffith and was examined at the molecular level in 1944 by O. Avery and his colleagues, who used the process to demonstrate that DNA was the genetic material of bacteria. Chapter 9 presents the details of these experiments. In transformation, a cell takes up extraneous DNA found in the environment and incorporates it into its genome

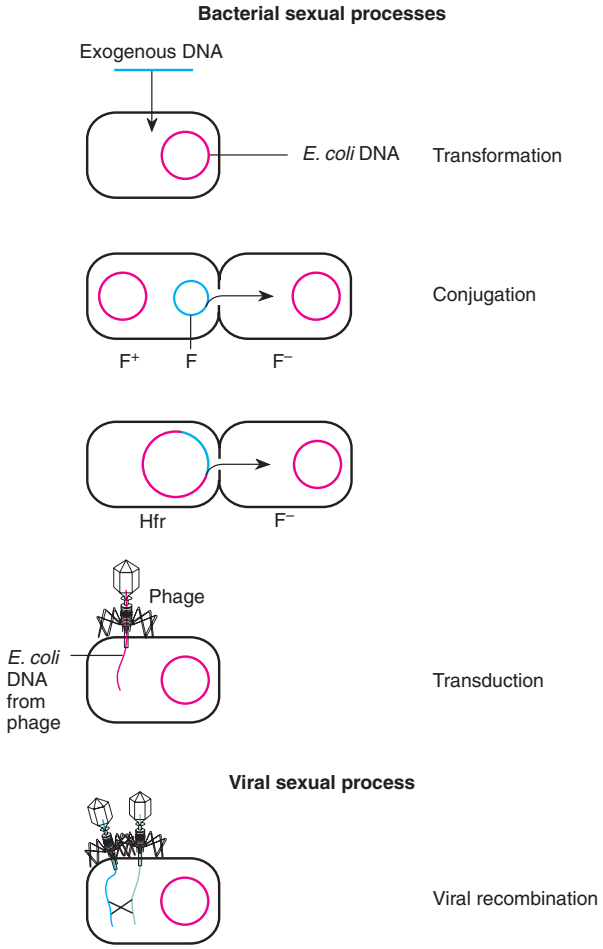


Figure 7.8 Summary of bacterial and viral sexual processes.

(genetic material) through recombination. Not all bacteria are competent to be transformed, and not all extracellular DNA is competent to transform. To be competent to transform, the extracellular DNA must be double-stranded. To be competent to be transformed, a cell must have the surface protein, **competence factor,** which binds to the extracellular DNA in an energy-requiring reaction. However, bacteria that are not naturally competent can be treated to make them competent, usually by treatment with calcium chloride, which makes them more permeable.

Mechanisms of Transformation

Under natural conditions, only one of the strands of extracellular DNA is brought into the cell. The single strand brought into the cell can then be incorporated into the host genome by two crossovers (fig. 7.9). (The molecular mechanisms of crossing over are presented in chapter 12.) Note that unlike eukaryotic crossing over, this is not a reciprocal process. The bacterial chromosome incorporates part of the foreign DNA. The remaining single-stranded DNA, originally part of the bacterial chromosome, is degraded by host enzymes called exonucleases; linear DNA is degraded rapidly in prokaryotes.

Transformation is a very efficient method of mapping in some bacteria, especially those that are inefficient in other mechanisms of DNA intake (such as transduction, discussed later in this chapter). For example, a good deal of the mapping of the soil bacterium, *Bacillus subtilis,* has been done through the process of transformation; *E. coli,* however, is inefficient in transformation, so other methods are used to map its chromosome.

Transformation Mapping

The general idea of transformation mapping is to add DNA from a bacterial strain with known genotype to another strain, also with known genotype, but with different alleles at two or more loci. We then look for incorporation of the donor alleles into the recipient strain of bacteria. The more often alleles from two loci are incorporated together into the host, the closer together these loci must be to each other. Thus, we can use an index of co-occurrence that is in inverse relationship to map distance: the larger the co-occurrence of alleles of two loci, the closer together the loci must be. This is another way of looking at the mapping concepts we discussed in

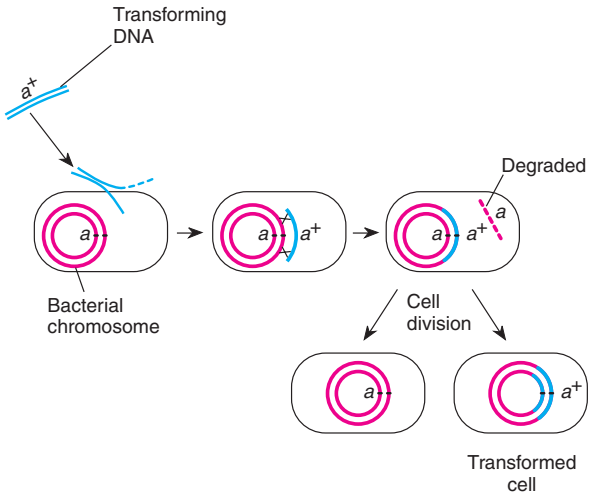


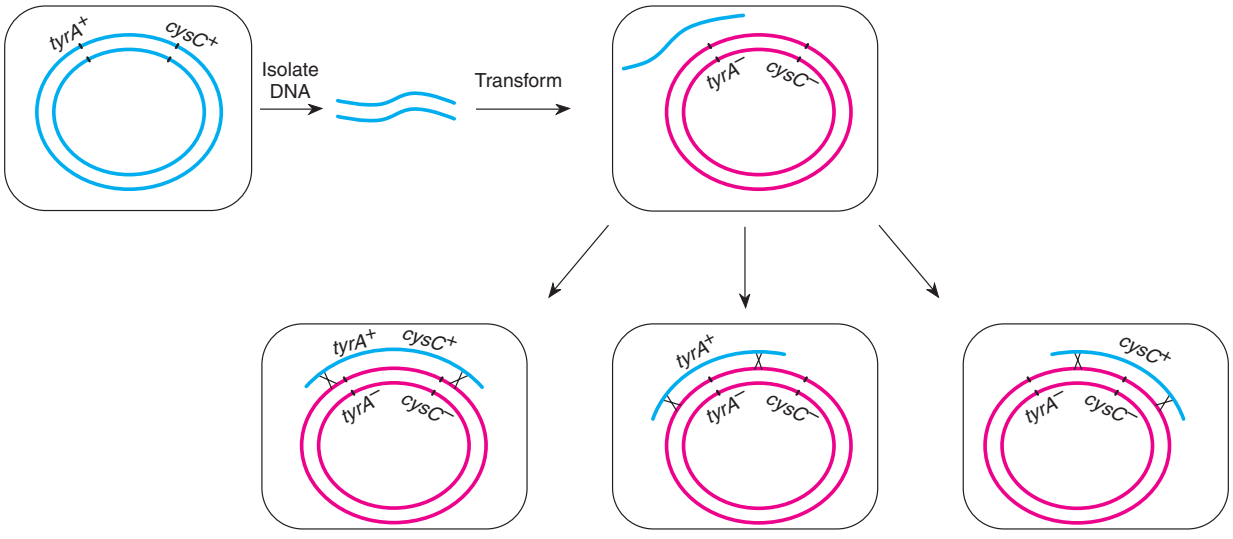
Figure 7.9 A single strand of transforming DNA (*blue* with a^+ allele) enters a bacterial cell (*red* chromosome with *a* allele). Two crossovers bring the foreign DNA into the bacterial chromosome. After DNA replication and cell division, one cell has the *a* allele and the other the a^+ allele. The chromosome is drawn as a double circle, symbolizing the double-stranded structure of DNA.

chapter 6, where we discovered that the closer two loci are, the fewer the recombinations between them and thus the higher the co-occurrence.

Now, we also must look at another concept, that is, selecting for recombinant cells. In fruit flies, every offspring of a mated pair represents a sampling of the meiotic tetrad, and thus a part of the total, whether or not recombination took place. Here, however, many cells are present that do not take part in the transformation process. In a bacterial culture, for example, only one cell in a thousand might be transformed. We must thus always be sure when working with bacterial gene transfer that we count only those cells that have taken part in the process. Let us look at an example.

A recipient strain of *B. subtilis* is auxotrophic for the amino acids tyrosine ($tyrA^-$) and cysteine ($cysC^-$). We are interested in how close these loci are on the bacterial chromosome. We thus isolate DNA from a prototrophic strain of bacteria ($tyrA^+$ $cysC^+$). We add this donor DNA to the auxotrophic strain and allow time for transformation to take place (fig. 7.10). If the experiment is successful, and the loci are close enough together, then some of the recipient bacteria may incorporate donor DNA that has either both donor alleles or one or the other donor allele. Thus, some of the recipient cells will now have the $tyrA^+$ and $cysC^+$ alleles, some will have just the donor $tyrA^+$ allele, some will have just the donor $cysC^+$ allele, and the overwhelming majority will be of the untransformed auxotrophic genotype, $tyrA^-$ $cysC^-$. We thus need to count the transformed cells.

We do this by removing any extraneous transforming DNA and then pouring the cells out onto a complete medium so that all cells can grow. These cells are then replica-plated onto three plates—a minimal medium plate, a minimal medium plus tyrosine plate, and a minimal medium plus cysteine plate—and allowed to grow overnight in an incubator at 37° C. We then count colonies (fig. 7.11). Those growing on minimal medium are of genotype $tyrA^+$ $cysC^+$; those growing on minimal medium with tyrosine but not growing on minimal medium are $tyrA^-$ $cysC^+$; and those growing on minimal medium with cysteine but not growing on minimal medium are $tyrA^+$ $cysC^-$. The overwhelming majority will grow on complete medium, but not on minimal medium or minimal media with just tyrosine or cysteine added. This majority is made up of the nontransformants, that is, auxotrophs that were not involved in a transformation event—they took up no foreign DNA.



Growth	$tyrA^+$ $cysC^+$	$tyrA^+$ $cysC^-$	$tyrA^-$ $cysC^+$
Complete medium	+	+	+
Minimal medium	+	–	–
Minimal + tyrosine	+	–	+
Minimal + cysteine	+	+	–

Figure 7.10 Transformation experiment with *B. subtilis*. A $tyrA^-$ $cysC^-$ strain is transformed with DNA from a $tyrA^+$ $cysC^+$ strain. Nontransformants as well as three types of transformants (two single and one double) result. Genotypes are determined by growth characteristics on four different types of petri plates (see fig. 7.11).

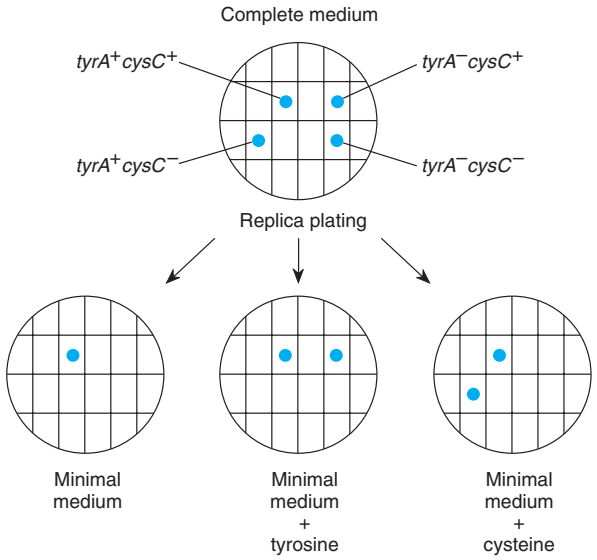


Figure 7.11 Four patterns of growth on different media reveal the genotypes of transformed and untransformed cells. Only four colonies are shown, and a grid is added for ease of identification. After transformation (see fig. 7.10), cells are plated on complete medium and then replica-plated onto minimal medium with either tyrosine or cysteine added.

As a control against reversion, the normal mutation of $tyrA^-$ to $tyrA^+$ or $cysC^-$ to $cysC^+$, we grow several plates of auxotrophs in minimal medium and minimal medium with tyrosine or cysteine added. These are auxotrophs that were not exposed to prototrophic donor DNA. We then count the number of natural revertants and correct our experimental numbers by the natural reversion rate. Thus, we are sure that what we measure is the actual transformation rate rather than just a mutation rate that we mistake for transformation. This control should *always* be carried out.

From the experiment (see figs. 7.10 and 7.11), we count twelve double transformants ($tyrA^+$ $cysC^+$), thirty-one $tyrA^+$ $cysC^-$, and twenty-seven $tyrA^-$ $cysC^+$. From these data, we calculate the co-occurrence, or cotransfer index, (r) as

$$r = \frac{\text{number of double transformants}}{\text{number of double transformants} + \text{number of single transformants}}$$

From our data

$$r = 12/(12 + 31 + 27) = 0.17.$$

This is a relative number indicating the co-occurrence of the two loci and thus their relative distance apart on the bacterial chromosome. Remember that as this number increases for different pairs of loci, the loci are closer and closer together.

By systematically examining many loci, we can establish their relative order. For example, if locus *A* is closely linked to locus *B* and *B* to *C,* we can establish the order *A B C.* It is not possible by this method to determine exact order for very closely linked genes. For this information we need to rely upon transduction, which we will consider shortly. However, transformation has allowed us to determine that the map of *B. subtilis* is circular, a phenomenon found in all prokaryotes and many phages. (The *E. coli* map is shown later.)

Conjugation

In 1946, Joshua Lederberg and Edward L. Tatum (later to be Nobel laureates) discovered that *E. coli* cells can exchange genetic material through the process of conjugation. They mixed two auxotrophic strains of *E. coli.* One strain required methionine and biotin (Met^- Bio^-), and the other required threonine and leucine (Thr^- Leu^-). This cross is shown in figure 7.12. Remember that if a strain is Met^- Bio^-, it is, without saying, wild-type for all other loci. Thus, a cell with the Met^- Bio^- phenotype actually has the genotype of met^- bio^- thr^+ leu^+. Similarly, the Thr^- Leu^- strain is actually met^+ bio^+ thr^- leu^-. (Note that symbols such as "Thr^-" represent phenotypes; symbols such as "thr^-" represent genotypes.)

Lederberg and Tatum used multiple auxotrophs in order to rule out spontaneous reversion (mutation). About one in 10^6 Met^- cells will spontaneously become prototrophic (Met^+) every generation. However, with multiple auxotrophs, the probability that several loci will simultaneously and spontaneously revert (e.g., $met^- \rightarrow met^+$) becomes vanishingly small. (In fact, the control plates in the experiment, illustrated in fig. 7.12, showed no growth for parental double mutants.) After mixing the strains, Lederberg and Tatum found that about one cell in 10^7 was prototrophic (met^+ bio^+ thr^+ leu^+).

To rule out transformation, one strain was put in each arm of a U-tube with a sintered glass filter at the bottom. (fig. 7.13). The liquid and large molecules, including DNA, were mixed by alternate application of pressure and suction to one arm of the tube; whole cells did not pass through the filter. The result was that the fluids surrounding the cells, as well as any large molecules (e.g., DNA), could be freely mixed while the cells were kept separate. After cell growth stopped in the two arms (in complete medium), the contents were plated out on minimal medium. There were no prototrophs in either arm. Therefore, cell-to-cell contact was required for the genetic material of the two cells to recombine.

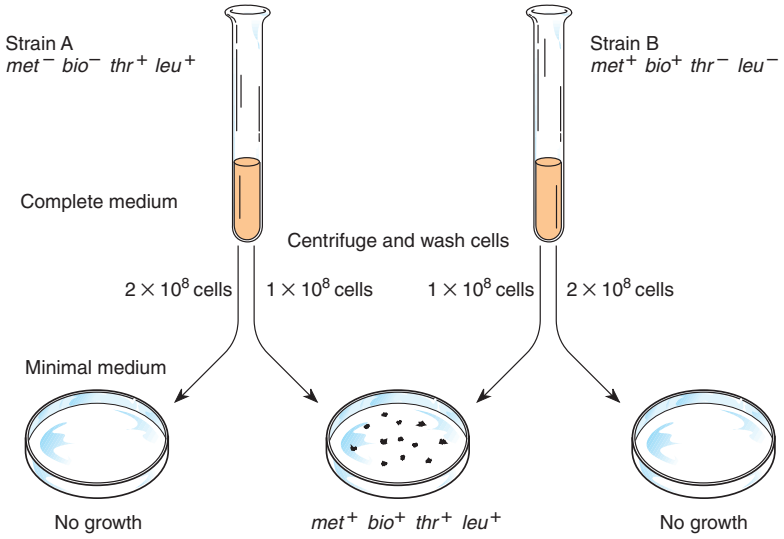


Figure 7.12 Lederberg and Tatum's cross showing that *E. coli* undergoes genetic recombination.

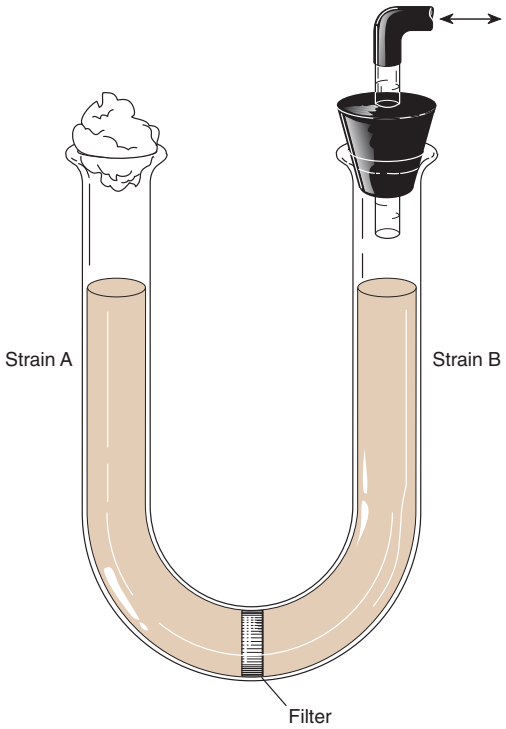


Figure 7.13 The U-tube experiment. Alternating suction and pressure force liquid and macromolecules back and forth across the filter.

F Factor

In bacteria, conjugation is a one-way transfer, with one strain acting as donor and the other as recipient. Sometimes donor cells, if stored for a long time, lose the ability to be donors, but they can regain the ability if they are mated with other donor strains. This discovery led to the hypothesis that a **fertility factor, F,** made any strain that carried it a male (donor) strain, termed F^+. The strain that did not have the F factor, referred to as a female or F^- strain, served as a recipient for genetic material during conjugation. Research supports this hypothesis.

The F factor is a *plasmid,* a term originally coined by Lederberg to refer to independent, self-replicating genetic particles. Plasmids are usually circles of double-stranded DNA. (Plasmids are at the heart of recombinant DNA technology, which is discussed in detail in chapter 13.) They are auxiliary circles of DNA that many bacteria carry. They are usually much smaller than the bacterial chromosome.

Researchers found that the transfer of the F factor occurred far more frequently than the transfer of other genes from the donor. That is, during conjugation, about one recombinant occurred in 10^7 cells, whereas transfer of the F factor occurred at a rate of about one conversion of F^- to F^+ in every five conjugations. An *E. coli* strain was then discovered that transferred its genetic material at a rate about one thousand times that of the normal F^+ strain. This strain was called **Hfr,** for *h*igh *f*requency of *r*ecombination. Several other phenomena occurred with this high rate of transfer. First, the ability to transfer the F factor itself dropped to almost zero in this strain. Second,

not all loci were transferred at the same rate. Some loci were transferred much more frequently than others.

Escherichia coli cells are normally coated with hair-like **pili (fimbriae).** F^+ and Hfr cells have one to three additional pili (singular: pilus) called **F-pili,** or sex pili. During conjugation, these sex pili form a connecting bridge between the F^+ (or Hfr) and F^- cells (fig. 7.14). Once a connection is made, the sex pilus then contracts to bring the two cells into contact. DNA transfer takes place through a nick in either the plasmid (in F^+ cells) or the bacterial chromosome (in Hfr cells). A single strand of the DNA double-stranded donor DNA then passes from the F^+ or Hfr cell to the F^- cell across the cell membranes. DNA replication in both the donor and recipient cells reestablishes double-stranded DNA in both. The F factor itself has the genes for sex-pilus formation and DNA transfer to a conjugating F^- cell. At least twenty-two genes are involved in the transfer process, including genes for the pilus protein, nicking the DNA, and regulation of the process.

In the transfer process of conjugation, the donor cell does not lose its F factor or its chromosome because only a single strand of the DNA double helix is transferred; the remaining single strand is quickly replicated. (The process of DNA replication is described in chapter 9.) For a short while, the F^- cell that has conjugated with an Hfr cell has two copies of whatever chromosomal loci were transferred: one copy of its own and one transferred in. With these two copies, the cell is a partial diploid, or a **merozygote.** The new foreign DNA **(exogenote)** can be incorporated into the host chromosome **(endogenote)** by an even number of breakages and reunions between the two, just as in transformation. The unincorporated linear DNA is soon degraded by enzymes. The conjugation process is diagrammed in figure 7.15.

Interrupted Mating

To demonstrate that the transfer of genetic material from the donor to the recipient cell during conjugation is a linear event, F. Jacob and E. Wollman devised the technique of **interrupted mating.** In this technique, F^- and Hfr strains were mixed together in a food blender.

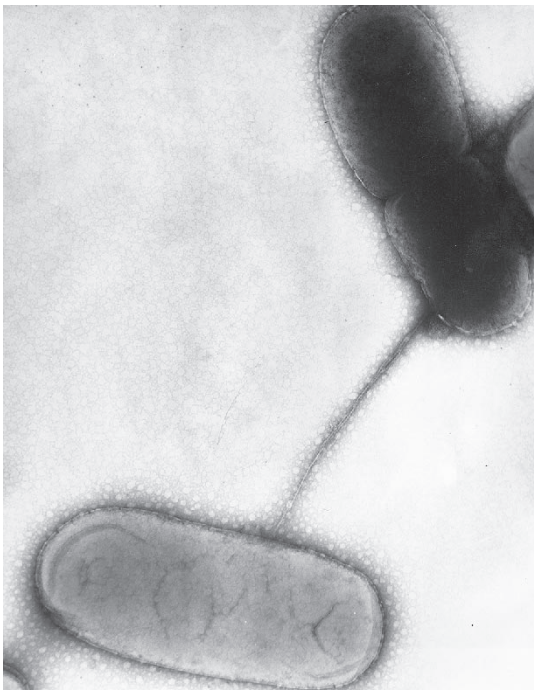


Figure 7.14 Electron micrograph of conjugation between an F^+ (*upper right*) and an F^- (*lower left*) cell with the F-pilus between them. Magnification 3,700×. (Courtesy of Wayne Rosenkrans and Dr. Sonia Guterman.)

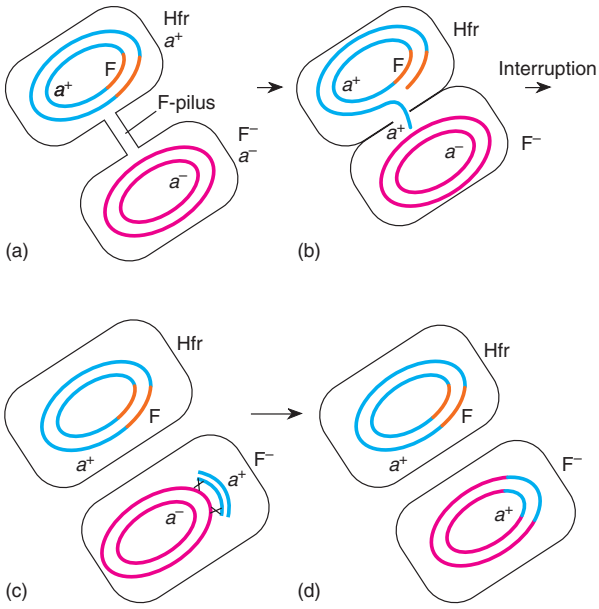


Figure 7.15 Bacterial conjugation. (*a*) The F-pilus draws an Hfr and an F^- cell close together. (*b*) The Hfr chromosome then begins to pass into the F^- cell, beginning at the F region of the Hfr chromosome but in the direction away from the F factor. Only a single strand passes into the F^- cell; this strand and the single strand remaining in the Hfr cell are replicated. After the process is interrupted (*c*), two crossovers bring the a^+ allele into the F^- a^- chromosome (*d*).

Elie Wollman (1917–). (Courtesy of Dr. Elie Wollman and the Pasteur Institute.)

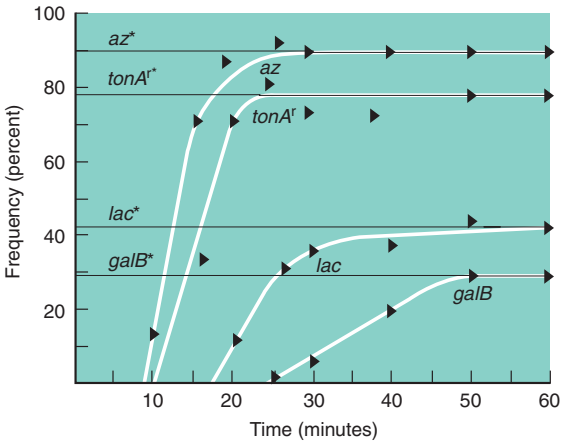
After waiting a specific amount of time, Jacob and Wollman turned the blender on. The spinning motion separated conjugating cells and thereby interrupted their mating. Then the researchers tested the F^- cells for various alleles originally in the Hfr cell. In an experiment like this, the Hfr strain is usually sensitive to an antibiotic such as streptomycin. After conjugation is interrupted, the cells are plated onto a medium containing the antibiotic, which kills all the Hfr cells. Then the genotypes of the F^- cells can be determined by replica-plating without fear of contamination by Hfr cells.

The mating outlined in table 7.3 was carried out. In the food blender, an Hfr strain sensitive to streptomycin (*str*^{s}) but resistant to azide (*azi*^{r}), resistant to phage T1 (*tonA*^{r}), and prototrophic for the amino acid leucine (*leu*$^+$) and the sugars galactose (*galB*$^+$) and lactose (*lac*$^+$) was added to an F^- strain that was resistant to streptomycin (*str*^{r}), sensitive to azide (*azi*^{s}), sensitive to T1 (*tonA*^{s}), and auxotrophic for leucine, galactose, and lactose (*leu*$^-$, *galB*$^-$, and *lac*$^-$). After a specific number of minutes (ranging from zero to sixty), the food blender was turned on. To kill all the Hfr cells, the cell suspension was plated on a medium containing streptomycin. The remaining cells were then plated on medium without leucine. The only colonies that resulted were F^- recombinants. They must have received the *leu*$^+$ allele from the Hfr in order to grow on a medium lacking leucine. Hence, all colonies had been selected to be F^- recombinants. By replica-plating onto specific media, investigators were able to determine the *azi*, *tonA*, *lac*, and *galB* alleles and the percentage of recombinant colonies that had the original Hfr allele (*leu*$^+$). (Note that by trial and error, it was determined that leucine should be the locus to use to select for recombinants. As we will see, the leucine locus entered first.)

Figure 7.16 shows that as time of mating increases, two things happen. First, new alleles enter the F^- cells from the Hfr cells. The *tonA*^{r} allele first appears among recombinants after about ten minutes of mating, whereas *galB*$^+$ first enters the F^- cells after about twenty-five minutes. This suggests a sequential entry of loci into the F^- cells from the Hfr (fig. 7.17). Second, as time proceeds, the percentage of recombinants with a given allele from the Hfr increases. At ten minutes, *tonA*^{r} is first found among recombinants. After fifteen minutes, about 40% of recombinants have the *tonA*^{r} allele from the Hfr; and after about twenty-five minutes, about 80% of the recombinants have the *tonA*^{r} allele. This limiting percent-

Table 7.3 **Genotypes of Hfr and F^- Cells Used in an Interrupted Mating Experiment**

Hfr	F^-
str^s	*str*^{r}
azi^r	*azi*^{s}
tonA^r	*tonA*^{s}
leu$^+$	*leu*$^-$
galB$^+$	*galB*$^-$
lac$^+$	*lac*$^-$



*Limiting percentage for *az*, *tonA*^{r}, *lac*, and *galB* loci.

Figure 7.16 Frequency of Hfr genetic characters among recombinants after interrupted mating. As time proceeds, new alleles appear and then increase in frequency. Interruption of the mating limits the frequency of successful passage. (From F. Jacobs and E. L. Wollman, *Sexuality and the Genetics of Bacteria,* Academic Press, 1961.)

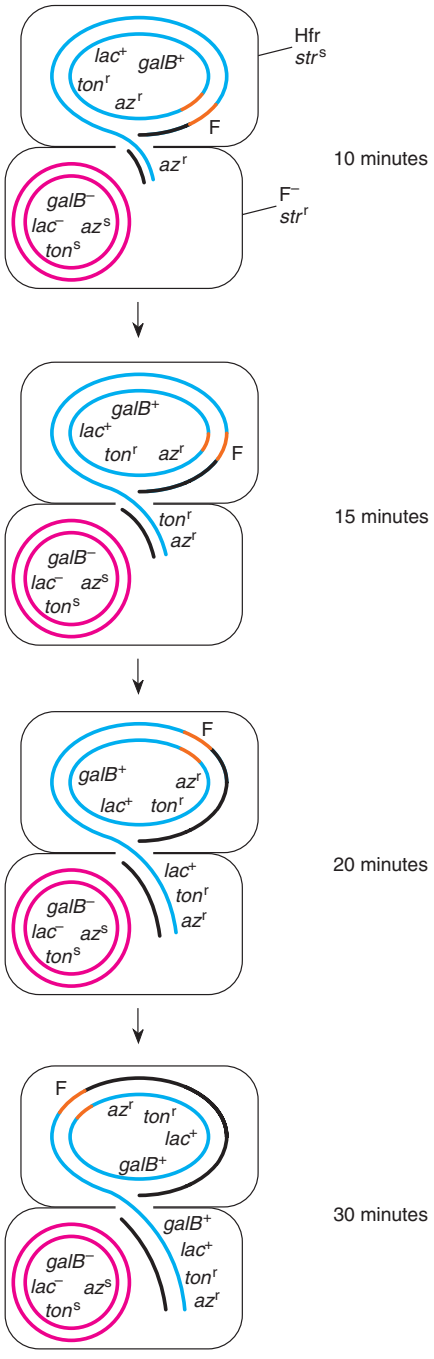


Figure 7.17 Conjugation in *E. coli*. Hfr chromosome is *blue;* F^- chromosome is *red;* and new DNA replication is *black*. As time proceeds, alleles from the Hfr enter the F^- cell in an orderly, sequential fashion. After the cells separate, two crossovers can bring Hfr alleles into the F^- chromosome. The F factor (*orange*) is the last part of the Hfr chromosome to enter the F^- cell.

age does not increase with additional time. The limiting percentage is lower for loci that enter later, a fact explained by the assumption that even without the food blender, mating is usually interrupted before completion by normal agitation alone.

Mapping and Conjugation

Jacob and Wollman, working with several different Hfr strains, collected data that indicated that the bacterial chromosome was circular. The strains were of independent origin, and the results were quite striking (table 7.4).

If we ponder this table for a short while, one fact becomes obvious: The relative order of the loci is always the same. What differs is the point of origin and the direction of the transfer. Jacob and Wollman proposed that normally the F factor is an independent circular DNA entity in the F^+ cell, and that during conjugation only the F factor is passed to the F^- cell. Since it is a small fragment of DNA, it can be passed entirely in a high proportion of conjugations before the cells separate. Every once in a while, however, the F factor becomes integrated into the chromosome of the host, which then becomes an Hfr cell. The point of integration can be different in different strains. However, once the F factor is integrated, it determines the initiation point of transfer for the *E. coli* chromosome, as well as the direction of transfer.

The F factor is the last part of the *E. coli* chromosome to be passed from the Hfr cell. This explains why an Hfr, in contrast to an F^+, rarely passes the F factor itself. In the original work of Lederberg and Tatum, the one recombinant in 10^7 cells most likely came from a conjugation between an F^- cell and an Hfr that had formed spontaneously from an F^+ cell. Integration of the F factor is diagrammed in figure 7.18. The F factor can also reverse this process and loop out of the *E. coli* chromosome. (Sometimes the F factor loops out incorrectly, as in figure 7.19, forming an F′ [F-prime] factor. The passage of this F′ factor to an F^- cell is called **F-duction** or **sexduction.** Not really useful in mapping, the process has proved exceptionally useful in studies of gene expression because of the formation of stable merozygotes, which we will examine in chapter 14.)

We could now diagram the *E. coli* chromosome and show the map location of all known loci. The map units would be in minutes, obtained by interrupted mating. However, at this point, the map would not be complete. Interrupted mating is most accurate in giving the relative position of loci that are not very close to each other. With this method alone, a great deal of ambiguity would arise as to the specific order of very close genes on the chromosome. The remaining sexual process in bacteria, transduction, provides the details that interrupted mating or transformation don't explain.

Table 7.4 Gene Order of Various Hfr Strains Determined by Means of Interrupted Mating

Types of Hfr	Order of Transfer of Genetic Characters*																		
HfrH	0	T	L	Az	T_1	Pro	Lac	Ad	Gal	Try	H	S-G	Sm	Mal	Xyl	Mtl	Isol	M	B_1
1	0	L	T	B_1	M	Isol	Mtl	Xyl	Mal	Sm	S-G	H	Try	Gal	Ad	Lac	Pro	T_1	Az
2	0	Pro	T_1	Az	L	T	B_1	M	Isol	Mtl	Xyl	Mal	Sm	S-G	H	Try	Gal	Ad	Lac
3	0	Ad	Lac	Pro	T_1	Az	L	T	B_1	M	Isol	Mtl	Xyl	Mal	Sm	S-G	H	Try	Gal
4	0	B_1	M	Isol	Mtl	Xyl	Mal	Sm	S-G	H	Try	Gal	Ad	Lac	Pro	T_1	Az	L	T
5	0	M	B_1	T	L	Az	T_1	Pro	Lac	Ad	Gal	Try	H	S-G	Sm	Mal	Xyl	Mtl	Isol
6	0	Isol	M	B_1	T	L	Az	T_1	Pro	Lac	Ad	Gal	Try	H	S-G	Sm	Mal	Xyl	Mtl
7	0	T_1	Az	L	T	B_1	M	Isol	Mtl	Xyl	Mal	Sm	S-G	H	Try	Gal	Ad	Lac	Pro
AB311	0	H	Try	Gal	Ad	Lac	Pro	T_1	Az	L	T	B_1	M	Isol	Mtl	Xyl	Mal	Sm	S-G
AB312	0	Sm	Mal	Xyl	Mtl	Isol	M	B_1	T	L	Az	T_1	Pro	Lac	Ad	Gal	Try	H	S-G
AB313	0	Mtl	Xyl	Mal	Sm	S-G	H	Try	Gal	Ad	Lac	Pro	T_1	Az	L	T	B_1	M	Isol

Source: From F. Jacobs and E. L. Wollman, *Sexuality and the Genetics of Bacteria,* Academic Press, 1961.

* The 0 refers to the origin of transfer.

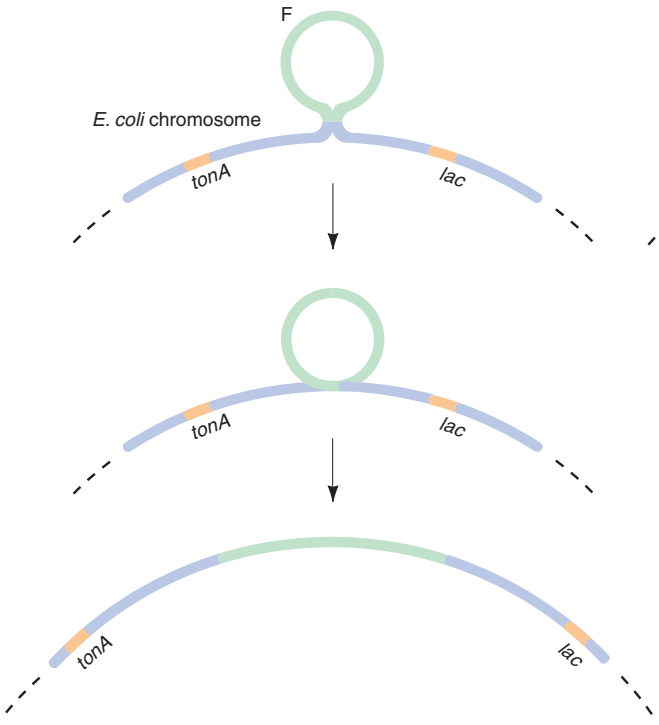


Figure 7.18 Integration of the F factor by a single crossover. After a simultaneous breakage in both the F factor and the *E. coli* chromosome, the two broken circles reunite to make one large circle, the Hfr chromosome. In this case, the integration occurs between the *tonA* and *lac* loci.

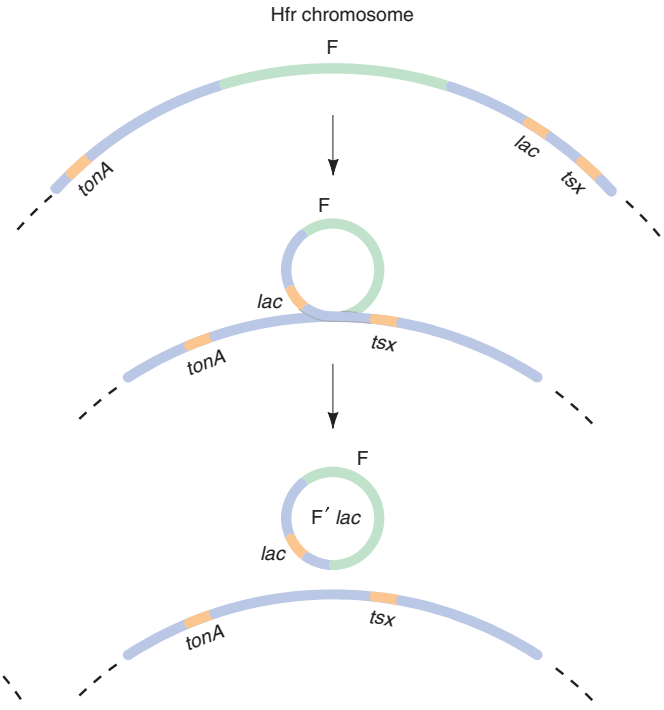


Figure 7.19 Occasionally, the F factor loops out imprecisely, taking part of the cell's genome in the loop. The circular F factor is freed by a single recombination (crossover) at the loop point.

LIFE CYCLES OF BACTERIOPHAGES

Phages are obligate intracellular parasites. Phage genetic material enters the bacterial cell after the phage has adsorbed to the cell surface. Once inside, the viral genetic material takes over the metabolism of the host cell. During the infection process, the cell's genetic material is destroyed, while the viral genetic material is replicated many times. The viral genetic material then controls the mass production of various protein components of the virus. New virus particles are assembled within the host cell, which bursts open (is lysed), releasing a **lysate** of hundreds of viral particles to infect other bacteria. This life cycle appears in figure 7.20.

Recombination

Much genetic work on phages has been done with a group of seven *E. coli* phages called the T series (T-odd: T1, T3, T5, T7; T-even: T2, T4, and T6) and several others, including phage λ (lambda; fig. 7.21). Figure 7.1 diagrammed the complex structure of T2. Phages can undergo recombination processes when a cell is infected with two genetically distinct virions. Hence, the phage genome can be mapped by recombination. As an example, consider the host-range and rapid-lysis loci. Rapid-lysis mutants (*r*) of the T-even phages produce large, sharp-edged plaques. The wild-type produces a smaller, more fuzzy-edged plaque (see fig. 7.7).

Alternative alleles are known also for host-range loci, phage loci that determine the strains of bacteria the phage can infect. For example, T2 can infect *E. coli* cells. These phages can be designated as T2h$^+$ for the normal host range. The *E. coli* is then called Tto^s, referring to their sensitivity to the T2 phage. In the course of evolution, an *E. coli* mutant arose that is resistant to the normal phage. This mutant strain is named Tto^r for T2 resistance. In the further course of evolution, the phages have produced mutant forms that can grow on the Tto^r strain of *E. coli.* These phage mutants are designated as T2h for host-range mutant. Remember, *host-range* signifies a mutation in the phage genome, whereas *phage resistance* indicates a mutation in the bacterial genome.

In 1945, Max Delbrück (a 1969 Nobel laureate) developed mixed indicators, which can be used to demonstrate four phage phenotypes on the same petri plate (fig. 7.22). A bacterial lawn of mixed Tto^r and Tto^s is grown. On this lawn, the rapid-lysis phage mutants (*r*) produce large plaques, whereas the wild-type (*r*$^+$) produce smaller plaques. Phages with host-range mutation (*h*) lyse both Tto^r and Tto^s bacteria. They produce the plaques that are clear (but appear dark) in figure 7.22. Since phages with the wild-type host-range allele (*h*$^+$) can only infect the Tto^s bacteria, they produce turbid plaques. The Tto^r bacteria growing within these plaques (which appear light-colored in fig. 7.22) produce the turbidity.

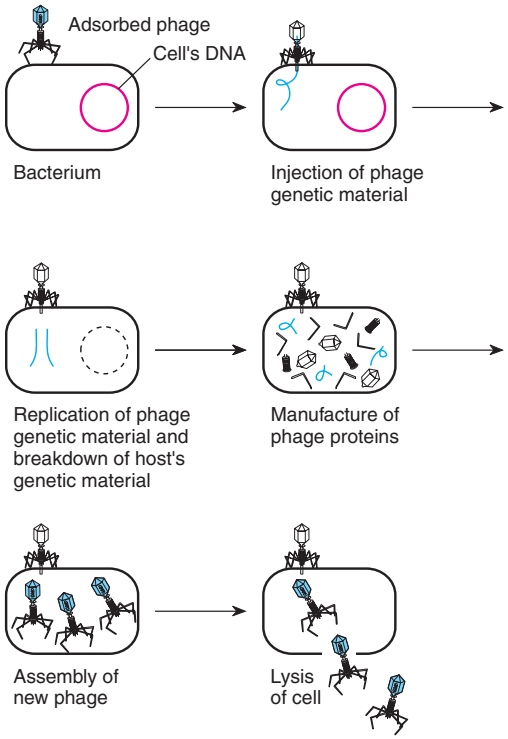


Figure 7.20 The viral life cycle, using T4 infection of *E. coli* as an example.

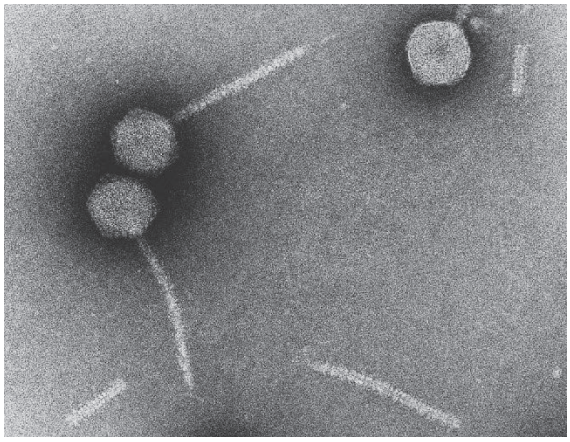


Figure 7.21 Phage λ. Magnification 167,300×. Note that phage λ lacks the tail fibers and base plate of phage T2 (see fig. 7.1). (Courtesy of Robley Williams.)

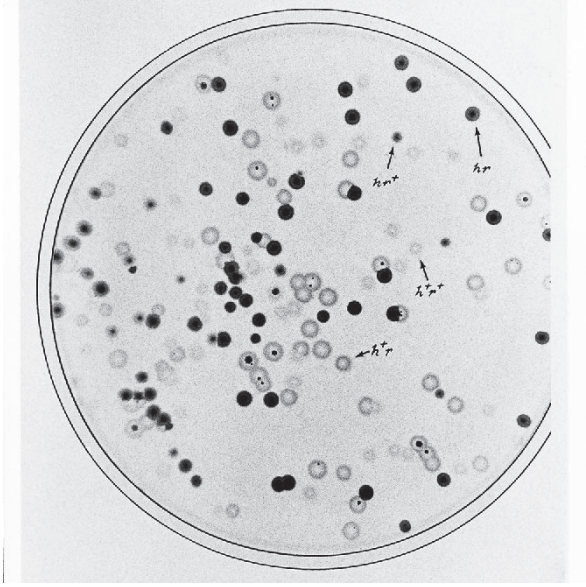


Figure 7.22 Four types of plaques produced by mixed phage T2 on a mixed lawn of *E. coli*. (From *Molecular Biology of Bacterial Viruses* by Gunther S. Stent, © 1963, 1978 by W. H. Freeman & Company. Used with permission.)

From the wild stock of phages, we can isolate host-range mutants by looking for plaques on a Tto^r bacterial lawn. Only *h* mutants will grow. These phages can then be tested for the *r* phenotype and the double mutants isolated. Once the two strains (double mutant and wild-type) are available, they can be added in large numbers to sensitive bacteria (fig. 7.23). Large numbers of phages are used to ensure that each bacterium is infected by at least one of each phage type, creating the possibility of recombination within the host bacterium. After a round of phage multiplication, the phages are isolated and plated out on Delbrück's mixed-indicator stock. From this growth, the phenotype (and, hence, genotype) of each phage can be recorded. The percentage of recombinants can be read directly from the plate. For example, on a given petri plate (e.g., fig. 7.22) there might be

hr	46	h^+r^+	52
h^+r	34	hr^+	26

The first two, *hr* and h^+r^+, are the original, or parental, phage genotypes. The second two categories result from recombination between the *h* and *r* loci on the phage chromosome. A single crossover in this region produces the recombinants. Note that with phage recombination, parental phages are counted, since every opportunity was provided for recombination within each bacterium. Thus, every progeny phage arises from a situation in

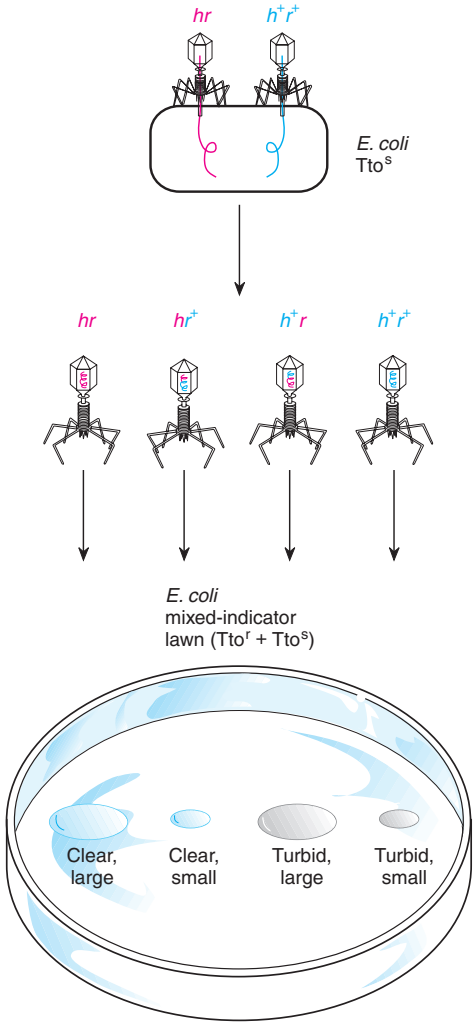


Figure 7.23 Crossing *hr* and h^+r^+ phage. Enough of both types are added to sensitive bacterial cells (Tto^s) to ensure multiple infections. The lysate, consisting of four genotypes, is grown on a mixed-indicator bacterial lawn (Tto^s and Tto^r). Plaques of four types appear (see fig. 7.22), indicating the genotypes of the parental and recombinant phages.

which recombination could have taken place. The proportion of recombinants is

$$(34 + 26)/(46 + 52 + 34 + 26)$$
$$= 60/158 = 0.38 \text{ or } 38\% \text{ or } 38 \text{ map units}$$

This percentage recombination is the map distance, which (as in eukaryotes) is a relative index of distance between loci: The greater the physical distance, the greater the amount of recombination, and thus the larger the map distance. One map unit (1 centimorgan) is equal to 1% recombinant offspring.

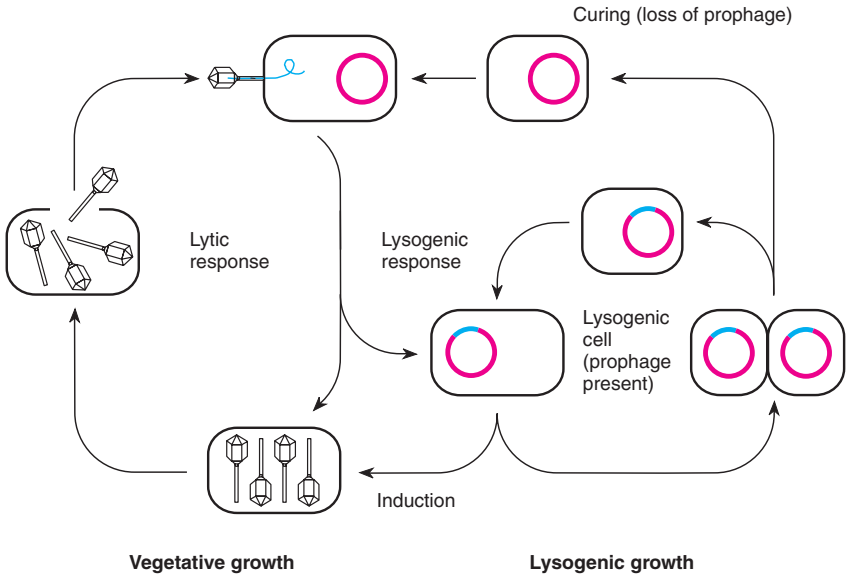


Figure 7.24 Alternative life-cycle stages of a temperate phage (lysogenic and vegetative growth).

Lysogeny

Certain phages are capable of two different life-cycle stages. Some of the time, they replicate in the host cytoplasm and destroy the host cell. At other times, these phages are capable of surviving in the host cell. The host is then referred to as **lysogenic** and the phage as **temperate.** (The term *lysogeny* means "giving birth to lysis." A lysogenic bacterium can be induced to initiate the virulent phase of the phage life cycle.)

The majority of research on lysogeny has been done on phage λ (see fig. 7.21). The λ prophage integrates into the host chromosome; other prophages, like P1, exist as independent plasmids. Phage λ, unlike the F factor, attaches at a specific point, termed *att*λ. This locus can be mapped on the *E. coli* chromosome; it lies between the galactose (*gal*) and biotin (*bio*) loci. When the phage is integrated, it protects the host from further infection (superinfection) by other λ phages. The integrated phage is now termed a **prophage.** Presumably it becomes integrated by a single crossover between itself and the host after apposition at the *att*λ site. (This process resembles the F-factor integration shown in fig. 7.18.)

A prophage can enter the lytic cycle of growth by a process of **induction,** which involves the excision of the prophage followed by the virulent or lytic stage of the viral life cycle. We consider the interesting and complex control mechanisms of life cycle in detail in chapter 14. Induction can take place through a variety of mechanisms, including UV irradiation and passage of the integrated prophage during conjugation **(zygotic induction).** The complete life cycle of a temperate phage is shown in figure 7.24.

TRANSDUCTION

Before lysis, when phage DNA is being packaged into phage heads, an occasional error occurs that causes bacterial DNA to be incorporated into the phage head instead. When this happens, bacterial genes can be transferred to another bacterium via the phage coat. This process, called transduction, has been of great use in mapping the bacterial chromosome. Transduction occurs in two patterns: specialized and generalized.

Specialized Transduction

The process of **specialized** or **restricted transduction** was first discovered in phage λ by Lederberg and his students. Specialized transduction is analogous to sexduction—it depends upon a mistake made during a looping-out process. In sexduction, the error is in the F factor. In specialized transduction, the error is in the λ prophage. Figure 7.25 shows the λ prophage looping out incorrectly to create a defective phage carrying the adjacent *gal* locus. Since only loci adjacent to the phage attachment site can be transduced in this process, specialized transduction has not proven very useful for mapping the host chromosome.

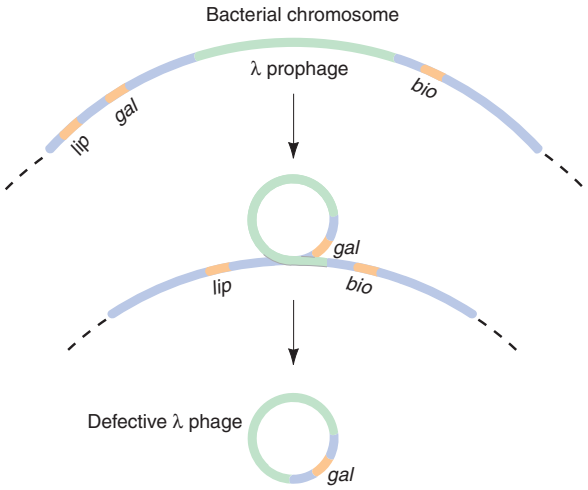


Figure 7.25 Imprecise excision, or looping out, of the λ prophage, resulting in a defective phage carrying the *gal* locus.

Generalized Transduction

Generalized transduction, which Zinder and Lederberg discovered, was the first mode of transduction discovered. The bacterium was *Salmonella typhimurium* and the phage was P22. Virtually any locus can be transduced by generalized transduction. The mechanism, therefore, does not depend on a faulty excision, but rather on the random inclusion of a piece of the host chromosome within the phage protein coat. A defective phage, one that carries bacterial DNA rather than phage DNA, is called a **transducing particle.** Transduction is complete when the genetic material from the transducing particle is injected into a new host and enters the new host's chromosome by recombination.

For P22, the rate of transduction is about once for every 10^5 infecting phages. Since a transducing phage can carry only 2 to 2.5% of the host chromosome, only genes very close to each other can be transduced together **(cotransduced).** Cotransduction can thus help to fill in the details of gene order over short distances after interrupted mating or transformation is used to ascertain the general pattern. Transduction is similar to transformation in that cotransduction, like co-occurrence in transformation, is a relative indicator of map distance.

Mapping with Transduction

Transduction can be used to establish gene order and map distance. Gene order can be established by two-factor transduction. For example, if gene *A* is cotransduced with gene *B* and *B* with gene *C*, but *A* is never cotransduced with *C*, we have established the order *A B C*

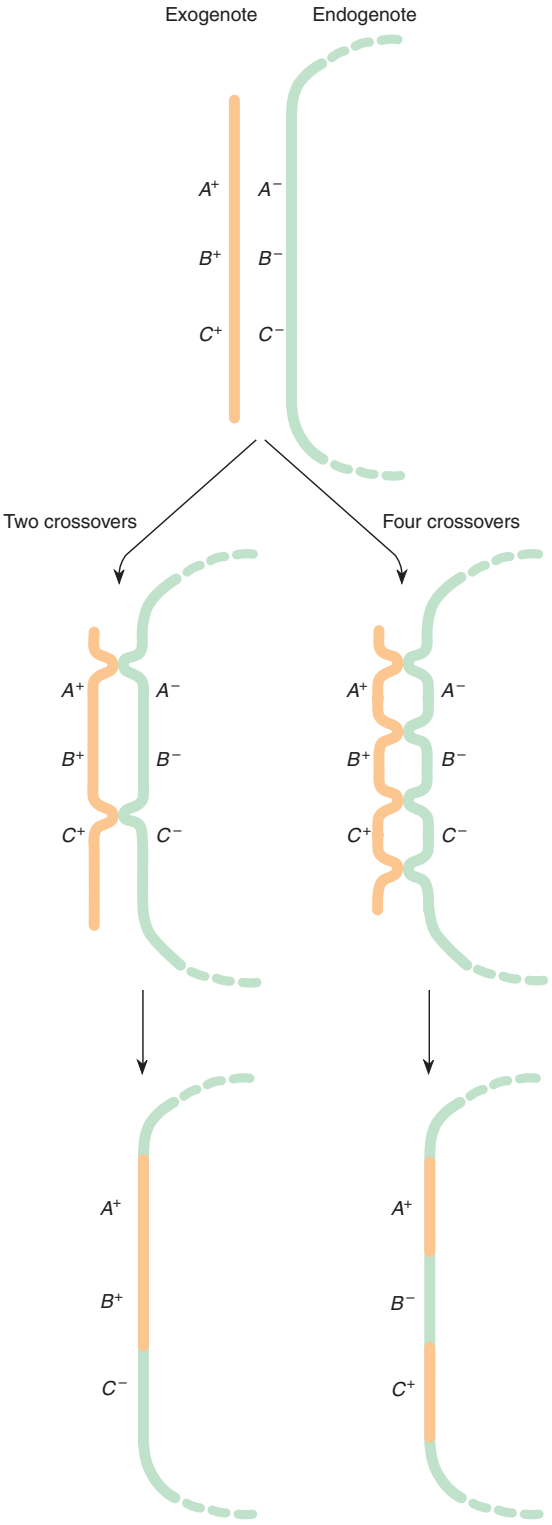


Figure 7.26 The rarest transductant requires four crossovers.

Table 7.5 **Gene Order Established by Two-Factor Cotransduction***

Transductants	Number
A^+B^+	30
A^+C^+	0
B^+C^+	25
$A^+\ B^+\ C^+$	0

* An $A^+\ B^+\ C^+$ strain of bacteria is infected with phage. The lysate is used to infect an $A^-\ B^-\ C^-$ strain. The transductants are scored for the wild-type alleles they contain. These data include only those bacteria transduced for two or more of the loci. Since *AB* cotransductants and *BC* cotransductants occur, but no *AC* types, we can infer the *A B C* order.

(table 7.5). This would also apply to quantitative differences in cotransduction. For example, if *E* is often cotransduced with *F* and *F* often with *G*, but *E* is very rarely cotransduced with *G*, then we have established the order *E F G*.

However, even more valuable is three-factor transduction, in which we can simultaneously establish gene order and relative distance. Three-factor transduction is especially valuable when the three loci are so close that it is very difficult to make ordering decisions on the basis of two-factor transduction or interrupted mating. For example, if genes *A*, *B*, and *C* are usually cotransduced, we can find the order and relative distances by taking advantage of the rarity of multiple crossovers. Let us use the prototroph ($A^+\ B^+\ C^+$) to make transducing phages that then infect the $A^-\ B^-\ C^-$ strain of bacteria.

To detect cells that have been transduced for one, two, or all three of the loci, we need to eliminate the nontransduced cells. In other words, after transduction, there will be $A^-\ B^-\ C^-$ cells in which no transduction event has taken place. There will also be seven classes of bacteria that have been transduced for one, two, or all three loci ($A^+\ B^+\ C^+$, $A^+\ B^+\ C^-$, $A^+\ B^-\ C^+$, $A^-\ B^+\ C^+$, $A^+\ B^-\ C^-$, $A^-\ B^+\ C^-$, and $A^-\ B^-\ C^+$). The simplest way to select for transduced bacteria is to select bacteria in which the wild-type has replaced at least one of the loci. For example, if, after transduction, we grow the bacteria in minimal media with the requirements of B^- and C^- added, all the bacteria that are A^+ will grow. (Without the requirement of A^- bacteria, no A^- bacteria will grow.) Hence, although we lose the $A^-\ B^+\ C^+$, $A^-\ B^+\ C^-$, and $A^-\ B^-\ C^+$ categories, we also lose the $A^-\ B^-\ C^-$, untransduced bacteria. In this example, the *A* locus is the selected locus; we must keep in mind that we have an incomplete, although informative, data set. Replica-plating allows us to determine genotypes at the *B* and *C* loci for the A^+ bacteria.

In this example, colonies that grow on complete medium without the requirement of the *A* mutant are replica-plated onto complete medium without the requirement of the *B* mutant and then onto complete medium without the requirement of the *C* mutant. In this way, each transductant can be scored for the other two loci (table 7.6). Now let us take all these selected transductants in which the A^+ allele was incorporated. These can be of four categories: $A^+\ B^+\ C^+$, $A^+\ B^+\ C^-$, $A^+\ B^-\ C^+$, and $A^+\ B^-\ C^-$. We can now compare the relative numbers of each of these four categories. The rarest category will be caused by the event that brings in the outer two markers, but not the center one, because this event requires four crossovers (fig. 7.26). Thus, by looking at the number of transductants in the various categories, we can determine that the gene order is *A B C* (table 7.7), since the $A^+\ B^-\ C^+$ category is the rarest.

Table 7.6 **Method of Scoring Three-Factor Transductants**

	Minimal Medium			
Colony Number	Without *A* Requirement	Without *B* Requirement	Without *C* Requirement	Genotype
1	+	+	−	$A^+\ B^+\ C^-$
2	+	−	−	$A^+\ B^-\ C^-$
3	+	−	−	$A^+\ B^-\ C^-$
4	+	+	+	$A^+\ B^+\ C^+$
5	+	−	+	$A^+\ B^-\ C^+$
.	.	.	.	.
.	.	.	.	.
.	.	.	.	.

Note: The plus indicates growth, the minus lack of growth. An $A^-\ B^-\ C^-$ strain was transduced by phage from an $A^+\ B^+\ C^+$ strain.

Table 7.7 **Numbers of Transductants and Relative Cotransduction Frequencies in the Experiment Used to Determine the *A B C* Gene Order (Table 7.6)**

Class	Number
$A^+ B^+ C^+$	50
$A^+ B^+ C^-$	75
$A^+ B^- C^+$	1
$A^+ B^- C^-$	300
	426
Relative Cotransductance	
A-B: (50 + 75)/426 = 0.29	
A-C: (50 + 1)/426 = 0.12	

Table 7.7 also includes calculations of the relative cotransduction frequencies. Remember that in all organisms and viruses, the higher the frequency of cooccurrence between the alleles of two loci, the closer those loci are on the chromosome. We usually measure the separation of loci by crossing over between them; the closer together, the lower those crossing-over values are and, hence, the smaller the measure of map units apart. Here, as with transformation, we are measuring the co-occurrence directly; therefore, the measure—cotransductance—is the inverse of map distance. In other words, the greater the cotransduction rate, the closer the two loci are; the more frequently two loci are transduced together, the closer they are and the higher the cotransduction value will be.

The data in table 7.7 should not be used to calculate the *B-C* cotransduction rate because the data are selected values, all of which are A^+; they do not encompass the total data. Missing is the $A^- B^+ C^+$ group that would contribute to the *B-C* cotransductance rate. The $A^- B^+ C^-$ and $A^- B^- C^+$ groups, also missing, would contribute only to the totals in the denominator, not the numerator, of the cotransductance index.

From these sorts of transduction experiments, it is possible to round out the details of map relations in *E. coli* after obtaining the overall picture by interrupted mating. The partial map of *E. coli* appears in figure 7.27. Definitions of loci can be found in table 7.8. Unlike the measurements in eukaryotic mapping, prokaryotic map distances are not generally thought of in map units (centimorgans). Rather, the general distance between loci is determined in *minutes* with cotransduction values used for loci that are very close to each other. (In chapter 13, we discuss mapping methods that rely on directly sequencing the DNA.)

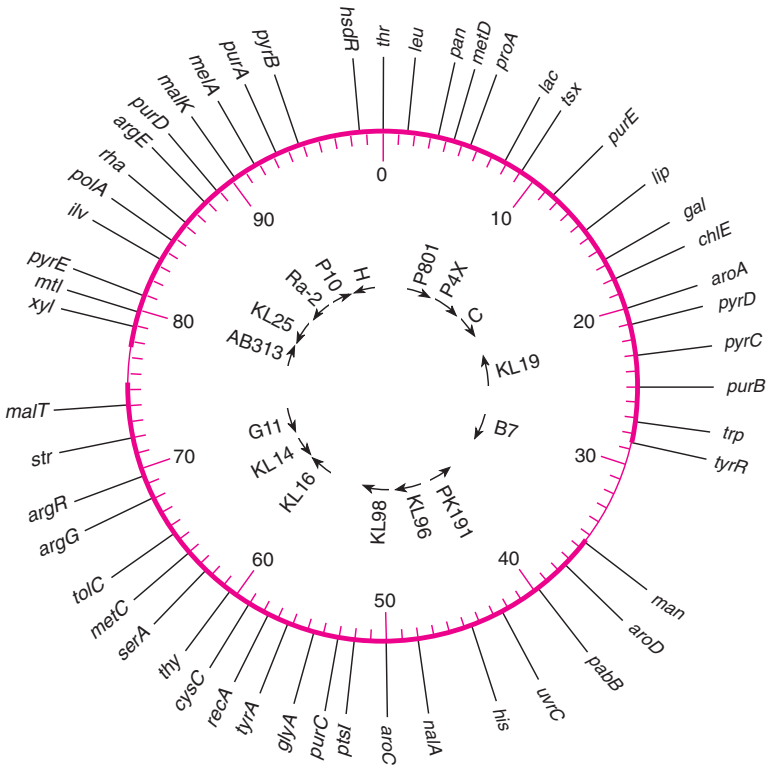


Figure 7.27 Selected loci on a circular map of *E. coli*. Definitions of loci not found in the text can be found in table 7.8. Units on the map are in minutes. *Arrows* within the circle refer to Hfr-strain transfer starting points, with directions indicated. The two thin regions on the outer circle are the only areas not covered by P1 transducing phages. (From B. J. Bachmann et al., "Recalibrated linkage map of *Escherichia coli* K-12," *Bacteriological Reviews,* 40:116–17. Copyright © 1976 American Society for Microbiology, Washington, D.C. Reprinted by permission.)

Table 7.8 Symbols Used in the Gene Map of the *E. coli* Chromosome

Genetic Symbols	Mutant Character	Enzyme or Reaction Affected
araD	Cannot use the sugar arabinose as a carbon source	L-Ribulose-5-phosphate-4-epimerase
araA		L-Arabinose isomerase
araB		L-Ribulokinase
araC		
argB	Requires the amino acid arginine for growth	N-Acetylglutamate synthetase
argC		N-Acetyl-γ-glutamokinase
argH		N-Acetylglutamic-γ-semialdehyde dehydrogenase
argG		Acetylornithine-*d*-transaminase
argA		Acetylornithinase
argD		Ornithine transcarbamylase
argE		Argininosuccinic acid synthetase
argF		Argininosuccinase
argR	Arginine operon regulator	
aroA, B, C	Requires several aromatic amino acids and vitamins for growth	Shikimic acid to 3-Enolpyruvyl-shikimate-5-phosphate
aroD		Biosynthesis of shikimic acid
azi	Resistant to sodium azide	
bio	Requires the vitamin biotin for growth	
carA	Requires uracil and arginine	Carbamate kinase
carB		
chlA-E	Cannot reduce chlorate	Nitrate-chlorate reductase and hydrogen lysase
cysA	Requires the amino acid cysteine for growth	3-Phosphoadenosine-5-phosphosulfate to sulfide
cysB		Sulfate to sulfide; four known enzymes
cysC		
dapA	Requires the cell-wall component diaminopimelic acid	Dihydrodipicolinic acid synthetase
dapB		N-Succinyl-diaminopimelic acid deacylase
dap + *hom*	Requires the amino acid precursor homoserine and the cell-wall component diaminopimelic acid for growth	Aspartic semialdehyde dehydrogenase
dnaA-Z	Mutation, DNA replication	DNA biosynthesis
Dsd	Cannot use the amino acid D-serine as a nitrogen source	D-Serine deaminase
fla	Flagella are absent	
galA	Cannot use the sugar galactose as a carbon source	Galactokinase
galB		Galactose-1-phosphate uridyl transferase
galD		Uridine-diphosphogalactose-4-epimerase
glyA	Requires glycine	Serine hydroxymethyl transferase
gua	Requires the purine guanine for growth	
H	The H antigen is present	
his	Requires the amino acid histidine for growth	Ten known enzymes*
hsdR	Host restriction	Endonuclease R
ile	Requires the amino acid isoleucine for growth	Threonine deaminase
ilvA	Requires the amino acids isoleucine and valine for growth	α-Hydroxy-β-keto acid rectoisomerase
ilvB		α, β-Dihydroxyisovaleric dehydrase*
ilvC		Transaminase B
ind (indole)	Cannot grow on tryptophan as a carbon source	Tryptophanase
λ (*att*λ)	Chromosomal location where prophage λ is normally inserted	
lacI	*Lac* operon regulator	
lacY	Unable to concentrate β-galactosides	Galactoside permease
lacZ	Cannot use the sugar lactose as a carbon source	β-Galactosidase

continued

Table 7.8 ***continued***

Genetic Symbols	Mutant Character	Enzyme or Reaction Affected
lacO	Constitutive synthesis of lactose operon proteins	Defective operator
leu	Requires the amino acid leucine for growth	Three known enzymes*
lip	Requires lipoate	
lon (long form)	Filament formation and radiation sensitivity are affected	
lys	Requires the amino acid lysine for growth	Diaminopimelic acid decarboxylase
lys + met	Requires the amino acids lysine and methionine for growth	
λrec, malT	Resistant to phage λ and cannot use the sugar maltose	Regulator for two operons
malK	Cannot use the sugar maltose as a carbon source	Maltose permease
man	Cannot use mannose sugar	Phosphomannose isomerase
melA	Cannot use melibiose sugar	Alpha-galactosidase
met A-M	Requires the amino acid methionine for growth	Ten or more genes
mtl	Cannot use the sugar mannitol as a carbon source	Two enzymes
muc	Forms mucoid colonies	Regulation of capsular polysaccharide synthesis
nalA	Resistant to nalidixic acid	
O	The O antigen is present	
pan	Requires the vitamin pantothenic acid for growth	
pabB	Requires *p*-aminobenzoate	
phe A, B	Requires the amino acid phenylalanine for growth	
pho	Cannot use phosphate esters	Alkaline phosphatase
pil	Has filaments (pili) attached to the cell wall	
plsB	Deficient phospholipid synthesis	Glycerol 3-phosphate acyltransferase
polA	Repairs deficiencies	DNA polymerase I
proA, *proB*, *proC*	Requires the amino acid proline for growth	
ptsI	Defective phosphotransferase system	Pts-system enzyme I
purA	Requires certain purines for growth	Adenylosuccinate synthetase
purB		Adenylosuccinase
purC, E		5-Aminoimidazole ribotide (AIR) to 5-aminoimidazole-4-(N-succino carboximide) ribotide
purD		Biosynthesis of AIR
pyrB	Requires the pyrimidine uracil for growth	Aspartate transcarbamylase
pyrC		Dihydroorotase
pyrD		Dihydroorotic acid dehydrogenase
pyrE		Orotidylic acid pyrophosphorylase
pyrF		Orotidylic acid decarboxylase
R gal	Constitutive production of galactose	Repressor for enzymes involved in galactose production
R1 pho, R2 pho	Constitutive synthesis of phosphatase	Alkaline phosphatase repressor
R try	Constitutive synthesis of tryptophan	Repressor for enzymes involved in tryptophan synthesis
RC (RNA control)	Uncontrolled synthesis of RNA	
recA	Cannot repair DNA radiation damage or recombine	
rhaA-D	Cannot use the sugar rhamnose as a carbon source	Isomerase, kinase, aldolase, and regulator
rpoA-D	Problems of transcription	Subunits of RNA polymerase
serA	Requires the amino acid serine for growth	3-Phosphoglycerate dehydrogenase
serB		Phosphoserine phosphatase
str	Resistant to or dependent on streptomycin	
suc	Requires succinic acid	

continued

Table 7.8 ***continued***

Genetic Symbols	Mutant Character	Enzyme or Reaction Affected
supB	Suppresses ochre mutations	t-RNA
tonA	Resistant to phages T1 and T5 (mutants called B/1, 5)	T1, T5 receptor sites absent
tonB	Resistant to phage T1 (mutants called B/1)	T1 receptor site absent
T6, colK rec	Resistant to phage T6 and colicine K	T6 and colicine receptor sites absent
T4 rec	Resistant to phage T4 (mutants called B/4)	T4 receptor site absent
tsx	T6 resistance	
thi	Requires the vitamin thiamine for growth	
tolC	Tolerance to colicine E1	
thr	Requires the amino acid threonine for growth	
thy	Requires the pyrimidine thymine for growth	Thymidylate synthetase
trpA	Requires the amino acid tryptophan for growth	Tryptophan synthetase, A protein
trpB		Tryptophan synthetase, B protein
trpC		Indole-3-glycerolphosphate synthetase
trpD		Phosphoribosyl anthranilate transferase
trpE		Anthranilate synthetase
tyrA	Requires the amino acid tyrosine for growth	Chorismate mutase T-prephenate dehydrogenase
tyrR		Regulates three genes
uvrA-E	Resistant to ultraviolet radiation	Ultraviolet-induced lesions in DNA are reactivated
valS	Cannot charge Valyl-tRNA	Valyl-tRNA synthetase
xyl	Cannot use the sugar xylose as a carbon source	

Source: B. J. Bachmann and K. B. Low, "Linkage map of *Escherichia coli* K-12," *Microbiological Reviews,* 44:1–56. Copyright © 1990 American Society for Microbiology, Washington, D.C. Reprinted by permission.

* Denotes enzymes controlled by the homologous gene loci of *Salmonella typhimurium.*

SUMMARY

STUDY OBJECTIVE 1: To define bacteria and bacterial viruses and learn about methods of studying them 149–154

Prokaryotes (bacteria) usually have a single circular chromosome of double-stranded DNA. A bacteriophage consists of a chromosome wrapped in a protein coat. Its chromosome can be DNA or RNA. Phenotypes of bacteria include colony morphology, nutritional requirements, and drug resistance. Phage phenotypes include plaque morphology and host range. Replica-plating is a rapid screening technique for assessing the phenotype of a bacterial clone.

STUDY OBJECTIVE 2: To study life cycles and sexual processes in bacteria and bacteriophages 154–166

In transformation, a competent bacterium can take up relatively large pieces of DNA from the medium. This DNA can be incorporated into the bacterial chromosome.

During the process of conjugation, the fertility factor, F, is passed from an F^+ to an F^- cell. If the F factor integrates into the host chromosome, an Hfr cell results that can pass its entire chromosome into an F^- cell. The F factor is the last region to cross into the F^- cell.

In transduction, a phage protein coat containing some of the host chromosome passes to a new host bacterium. Again, recombination with this new chromosomal segment can take place.

STUDY OBJECTIVE 3: To make use of the sexual processes of bacteria and their viruses to map their chromosomes 155–171

We can map the phage chromosome by measuring recombination after a bacterium has been simultaneously infected by two strains of the virus carrying different alleles. In *E. coli,* mapping is most efficiently accomplished via interrupted mating and transduction. The former provides information on general gene arrangement and the latter provides finer details.

SOLVED PROBLEMS

PROBLEM 1: A wild-type strain of *B. subtilis* is transformed by DNA from a strain that cannot grow on galactose (*gal*$^-$) and also needs biotin for growth (*bio*$^-$). Transformants are isolated by exposing the transformed cells to minimal medium with penicillin, killing the wild-type cells. After the penicillin is removed, replica-plating is used to establish the genotypes of 30 transformants:

Class 1 *gal*$^-$ *bio*$^-$	17
Class 2 *gal*$^-$ *bio*$^+$	4
Class 3 *gal*$^+$ *bio*$^-$	9

What is the relative co-occurrence of these two loci?

Answer: The three classes of colonies represent the three possible transformant groups. Classes 2 and 3 are single transformants and class 1 is the double transformant. We are interested in the relative co-occurrence of the two loci. Therefore we divide the number of double transformants by the total: $r = 17/(17 + 4 + 9) = 0.57$. This is a relative value inverse to a map distance; the larger it is, the closer the loci are to each other.

PROBLEM 2: A *gal*$^-$ *bio*$^-$ *att*λ$^-$ strain of *E. coli* is transduced by P22 phages from a wild-type strain. Transductants are selected for by growing the cells with galactose as the sole energy source. Replica-plating and testing for lysogenic ability gives the genotypes of 106 transformants:

Class 1 *gal*$^+$ *bio*$^-$ *att*λ$^-$	71
Class 2 *gal*$^+$ *bio*$^+$ *att*λ$^-$	0
Class 3 *gal*$^+$ *bio*$^-$ *att*λ$^+$	9
Class 4 *gal*$^+$ *bio*$^+$ *att*λ$^+$	26

What is the gene order, and what are the relative cotransduction frequencies?

Answer: We have selected all transductants that are *gal*$^+$. Class 2 is in the lowest frequency (0) and therefore represents the quadruple crossover between the transducing DNA and the host chromosome. From this, we see that *att*λ must be in the middle because this low-probability event is the one that would have switched only the middle locus. In other words, the two end loci would be recombinant, and the middle locus would have the host allele. We can only calculate two cotransduction frequencies because these are selected data. Note that in class 1, there is no cotransduction between *gal* and either of the other two loci; class 2 would show the cotransduction of *gal* and *bio;* class 3 represents the cotransduction of *gal* and *att*λ; and class 4 represents the cotransduction of *gal* and both other loci. Therefore, cotransduction values are

$$gal\text{–}att\lambda = (9 + 26)/106 = 35/106 = 0.33$$
$$gal\text{–}bio = (0 + 26)/106 = 26/106 = 0.25.$$

EXERCISES AND PROBLEMS*

BACTERIA AND BACTERIAL VIRUSES IN GENETIC RESEARCH

1. What is the nature and substance of prokaryotic chromosomes and viral chromosomes? Are viruses alive?

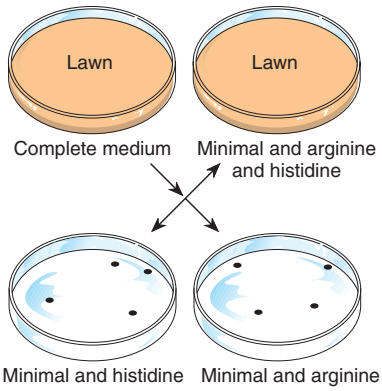
TECHNIQUES OF CULTIVATION

2. What are the differences between a heterotroph and an auxotroph? a minimal and a complete medium? an enriched and a selective medium?
3. What are the differences between a plaque and a colony?

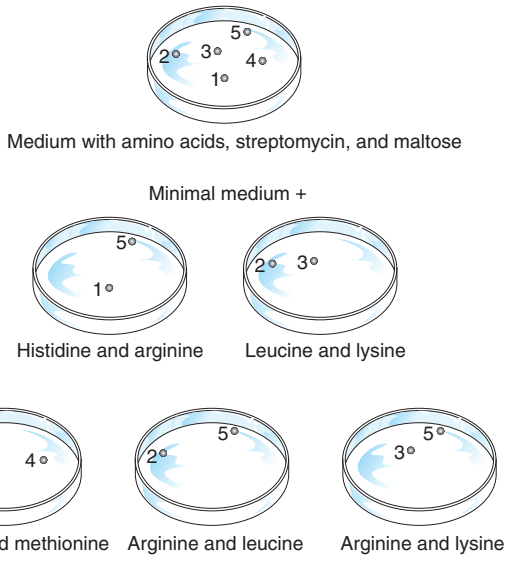
BACTERIAL PHENOTYPES

4. What genotypic notation indicates alleles that make a bacterium
   - **a.** resistant to penicillin?
   - **b.** sensitive to azide?
   - **c.** require histidine for growth?
   - **d.** unable to grow on galactose?
   - **e.** able to grow on glucose?
   - **f.** susceptible to phage T1 infection?
5. An *E. coli* cell is placed on a petri plate containing λ phages. It produces a colony overnight. By what mechanisms might it have survived?
6. An *E. coli* lawn is formed on a petri plate containing complete medium. Replica-plating is used to transfer material to plates containing minimal medium and combinations of the amino acids arginine and histidine (see the figure). Give the genotype of the original strain as well as the genotypes of the odd colonies found growing on the plates.

*Answers to selected exercises and problems are on page A-7.



7. Prototrophic Hfr *E. coli* strain G11, sensitive to streptomycin and *malT*$^+$ (can use maltose) is used in a conjugation experiment. The *str* locus is one of the last to be transferred, whereas the *malT* locus is one of the first. This strain is mated to an F$^-$ strain resistant to streptomycin, *malT*$^-$ (cannot utilize maltose), and requiring five amino acids (histidine, arginine, leucine, lysine, and methionine). Recombinants are selected for by plating on a medium with streptomycin, with maltose as the sole carbon source, and all five amino acids present. Thus, all recombinant F$^-$ cells will grow irrespective of their amino acid requirements. Five colonies are grown on the original plate with streptomycin, maltose, and all five amino acids in question (see the figure). These colonies are replica-plated onto minimal medium containing various amino acids. What are the genotypes of each of the five colonies?



8. A petri plate with complete medium has six colonies growing on it after one of the conjugation experiments described earlier. The colonies are numbered, and the plate is used as a master to replicate onto plates of glucose-containing selective (minimal) medium with various combinations of additives. From the following data, which show the presence (+) or absence (−) of growth, give your best assessment of the genotypes of the six colonies.

	Colony					
On Minimal Medium +	**1**	**2**	**3**	**4**	**5**	**6**
Nothing	−	−	+	−	−	−
Xylose + arginine	+	−	+	+	−	−
Xylose + histidine	−	−	+	−	−	−
Arginine + histidine	−	+	+	+	−	−
Galactose + histidine	−	−	+	−	−	+
Threonine + isoleucine + valine	−	−	+	−	+	−
Threonine + valine + lactose	−	−	+	−	−	−

VIRAL PHENOTYPES

9. Give possible genotypes of an *E. coli*-phage T1 system in which the phage cannot grow on the bacterium. Give genotypes for a T1 phage that can grow on the bacterium.

SEXUAL PROCESSES IN BACTERIA AND BACTERIOPHAGES

10. What is a plasmid? How does one integrate into a host's chromosome? How does it leave?

11. In conjugation experiments, one Hfr strain should carry a gene for some sort of sensitivity (e.g., *azt*^{s} or *str*^{s}) so that the Hfr donors can be eliminated on selective media after conjugation has taken place. Should this locus be near to or far from the origin of transfer point of the Hfr chromosome? What are the consequences of either alternative?

12. How does a geneticist doing interrupted mating experiments know that the locus for the drug-sensitivity allele, used to eliminate the Hfr bacteria after conjugation, has crossed into the F$^-$ strain?

13. Diagram the step-by-step events required to integrate foreign DNA into a bacterial chromosome in each of the three processes outlined in the chapter (transformation, conjugation, transduction). Do the same for viral recombination. (*See also* TRANSDUCTION)

14. The DNA from a prototrophic strain of *E. coli* is isolated and used to transform an auxotrophic strain deficient in the synthesis of purines (*purB*$^-$), pyrimidines (*pyrC*$^-$), and the amino acid tryptophan (*trp*$^-$). Tryptophan was used as the marker to determine whether transformation had occurred (the selected marker). What are the gene order and the relative co-occurrence frequencies between loci, given these data:

trp$^+$ *pyrC*$^+$ *purB*$^+$	86
trp$^+$ *pyrC*$^+$ *purB*$^-$	4
trp$^+$ *pyrC*$^-$ *purB*$^+$	67
trp$^+$ *pyrC*$^-$ *purB*$^-$	14

15. Using the data in figure 7.16, draw a tentative map of the *E. coli* chromosome.

16. Three Hfr strains of *E. coli* (P4X, KL98, and Ra-2) are mated individually with an auxotrophic F$^-$ strain using interrupted mating techniques. Using the following data, construct a map of the *E. coli* chromosome, including distances in minutes.

	Approximate Time of Entry		
Donor Loci	**Hfr P4X**	**Hfr KL98**	**Hfr Ra−2**
gal$^+$	11	67	70
thr$^+$	94	50	87
xyl$^+$	73	29	8
lac$^+$	2	58	79
his$^+$	38	94	43
ilv$^+$	77	33	4
argG$^+$	62	18	19

How many different petri plates and selective media are needed?

17. Design an experiment using interrupted mating and create a resulting possible data set that would correctly map five of the loci on the *E. coli* chromosome (fig. 7.27).

18. Lederberg and his colleagues (Nester, Schafer, and Lederberg, 1963, *Genetics* 48:529) determined gene order and relative distance between genes using three markers in the bacterium *Bacillus subtilis.* DNA from a prototrophic strain (*trp*$^+$ *his*$^+$ *tyr*$^+$) was used to transform the auxotroph. The seven classes of transformants, with their numbers, are tabulated as follows:

trp$^+$	*trp*$^-$	*trp*$^-$	*trp*$^+$	*trp*$^+$	*trp*$^-$	*trp*$^+$
his$^-$	*his*$^+$	*his*$^-$	*his*$^+$	*his*$^-$	*his*$^+$	*his*$^+$
tyr$^-$	*tyr*$^-$	*tyr*$^+$	*tyr*$^-$	*tyr*$^+$	*tyr*$^+$	*tyr*$^+$
2,600	418	685	1,180	107	3,660	11,940

Outline the techniques used to obtain these data. Taking the loci in pairs, calculate co-occurrences. Construct the most consistent linkage map of these loci.

19. In a transformation experiment, an $a^+ b^+ c^+$ strain is used as the donor and an $a^- b^- c^-$ strain as the recipient. One hundred a^+ transformants are selected and then replica-plated to determine whether b^+ and c^+ are present. What can you conclude about the relative positions of the genes, based on the listed genotypes?

$a^+ b^- c^-$	21
$a^+ b^- c^+$	69
$a^+ b^+ c^-$	3
$a^+ b^+ c^+$	7

20. In a transformation experiment, an $a^+ b^+ c^-$ strain is used as donor and an $a^- b^- c^+$ strain as recipient. If you select for a^+ transformants, the least frequent class is $a^+ b^+ c^+$. What is the order of the genes?

21. A mating between *his*$^+$, *leu*$^+$, *thr*$^+$, *pro*$^+$, *str*^{s} cells (Hfr) and *his*$^-$, *leu*$^-$, *thr*$^-$, *pro*$^-$, *str*^{r} cells (F$^-$) is allowed to continue for twenty-five minutes. The mating is stopped, and the genotypes of the recombinants are determined. What is the first gene to enter, and what is the probable gene order, based on the following data?

Genotype	**Number of Colonies**
his$^+$	0
leu$^+$	12
thr$^+$	27
pro$^+$	6

22. **a.** In a transformation experiment, the donor is *trp*$^+$ *leu*$^+$ arg$^+$, and the recipient is *trp*$^-$ *leu*$^-$ arg$^-$. The selection process is for *trp*$^+$ transformants, which are then further tested. Forty percent are *trp*$^+$ arg$^+$; 5% are *trp*$^+$ *leu*$^+$. In what two possible orders could the genes be arranged?

b. You can do only one more transformation to determine gene order. You must use the same donor and recipient, but you can change the selection procedure for the initial transformants. What should you do, and what results should you expect for each order you proposed in *a*?

23. DNA from a bacterial strain that is $a^+ b^+ c^+$ is used to transform a strain that is $a^- b^- c^-$. The numbers of each transformed genotype appear. What can we say about the relative position of the genes?

Genotype	**Number**
$a^+ b^- c^-$	214
$a^- b^+ c^-$	231
$a^- b^- c^+$	206
$a^+ b^+ c^-$	11
$a^+ b^+ c^+$	6
$a^+ b^- c^+$	93
$a^- b^+ c^+$	14

24. An Hfr strain that is $a^+ b^+ c^+ d^+ e^+$ is mated with an F^- strain that is $a^- b^- c^- d^- e^-$. The mating is interrupted every five minutes, and the genotypes of the F^- recombinants are determined. The results appear following. (A *plus* indicates appearance; a *minus* the lack of the locus.) Draw a map of the chromosome and indicate the position of the F factor, the direction of transfer, and the minutes between genes.

Time	*a*	*b*	*c*	*d*	*e*
5	−	−	−	−	−
10	+	−	−	−	−
15	+	−	−	−	−
20	+	−	−	−	−
25	+	−	−	−	−
30	+	−	−	+	−
35	+	−	−	+	−
40	+	+	−	+	−
45	+	+	−	+	−
50	+	+	−	+	−
55	+	+	−	+	−
60	+	+	−	+	−
65	+	+	+	+	−
70	+	+	+	+	−
75	+	+	+	+	+

25. A bacterial strain that is $lys^+ his^+ val^+$ is used as a donor, and $lys^- his^- val^-$ as the recipient. Initial transformants are isolated on minimal medium + histidine + valine.

a. What genotypes will grow on this medium?

b. These colonies are replicated to minimal medium + histidine, and 75% of the original colonies grow. What genotypes will grow on this medium?

c. The original colonies are also replicated to minimal medium + valine, and 6% of the colonies grow. What genotypes will grow on this medium?

d. Finally, the original colonies are replicated to minimal medium. No colonies grow. From this information, what genotypes will grow on minimal medium + histidine and on minimal medium + valine?

e. Based on this information, which gene is closer to *lys*?

f. The original transformation is repeated, but the original plating is on minimal medium + lysine + histidine. Fifty colonies appear. These colonies are replicated to determine their genotypes, with these results:

$val^+ his^+ lys^+$	0
$val^+ his^- lys^+$	37
$val^+ his^+ lys^-$	3

Based on all the results, what is the most likely gene order?

LIFE CYCLES OF BACTERIOPHAGES

26. Define *prophage, lysate, lysogeny,* and *temperate phage.*

27. Outline an experiment to demonstrate that two phages do not undergo recombination until a bacterium is infected simultaneously with both.

28. Doermann (1953, *Cold Spr. Harb. Symp. Quant. Biol.* 18:3) mapped three loci of phage T4: minute, rapid lysis, and turbid. He infected *E. coli* cells with both the triple mutant (*m r tu*) and the wild-type ($m^+ r^+ tu^+$) and obtained the following data:

m	m^+	*m*	*m*	m^+	m^+	*m*	m^+
r	*r*	r^+	*r*	r^+	*r*	r^+	r^+
tu	*tu*	*tu*	tu^+	*tu*	tu^+	tu^+	tu^+
3,467	474	162	853	965	172	520	3,729

What is the linkage relationship among these loci? In your answer include gene order, relative distance, and coefficient of coincidence.

29. Wild-type phage T4 (r^+) produce small, turbid plaques, whereas *r*II mutants produce large, clear plaques. Four *r*II mutants (*a*–*d*) are crossed. (Assume, for the purposes of this problem, that *a*–*d* are four closely linked loci. The actual structure of the *r*II region is presented in chapter 12. Here, assume that $a \times b$ means $a^- b^+ c^+ d^+ \times a^+ b^- c^+ d^+$.) These percentages of wild-type plaques are obtained in crosses:

$a \times b$	0.3
$a \times c$	1.0
$a \times d$	0.4
$b \times c$	0.7
$b \times d$	0.1
$c \times d$	0.6

Deduce a genetic map of these four mutants.

30. A phage cross is performed between $a^+ b^+ c^+$ and *a b c* phage. Based on these results, derive a complete map:

$a^+ b^+ c^+$	1,801
$a^+ b^+ c$	954
$a^+ b c^+$	371
$a^+ b c$	160
$a b^+ c^+$	178
$a b^+ c$	309
$a b c^+$	879
a b c	1,850
	6,502

31. The *r*II mutants of T4 phage will grow and produce large plaques on strain B; *r*II mutants will not grow on strain K12. Certain crosses are performed in strain B. (As with question 29, assume that the three mutants are of three separate loci in the *r*II region.) By diluting and plating on strain B, it is determined that each experiment generates about 250×10^7 phage. By dilution, approximately 1/10,000 of the progeny are plated on K12 to generate these wild-type recombinants (plaques on K12):

1×2	50
1×3	25
2×3	75

Draw a map of these three mutants (1, 2, and 3) and indicate the distances between them.

TRANSDUCTION

32. Define and illustrate *specialized* and *generalized transduction.*

33. In *E. coli,* the three loci *ara, leu,* and *ilvH* are within 1/2-minute map distance apart. To determine the exact order and relative distance, the prototroph (ara^+ leu^+ $ilvH^+$) was infected with transducing phage P1. The lysate was used to infect the auxotroph (ara^- leu^- $ilvH^-$). The ara^+ classes of transductants were selected to produce the following data:

ara^+	ara^+	ara^+	ara^+
leu^-	leu^+	leu^-	leu^+
$ilvH^-$	$ilvH^-$	$ilvH^+$	$ilvH^+$
32	9	0	340

Outline the specific techniques used to isolate the various transduced classes. What is the gene order and what are the relative cotransduction frequencies between genes? Why do some classes occur so infrequently?

34. Consider this portion of an *E. coli* chromosome:

thr *ara* *leu*

Three *ara* loci, *ara*-1, *ara*-2, and *ara*-3, are located in the *ara* region. A mutant of each locus (*ara*-1^-, *ara*-2^-, and *ara*-3^-) was isolated, and their order with respect to *thr* and *leu* was analyzed by transduction. The donor was always thr^+ leu^+ and the recipient was always thr^- leu^-. Each *ara* mutant was used as a donor in one cross and as a recipient in another; ara^+ transductants were selected in each case. The ara^+ transductants were then scored for leu^+ and thr^+. Based on the following results, determine the order of the ara^- mutants with respect to *thr* and *leu.*

Cross	Recipient	Donor	Ratio: $\frac{thr^- ara^+ leu^+}{thr^+ ara^+ leu^-}$
1	*ara*-1^-	*ara*-2^-	48.5
2	*ara*-2^-	*ara*-1^-	2.4
3	*ara*-1^-	*ara*-3^-	4.0
4	*ara*-3^-	*ara*-1^-	19.1
5	*ara*-2^-	*ara*-3^-	1.5
6	*ara*-3^-	*ara*-2^-	25.5

35. An *E. coli* strain that is leu^+ thr^+ azi^r is used as a donor in a transduction of a strain that is leu^- thr^- azi^s. Either leu^+ or thr^+ transductants are selected and then scored for unselected markers. The results are obtained:

Selected Marker	Unselected Markers
leu^+	48% azi^r
leu^+	2% thr^+
thr^+	3% leu^+
thr^+	0% azi^r

What is the order of the three loci?

CRITICAL THINKING QUESTIONS

1. Consider the data from table 7.4. Is there another way to interpret the data other than coming from a circular bacterial chromosome?

2. Why might transformation have evolved, given that the bacterium is importing DNA from a dead organism?

Suggested Readings for chapter 7 are on page B-4.