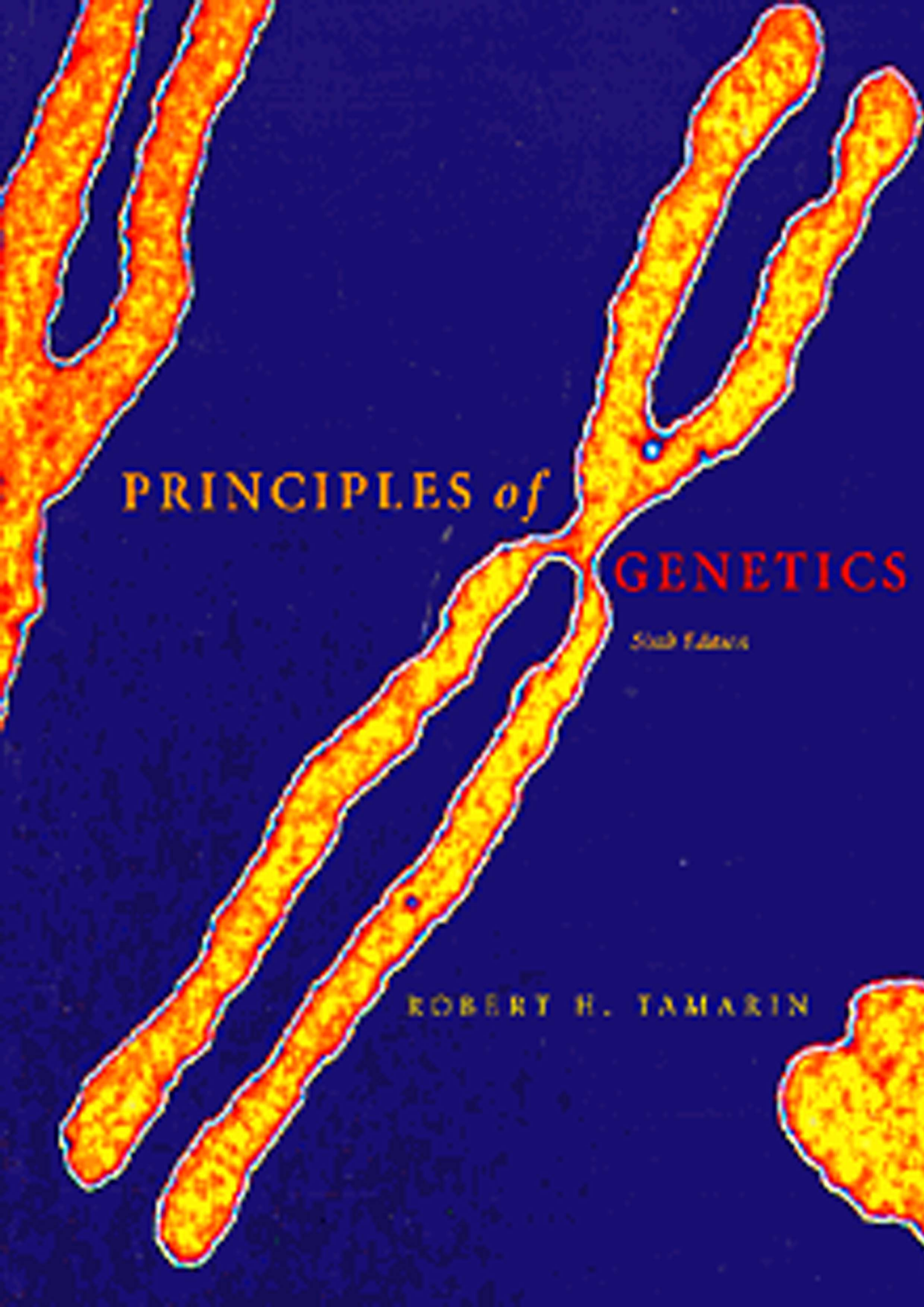
PRINCIPLES of
GENETICS
Sixth Edition
ROBERT H. TAMARIN

# 6

# LINKAGE AND MAPPING IN EUKARYOTES

## STUDY OBJECTIVES

1. To learn about analytical techniques for locating the relative positions of genes on chromosomes in diploid eukaryotic organisms 110
2. To learn about analytical techniques for locating the relative positions of genes on chromosomes in ascomycete fungi 122
3. To learn about analytical techniques for locating the relative positions of genes on human chromosomes 132

## STUDY OUTLINE



Scanning electron micrograph (false color) of a fruit fly, *Drosophila melanogaster.*

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After Sutton suggested the chromosomal theory of inheritance in 1903, evidence accumulated that genes were located on chromosomes. For example, Morgan showed by an analysis of inheritance patterns that the white-eye locus in Drosophila is located on the X chromosome. Given that any organism has many more genes than chromosomes, it follows that each chromosome has many loci. Since chromosomes in eukaryotes are linear, it also follows that genes are arranged in a linear fashion on chromosomes, like beads on a string. Sturtevant first demonstrated this in 1913. In this chapter, we look at analytical techniques for mapping chromosomes—techniques for determining the relationship between different genes on the same chromosome. These techniques are powerful tools that allow us to find out about the physical relationships of genes on chromosomes without ever having to see a gene or a chromosome. We determine that genes are on the same chromosome when the genes fail to undergo independent assortment, and then we use recombination frequencies to determine the distance between genes.

If loci were locked together permanently on a chromosome, allelic combinations would always be the same. However, at meiosis, crossing over allows the alleles of associated loci to show some measure of independence. A geneticist can use crossing over between loci to determine how close one locus actually is to another on a chromosome and thus to map an entire chromosome and eventually the entire **genome** (genetic complement) of an organism.

Loci carried on the same chromosome are said to be linked to each other. There are as many **linkage groups** ($l$) as there are autosomes in the haploid set plus sex chromosomes. *Drosophila* has five linkage groups ($2n$ = 8; $l$ = 3 autosomes + X + Y), whereas human beings have twenty-four linkage groups ($2n$ = 46; $l$ = 22 autosomes + X + Y). Prokaryotes and viruses, which usually have a single chromosome, are discussed in chapter 7.

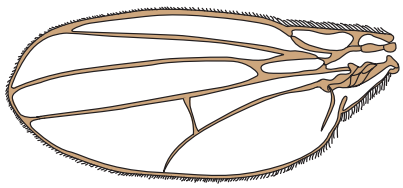
Historically, classical mapping techniques, as described in this chapter and the next, gave researchers their only tools to determine the relationships of particular genes and their chromosomes. When geneticists know the locations of specific genes, they can study them in relation to each other and begin to develop a comprehensive catalogue of the genome of an organism. Knowing the location of a gene also helps in isolating the gene and studying its function and structure. And mapping the genes of different types of organisms (diploid, haploid, eukaryotic, prokaryotic) gives geneticists insight into genetic processes. More recently, recombinant DNA technology has allowed researchers to sequence whole genomes, including the human and fruit fly genomes; this means they now know the exact locations of all the genes on all the chromosomes of these organisms (see chapter 13). Geneticists are now creating massive databases containing this information, much of it available for free or by subscription on the World Wide Web. Until investigators mine all this information for all organisms of interest, they will still use analytical techniques in the laboratory and field to locate genes on chromosomes.

## DIPLOID MAPPING

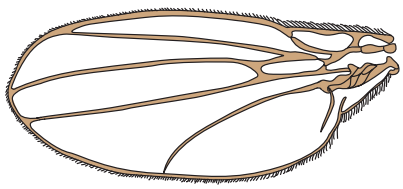
### *Two-Point Cross*

In *Drosophila,* the recessive band gene (*bn*) causes a dark transverse band on the thorax, and the detached gene (*det*) causes the crossveins of the wings to be either detached or absent (fig. 6.1). A banded fly was crossed with a detached fly to produce wild-type, dihybrid offspring in the $F_1$ generation. $F_1$ females were then testcrossed to banded, detached males (fig. 6.2). (There is no crossing over in male fruit flies; in experiments designed to detect linkage, heterozygous females—in which crossing over occurs—are usually crossed with homozygous recessive males.) If the loci were assorting independently, we would expect a 1:1:1:1 ratio of the four possible phenotypes. However, of the first one thousand offspring examined, experimenters recorded a ratio of 2:483: 512:3.

Several points emerge from the data in figure 6.2. First, no simple ratio is apparent. If we divide by two, we get a ratio of 1:241:256:1.5. Although the first and last categories seem about equal, as do the middle two, no simple numerical relation seems to exist between the middle and end categories. Second, the two cate-



Wild-type



Detached

**Figure 6.1** Wild-type ($det^+$) and detached (*det*) crossveins in *Drosophila*.

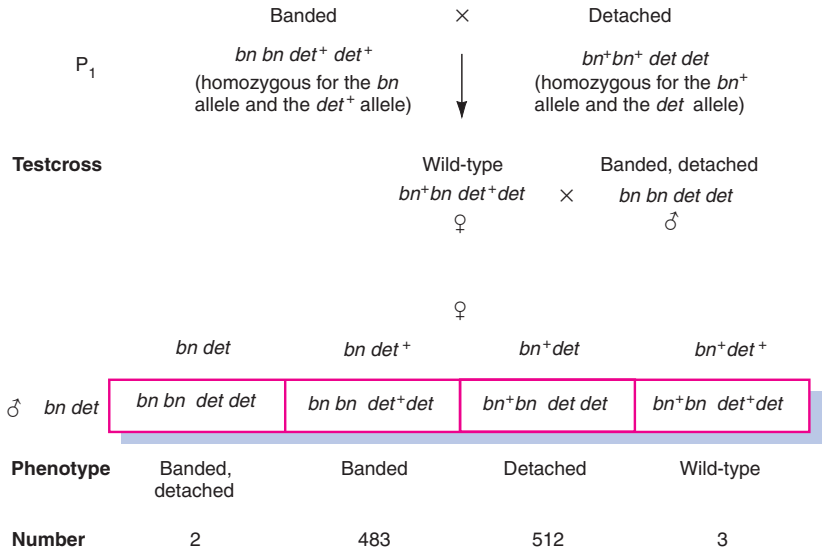



**Figure 6.2** Testcrossing a dihybrid *Drosophila.*

gories in very high frequency have the same phenotypes as the original parents in the cross ($P_1$ of fig. 6.2). That is, banded flies and detached flies were the original parents as well as the great majority of the testcross offspring. We call these phenotypic categories **parentals,** or **nonrecombinants.** On the other hand, the testcross offspring in low frequency combine the phenotypes of the two original parents ($P_1$). These two categories are referred to as **nonparentals,** or **recombinants.** The simplest explanation for these results is that the banded and detached loci are located near each other on the same chromosome (they are a linkage group), and therefore they move together as associated alleles during meiosis.

We can analyze the original cross by drawing the loci as points on a chromosome (fig. 6.3). This shows that 99.5% of the testcross offspring (the nonrecombinants) come about through the simple linkage of the two loci. The remaining 0.5% (the recombinants) must have arisen through a crossover of homologues, from a chiasma at meiosis, between the two loci (fig. 6.4). Note that since it is not possible to tell from these crosses which chromosome the loci are actually on or where the centromere is in relation to the loci, the centromeres are not included in the figures. The crossover event is viewed as a breakage and reunion of two chromatids lying adjacent to each other during prophase I of meiosis. Later in this chapter, we find cytological proof for this; in chapter 12, we explore the molecular mechanisms of this breakage and reunion process.

From the testcross in figure 6.3, we see that 99.5% of the gametes produced by the dihybrid are nonrecombinant, whereas only 0.5% are recombinant. This very small frequency of recombinant offspring indicates that the two loci lie very close to each other on their particular chromosome. In fact, we can use the recombination percentages of gametes, and therefore of testcross offspring, as estimates of distance between loci on a chromosome: 1% recombinant offspring is referred to as one **map unit** (or one **centimorgan,** in honor of geneticist T. H. Morgan, the first geneticist to win the Nobel Prize; box 6.1). Although a map unit is not a physical distance, it is a relative distance that makes it possible to know the order of and relative separation between loci on a chromosome. In this case, the two loci are 0.5 map units apart. (From sequencing various chromosomal segments—see chapter 13—we have learned that the relationship between centimorgans and DNA base pairs is highly variable, depending on species, sex, and region of the chromosome. For example, in human beings, 1 centimorgan can vary between 100,000 and 10,000,000 base pairs. In the fission yeast, *Schizosaccharomyces pombe,* 1 centimorgan is only about 6,000 base pairs.)

The arrangement of the *bn* and *det* alleles in the dihybrid of figure 6.3 is termed the **trans** configuration, meaning "across," because the two mutants are across from each other, as are the two wild-type alleles. The alternative arrangement, in which one chromosome carries both mutants and the other chromosome carries both wild-type alleles (fig. 6.5), is referred to as the **cis** configuration. (Two other terms, **repulsion** and **coupling,** have the same meanings as *trans* and *cis,* respectively.)

A cross involving two loci is usually referred to as a **two-point cross;** it gives us a powerful tool for dissecting the makeup of a chromosome. The next step in our

**BOX 6.1**

## Historical Perspectives

### *The Nobel Prize*

On 10 December each year, the king of Sweden awards the Nobel Prizes at the Stockholm Concert Hall. The date is the anniversary of Alfred Nobel's death. Awards are given annually in physics, chemistry, medicine and physiology, literature, economics, and peace. In 2000, each award was worth $900,000, although an award sometimes goes to two or three recipients. The prestige is priceless.

Winners of the Nobel Prize are chosen according to the will of Alfred Nobel, a wealthy Swedish inventor and industrialist, who held over three hundred patents when he died in 1896 at the age of sixty-three. Nobel developed a detonator and processes for detonation of nitroglycerine, a substance invented by Italian chemist Ascanio Sobrero in 1847. In the form Nobel developed, the explosive was patented as dynamite. Nobel also invented several other forms of explosives. He was a benefactor of Sobrero, hiring him as a consultant and paying his wife a pension after Sobrero died.

Nobel believed that dynamite would be so destructive that it would serve as a deterrent to war. Later, realizing that this would not come to pass, he instructed that his fortune be invested and the interest used to fund the awards. The first prizes were awarded in 1901. Each award consists of a diploma, medal, and check.

American, British, German, French, and Swedish citizens have earned the most prizes (table 1). Table 2 features some highlights of Nobel laureate achievements in genetics.


**The Nobel medal.** The medal is half a pound of 23-karat gold, measures about 2 1/2 inches across, and has Nobel's face and the dates of his birth and death on the front. The diplomas that accompany the awards are individually designed. (Reproduced by permission of the Nobel Foundation.)

**Table 1 Distribution of Nobel Awards to the Top Five Recipient Nations (Including 2000 Winners)**

| | Physics | Chemistry | Medicine and Physiology | Peace | Literature | Economics | Total |
|---|---|---|---|---|---|---|---|
| United States | 77 | 46 | 88 | 20 | 9 | 30 | 270 |
| Britain | 20 | 24 | 25 | 9 | 8 | 5 | 91 |
| Germany | 18 | 27 | 15 | 4 | 6 | 1 | 71 |
| France | 12 | 7 | 7 | 8 | 12 | 1 | 47 |
| Sweden | 4 | 4 | 7 | 5 | 7 | 2 | 29 |

**Table 2** Some Nobel Laureates in Genetics (Medicine and Physiology; Chemistry)

| Name | Year | Nationality | Cited for |
|---|---|---|---|
| Thomas Hunt Morgan | 1933 | USA | Discovery of how chromosomes govern heredity |
| Hermann J. Muller | 1946 | USA | X-ray inducement of mutations |
| George W. Beadle | 1958 | USA | Genetic regulation of biosynthetic pathways |
| Edward L. Tatum | 1958 | USA | |
| Joshua Lederberg | 1958 | USA | Bacterial genetics |
| Severo Ochoa | 1959 | USA | Discovery of enzymes that synthesize nucleic acids |
| Arthur Kornberg | 1959 | USA | |
| Francis H. C. Crick | 1962 | British | Discovery of the structure of DNA |
| James D. Watson | 1962 | USA | |
| Maurice Wilkins | 1962 | British | |
| François Jacob | 1965 | French | Regulation of enzyme biosynthesis |
| André Lwoff | 1965 | French | |
| Jacques Monod | 1965 | French | |
| Peyton Rous | 1966 | USA | Tumor viruses |
| Robert W. Holley | 1968 | USA | Unraveling of the genetic code |
| H. Gobind Khorana | 1968 | USA | |
| Marshall W. Nirenberg | 1968 | USA | |
| Max Delbrück | 1969 | USA | Viral genetics |
| Alfred Hershey | 1969 | USA | |
| Salvador Luria | 1969 | USA | |
| Renato Dulbecco | 1975 | USA | Tumor viruses |
| Howard Temin | 1975 | USA | Discovery of reverse transcriptase |
| David Baltimore | 1975 | USA | |
| Werner Arber | 1978 | Swiss | Discovery and use of restriction endonucleases |
| Hamilton Smith | 1978 | USA | |
| Daniel Nathans | 1978 | USA | |
| Walter Gilbert | 1980 | USA | Techniques of sequencing DNA |
| Frederick Sanger | 1980 | British | |
| Paul Berg | 1980 | USA | Pioneer work in recombinant DNA |
| Baruj Benacerraf | 1980 | USA | Genetics of immune reactions |
| Jean Dausset | 1980 | French | |
| George Snell | 1980 | USA | |
| Aaron Klug | 1982 | British | Crystallographic work on protein-nucleic acid complexes |
| Barbara McClintock | 1983 | USA | Transposable genetic elements |
| Cesar Milstein | 1984 | British/Argentine | Immunogenetics |
| Georges Koehler | 1984 | German | |
| Niels K. Jerne | 1984 | British/Danish | |
| Susumu Tonegawa | 1987 | Japanese | Antibody diversity |
| J. Michael Bishop | 1989 | USA | Proto-oncogenes |
| Harold E. Varmus | 1989 | USA | |
| Thomas R. Cech | 1989 | USA | Enzymatic properties of RNA |
| Sidney Altman | 1989 | Canada | |
| Kary Mullis | 1993 | USA | Polymerase chain reaction |
| Michael Smith | 1993 | Canada | Site-directed mutagenesis |
| Richard Roberts | 1993 | British | Discovery of intervening sequences in RNA |
| Phillip Sharp | 1993 | USA | |
| E. B. Lewis | 1995 | USA | Genes control development |

*continued*

**BOX 6.1 CONTINUED**

**Table 2** *continued*

| Name | Year | Nationality | Cited for |
|---|---|---|---|
| Christiane Nüsslein-Volhard | 1995 | German | |
| Eric Wieschaus | 1995 | USA | |
| Stanley B. Prusiner | 1997 | USA | Discovery of prions |
| Günter Blobel | 1999 | German | Signal recognition during protein synthesis |

analysis is to look at three loci simultaneously so that we can determine their relative order on the chromosome. More important, we can also analyze the effects of multiple crossovers, which cannot be detected in a two-point cross, on map distances. Two crossovers between two loci can cause the chromosome to look as if no crossovers took place, causing us to underestimate map distances. Thus we need a third locus, between the first two, to detect multiple crossover events.

## *Three-Point Cross*

Analysis of three loci, each segregating two alleles, is referred to as a **three-point cross.** We will examine wing morphology, body color, and eye color in *Drosophila.* Black body (*b*), purple eyes (*pr*), and curved wings (*c*) are all recessive genes. Since the most efficient way to study linkage is through the testcross of a multihybrid, we will study these three loci by means of the crosses shown in

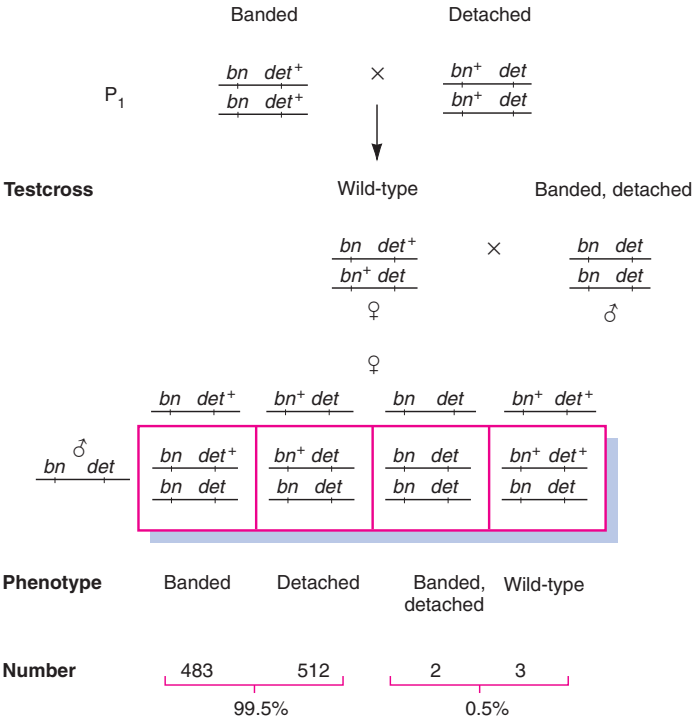



**Figure 6.3** Chromosomal arrangement of the two loci in the crosses of figure 6.2. A line arbitrarily represents the chromosomes on which these loci are actually situated.

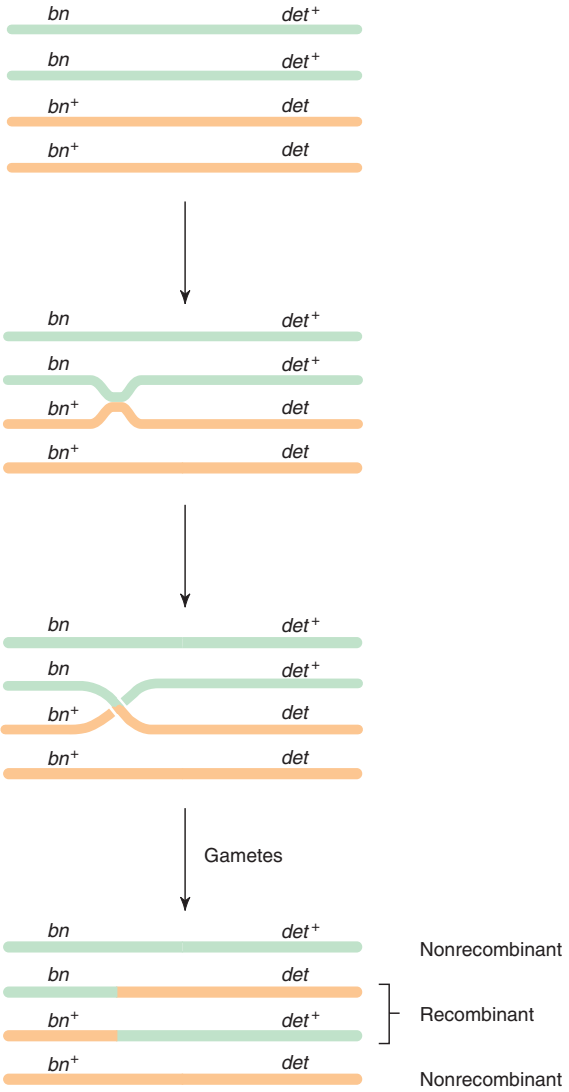



**Figure 6.4** Crossover of homologues during meiosis between the *bn* and *det* loci in the tetrad of the dihybrid female.

figure 6.6. One point in this figure should be clarified. Since the organisms are diploid, they have two alleles at each locus. Geneticists use various means to present this situation. For example, the recessive homozygote can be pictured as

1. *bb prpr cc*
2. *b/b pr/pr c/c* or $\frac{b}{b}\ \frac{pr}{pr}\ \frac{c}{c}$
3. *b pr c/b pr c* or $\frac{b\ pr\ c}{b\ pr\ c}$

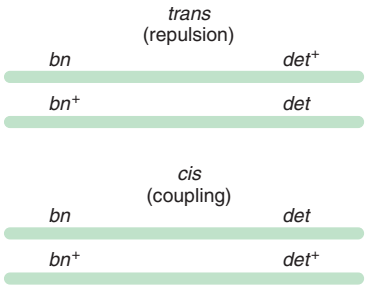



**Figure 6.5** *Trans* (repulsion) and *cis* (coupling) arrangements of dihybrid chromosomes.

A slash (also called a rule line) is used to separate alleles on homologous chromosomes. Thus (*1*) is used tentatively, when we do not know the linkage arrangement of the loci, (*2*) is used to indicate that the three loci are on different chromosomes, and (*3*) indicates that all three loci are on the same chromosome.

In figure 6.6, the trihybrid organism is testcrossed. If independent assortment is at work, the eight types of resulting gametes should appear with equal frequencies, and thus the eight phenotypic classes would each make up one-eighth of the offspring. However, if there were *complete linkage,* so that the loci are so close together on the same chromosome that virtually no crossing over takes place, we would expect the trihybrid to produce only two gamete types in equal frequency and to yield two phenotypic classes identical to the original parents. This would occur because, under complete linkage, the trihybrid would produce only two chromosomal types in gametes: the *b pr c* type from one parent and the $b^+$ $pr^+$ $c^+$ type from the other. Crossing over between linked loci would produce eight phenotypic classes in various proportions depending on the distances between loci. The actual data appear in table 6.1.

The data in the table are arranged in reciprocal classes. Two classes are reciprocal if between them they contain each mutant phenotype just once. Wild-type and black, purple, curved classes are thus reciprocal, as are the purple, curved and the black classes. Reciprocal classes occur in roughly equal numbers: 5,701 and 5,617; 388 and 367; 1,412 and 1,383; and 60 and 72. As we shall see, a single meiotic recombinational event produces reciprocal classes. Wild-type and black, purple, curved are the two nonrecombinant classes. The purple, curved class of 388 is grouped with the black class of 367. These two would be the products of a crossover between the *b* and the *pr* loci if we assume that the three loci are linked and that the gene order is *b pr c* (fig. 6.7). The next two classes, of 1,412 and 1,383 flies, would result from a crossover between *pr* and *c,* and the last set, 60 and 72, would result from two crossovers, one between *b* and *pr* and the other between

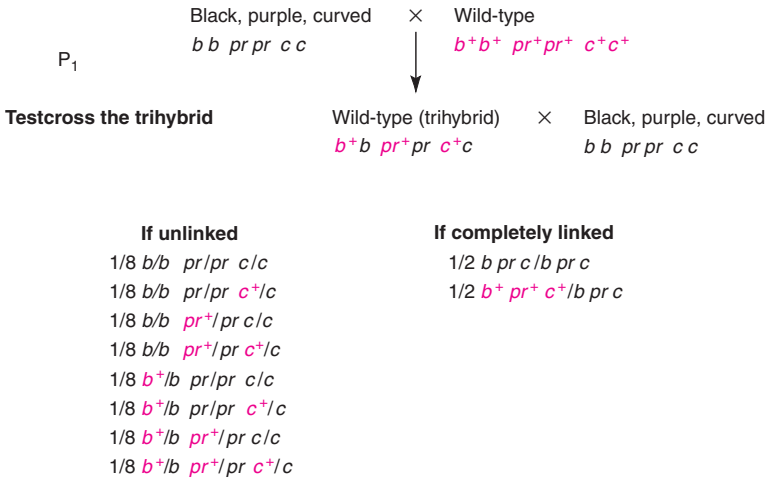



**Figure 6.6** Possible results in the testcross progeny of the *b pr c* trihybrid.

*pr* and *c* (fig. 6.8). Groupings according to these recombinant events are shown at the right in table 6.1.

In the final column of table 6.1, recombination between *b* and *c* is scored. Only those recombinant classes that have a new arrangement of *b* and *c* alleles, as compared with the parentals, are counted. This last column shows us what a *b–c,* two-point cross would have revealed had we been unaware of the *pr* locus in the middle.

### *Map Distances*

The percent row in table 6.1 reveals that 5.9% (887/15,000) of the offspring in the *Drosophila* trihybrid testcross resulted from recombination between *b* and *pr,* 19.5% between *pr* and *c,* and 23.7% between *b* and *c.* These numbers allow us to form a tentative map of the loci (fig. 6.9). There is, however, a discrepancy. The distance between *b* and *c* can be calculated in two ways. By adding the two distances, *b–pr* and *pr–c,* we get 5.9 + 19.5 = 25.4 map units; yet by directly counting the recombinants (the last column of table 6.1), we get a distance of only 23.7 map units. What causes this discrepancy of 1.7 map units?

Returning to the last column of table 6.1, we observe that the double crossovers (60 and 72) are not counted, yet each actually represents two crossovers in this re-

**Table 6.1** **Results of Testcrossing Female *Drosophila* Heterozygous for Black Body Color, Purple Eye Color, and Curved Wings ($b^+b\ pr^+pr\ c^+c \times bb\ prpr\ cc$)**

| Phenotype | Genotype | Number | Alleles from Trihybrid Female | Number Recombinant Between | | |
|---|---|---|---|---|---|---|
| | | | | *b* and *pr* | *pr* and *c* | *b* and *c* |
| Wild-type | $b^+b\ pr^+pr\ c^+\ c$ | 5,701 | $b^+\ pr^+\ c^+$ | | | |
| Black, purple, curved | *bb prpr cc* | 5,617 | *b pr c* | | | |
| Purple, curved | $b^+b\ prpr\ cc$ | 388 | $b^+\ pr\ c$ | 388 | | 388 |
| Black | $bb\ pr^+pr\ c^+c$ | 367 | $b\ pr^+\ c^+$ | 367 | | 367 |
| Curved | $b^+b\ pr^+pr\ cc$ | 1,412 | $b^+\ pr^+\ c$ | | 1,412 | 1,412 |
| Black, purple | $bb\ prpr\ c^+c$ | 1,383 | $b\ pr\ c^+$ | | 1,383 | 1,383 |
| Purple | $b^+b\ prpr\ c^+c$ | 60 | $b^+pr\ c^+$ | 60 | 60 | |
| Black, curved | $bb\ pr^+pr\ cc$ | 72 | $b\ pr^+\ c$ | 72 | 72 | |
| Total | | 15,000 | | 887 | 2,927 | 3,550 |
| Percent | | | | 5.9 | 19.5 | 23.7 |

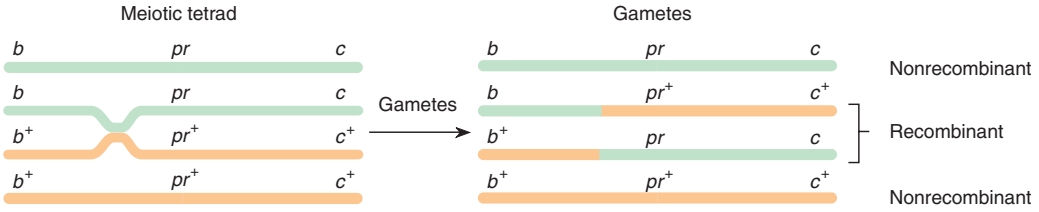



**Figure 6.7** Results of a crossover between the black and purple loci in *Drosophila*.

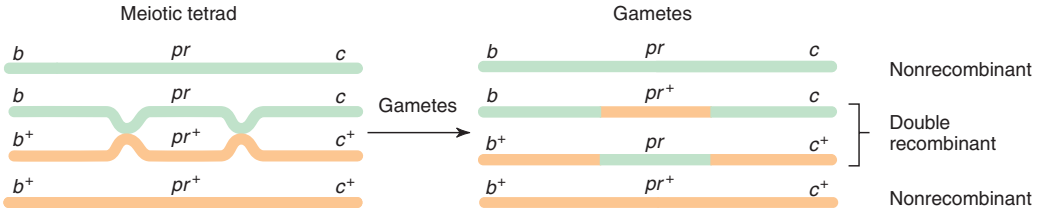



**Figure 6.8** Results of a double crossover in the *b pr c region of the Drosophila* chromosome.

gion. The reason they are not counted is simply that if we observed only the end loci of this chromosomal segment, we would not detect the double crossovers; the first one of the two crossovers causes a recombination between the two end loci, whereas the second one returns these outer loci to their original configuration (see fig. 6.8). If we took the 3,550 recombinants between *b* and *c* and added in twice the total of the double recombinants, 264, we would get a total of 3,814. This is 25.4 map units, which is the more precise figure we calculated before. The farther two loci are apart on a chromosome, the more double crossovers occur between them. Double crossovers tend to mask recombinants, as in our example, so that distantly linked loci usually appear closer than they really are. Thus, the most accurate map distances are those established on very closely linked loci. In other words, summed short distances are more accurate than directly measured larger distances.

The results of the previous experiment show that we can obtain at least two map distances between any two loci: measured and actual. Measured map distance between two loci is the value obtained from a two-point cross. Actual map distance is an idealized, more accurate value obtained from summing short distances between many intervening loci. We obtain the short distances from crosses involving other loci between the original two. When we plot measured map distance against actual map distance, we obtain the curve in figure 6.10. This curve is called a **mapping function.** This graph is of both practical and theoretical value. Pragmatically, it allows

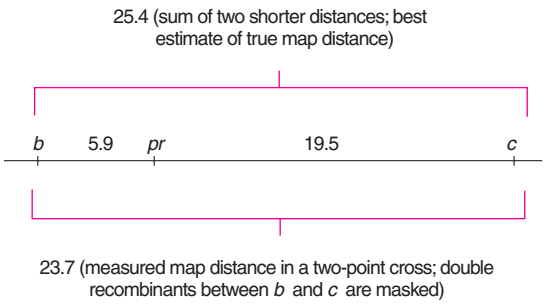



**Figure 6.9** Tentative map of the black, purple, and curved chromosome in *Drosophila*. Numbers are map units (centimorgans).

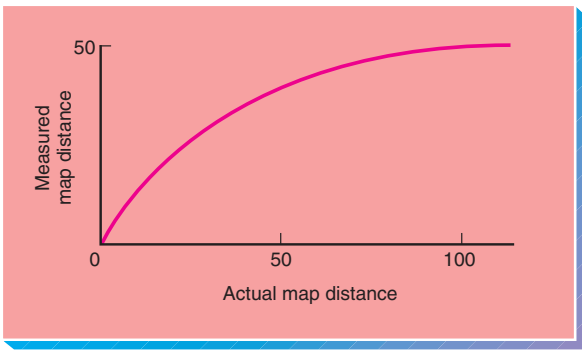



**Figure 6.10** Mapping function.

us to convert a measured map distance into a more accurate one. Theoretically, it shows that measured map distance never exceeds 50 map units in any one cross. Multiple crossovers reduce the apparent distance between two loci to a maximum of 50 map units, the value that independent assortment produces (50% parentals, 50% recombinants).

### *Gene Order*

Although we performed the previous analysis merely assuming that *pr* was in the middle, the data in table 6.1 confirm our original assumption that the gene order is *b pr c.* Of the four pairs of reciprocal phenotypic classes in table 6.1, one pair has the highest frequency (5,701 and 5,617) and one pair has the lowest (60 and 72). The pair with highest frequency is the nonrecombinant group. The one with the lowest frequency is the double recombinant group, the one in which only the middle locus has been changed from the parental arrangement. A comparison of either of the double recombinant classes with either of the nonrecombinant classes shows the gene that is in the middle and, therefore, the gene order. In other words, the data allow us to determine gene order. Since $b^+$ $pr^+$ $c^+$ was one of the nonrecombinant gametes, and $b^+$ *pr* $c^+$ was one of the double recombinant gametes, *pr* stands out as the changed locus, or the one in the middle. In a similar manner, comparing *b* $pr^+$ *c* with *b pr c* would also point to *pr* as the inside locus (or **inside marker**). So would comparing $b^+$ *pr* $c^+$ with *b pr c* or *b* $pr^+$ *c* with $b^+$ *pr* $c^+$. In each case, the middle locus, *pr,* displays the different pattern, whereas the allelic arrangements of the outside markers, *b* and *c,* behave in concert.

If this seems confusing, simply compare the double crossovers and nonrecombinants to find one of each in which two alleles are identical. For example, the double recombinant $b^+$ *pr* $c^+$ and the nonrecombinant $b^+$ $pr^+$ $c^+$ share the $b^+$ and $c^+$ alleles. The *pr* locus is mutant in one case and wild-type in the other. Hence, *pr* is the locus in the middle.

From the data in table 6.1, we can confirm the association of alleles in the trihybrid parent. That is, since the data came from testcrossing a trihybrid, the allelic configuration in that trihybrid is reflected in the nonrecombinant classes of offspring. In this case, one is the result of a $b^+$ $pr^+$ $c^+$ gamete, the other, of a *b pr c* gamete. Thus, the trihybrid had the genotype *b pr c*/$b^+$ $pr^+$ $c^+$: all alleles were in the *cis* configuration.

### *Coefficient of Coincidence*

The next question in our analysis of this three-point cross is, are crossovers occurring independently of each other? That is, does the observed number of double recombinants equal the expected number? In the example, there were 132/15,000 double crossovers, or 0.88%. The expected number is based on the independent occurrence of crossing over in the two regions measured. That is, 5.9% of the time there is a crossover in the *b–pr* region, which we can express as a probability of occurrence of 0.059. Similarly, 19.5% of the time there is a crossover in the *pr–c* region, or a probability of occurrence of 0.195. A double crossover should occur as a product of the two probabilities: $0.059 \times 0.195 = 0.0115$. This means that 1.15% of the gametes (1.15% of 15,000 = 172.5) should be double recombinants. In our example, the observed number of double recombinant offspring is lower than expected (132 observed, 172.5 expected). This implies a **positive interference,** in which the occurrence of the first crossover reduces the chance of the second. We can express this as a **coefficient of coincidence,** defined as

$$\frac{\text{observed number of double recombinants}}{\text{expected number of double recombinants}}$$

In the example, the coefficient of coincidence is $132/172.5 = 0.77$. In other words, only 77% of the expected double crossovers occurred. Sometimes we express this reduced quantity of double crossovers as the *degree of interference,* defined as

$$\text{interference} = 1 - \text{coefficient of coincidence}$$

In our example, the interference is 23%.

It is also possible to have **negative interference,** in which we observe more double recombinants than expected. In this situation, the occurrence of one crossover seems to enhance the probability that crossovers will occur in adjacent regions.

### *Another Example*

Let us work out one more three-point cross, in which neither the middle gene nor the *cis-trans* relationship of the alleles in the trihybrid $F_1$ parent is given. On the third chromosome of *Drosophila,* hairy (*h*) causes extra bristles on the body, thread (*th*) causes a thread-shaped arista (antenna tip), and rosy (*ry*) causes the eyes to be reddish brown. All three traits are recessive. Trihybrid females were testcrossed; the phenotypes from one thousand offspring are listed in table 6.2. At this point, it is possible to use the data to determine the parental genotypes (the $P_1$ generation, assuming that they were homozygotes), the gene order, the map distances, and the coefficient of coincidence. The table presents the data in no particular order, as an experimenter might have recorded them. Phenotypes are tabulated and, from these, the genotypes can be reconstructed. Notice that the data can be put into the form found in table 6.1;

**Table 6.2** **Offspring from a Trihybrid ($h^+h\ ry^+ry\ th^+th$) Testcross ($x\ hh\ ryry\ thth$) in *Drosophila***

| Phenotype | Genotype (order unknown) | Number |
|---|---|---|
| Thread | $h^+ry^+th/h\ ry\ th$ | 359 |
| Rosy, thread | $h^+ry\ th/h\ ry\ th$ | 47 |
| Hairy, rosy, thread | $h\ ry\ th/h\ ry\ th$ | 4 |
| Hairy, thread | $h\ ry^+th/h\ ry\ th$ | 98 |
| Rosy | $h^+ry\ th^+/h\ ry\ th$ | 92 |
| Hairy, rosy | $h\ ry\ th^+/h\ ry\ th$ | 351 |
| Wild-type | $h^+ry^+th^+/h\ ry\ th$ | 6 |
| Hairy | $h\ ry^+th^+/h\ ry\ th$ | 43 |

**Table 6.3** **Data from Table 6.2 Arranged to Show Recombinant Regions**

| Trihybrid's Gamete | Number | *h–th* | *th–ry* | *h–ry* |
|---|---|---|---|---|
| $h^+th\ ry^+$ | 359 | | | |
| $h\ th^+ry$ | 351 | | | |
| $h\ th\ ry^+$ | 98 | 98 | | 98 |
| $h^+th^+ry$ | 92 | 92 | | 92 |
| $h^+th\ ry$ | 47 | | 47 | 47 |
| $h\ th^+ry^+$ | 43 | | 43 | 43 |
| $h\ th\ ry$ | 4 | 4 | 4 | |
| $h^+th^+ry^+$ | 6 | 6 | 6 | |
| Total | 1,000 | 200 | 100 | 280 |

we see a large reciprocal set (359 and 351), a small reciprocal set (4 and 6), and large and small intermediate sets (98 and 92, 47 and 43).

From the data presented, is it obvious that the three loci are linked? The pattern, as just mentioned, is identical to that of the previous example, in which the three loci were linked. (What pattern would appear if two of the loci were linked and one assorted independently? See problem 6 at the end of the chapter.) Next, what is the allelic arrangement in the trihybrid parent? The offspring with the parental, or nonrecombinant, arrangements are the reciprocal pair in highest frequency. Table 6.2 shows that thread and hairy, rosy offspring are the nonrecombinants. Thus, the nonrecombinant gametes of the trihybrid $F_1$ parent were *h ry* $th^+$ and $h^+$ $ry^+$ *th,* which is the allelic arrangement of the trihybrid with the actual order still unknown—*h ry* $th^+/h^+$ $ry^+$ *th.* (What were the genotypes of the parents of this trihybrid, assuming they were homozygotes?) Continuing, which gene is in the middle? From table 6.2, we know that *h ry th* and $h^+$ $ry^+$ $th^+$ are the double recombinant gametes of the trihybrid parent because they occur in such low numbers. Comparison of these chromosomes with either of the nonrecombinant chromosomes ($h^+$ $ry^+$ *th* or *h ry* $th^+$) shows that the thread (*th*) locus is in the middle. We now know that the original trihybrid had the following chromosomal composition: *h* $th^+$ *ry*/$h^+$ *th* $ry^+$. The *h* and *ry* alleles are in the *cis* configuration, with *th* in the *trans* configuration.

We can now compare the chromosome from the trihybrid in each of the eight offspring categories with the parental arrangement and determine the regions that had crossovers. Table 6.3 does this. We can see that the *h–th* distance is 20 map units, the *th–ry* distance is 10 map units, and the apparent *h–ry* distance is 28 map units (fig. 6.11). As in the earlier example, the *h–ry* discrepancy is from not counting the double crossovers twice each: 280 + 2(10) = 300, which is 30 map units and the more accurate figure. Last, we wish to know what the coefficient of coincidence is. The expected occurrence of double recombinants is 0.200 × 0.100 = 0.020, or 2%. Two percent of 1,000 = 20. Thus

$$\text{coefficient of coincidence} = \frac{\text{observed number of double recombinants}}{\text{expected number of double recombinants}} = 10/20 = 0.50$$

Only 50% of the expected double crossovers occurred.

Geneticists have mapped the chromosomes of many eukaryotic organisms from three-point crosses of this type—those of *Drosophila* are probably the most extensively studied. *Drosophila* and other species of flies have giant **polytene** salivary gland chromosomes, which arise as a result of **endomitosis.** In this process,

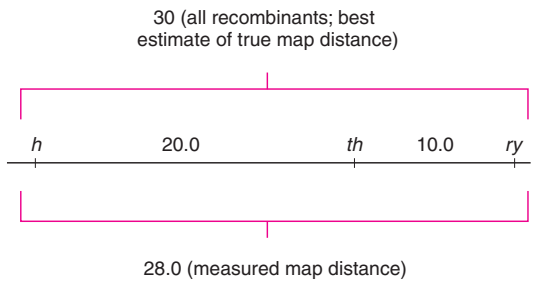



**Figure 6.11** Map of the *h th ry* region of the *Drosophila* chromosome, with numerical discrepancy in distances. Numbers are map units (centimorgans).

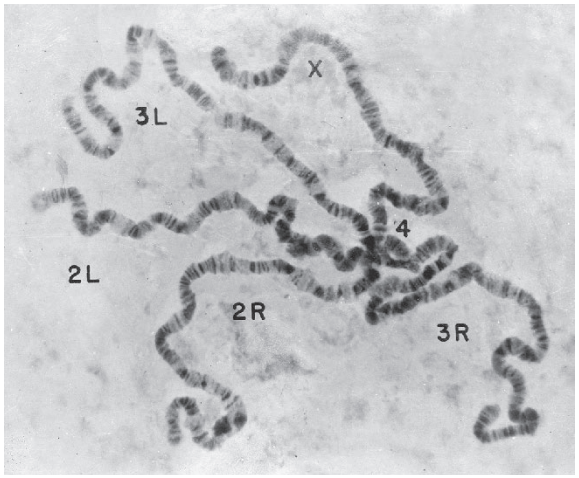



**Figure 6.12** Giant salivary gland chromosomes of *Drosophila.* X, 2, 3, and 4 are the four nonhomologous chromosomes. *L* and *R* indicate the *left* and *right* arms (in relation to the centromere). The dark bands are chromomeres. (B. P. Kaufman, "Induced Chromosome Rearrangements in *Drosophila melanogaster,*" *Journal of Heredity,* 30:178–90, 1939. Reproduced by permission of Oxford University Press.)

the chromosomes replicate, but the cell does not divide. In the salivary gland of the fruit fly, homologous chromosomes synapse and then replicate to make about one thousand copies, forming very thick structures with a distinctive pattern of bands called chromomeres (fig. 6.12). Using methods chapter 8 will discuss, scientists have mapped many loci to particular bands. Part of the *Drosophila* chromosomal map is presented in figure 6.13 (see also box 6.2). Locate the loci we have mapped so far to verify the map distances.

In summary, we know that two or more loci are linked if offspring do not fall into simple Mendelian ratios. Map distances are the percentage of recombinant offspring in a testcross. With three loci, determine the parental (nonrecombinant) and double recombinant groups first. Then establish the locus in the middle, and recast the data in the correct gene order. The most accurate map distances are those obtained by summing shorter distances. Determine a coefficient of coincidence by comparing observed number of double recombinants to expected number.

## *Cytological Demonstration of Crossing Over*

If we are correct that a chiasma during meiosis is the visible result of a physical crossover, then we should be able to demonstrate that genetic crossing over is accompanied by cytological crossing over. That is, the recombination event should entail the exchange of physical parts of homologous chromosomes. This can be demonstrated if we can distinguish between two homologous chromosomes, a technique Creighton and McClintock first used in maize (corn) and Stern first applied to *Drosophila,* both in 1931. We will look at Creighton and McClintock's experiment.

Harriet Creighton and Barbara McClintock worked with chromosome 9 in maize ($n = 10$). In one strain, they found a chromosome with abnormal ends. One end had a knob, and the other had an added piece of chromatin from another chromosome (fig. 6.14). This knobbed chromosome was thus clearly different from its normal homologue. It also carried the dominant colored (*C*) allele and the recessive waxy texture (*wx*) allele. After mapping studies showed that *C* was very close to the knob and *wx* was close to the added piece of chromatin, Creighton and McClintock made the cross shown in figure 6.14. The dihybrid plant with heteromorphic chromosomes was crossed with the normal homomorphic plant (only normal chromosomes) that had the genotype of *c Wx/c wx* (colorless and nonwaxy phenotype). If a crossover occurred during meiosis in the dihybrid in the region between *C* and *wx*, a physical crossover, visible cytologically (under the microscope), should also occur, causing the knob to become associated with an otherwise normal chromosome and the extra piece of chromosome 9 to be associated with a knobless chromosome. Four types of gametes would result (fig. 6.14).

Barbara McClintock (1902–1992). (Courtesy of Cold Spring Harbor Research Library Archives. Photographer, David Miklos.)

Harriet B. Creighton (1909– ). (Courtesy of Harriet B. Creighton.)

**BOX 6.2**

## Historical Perspectives

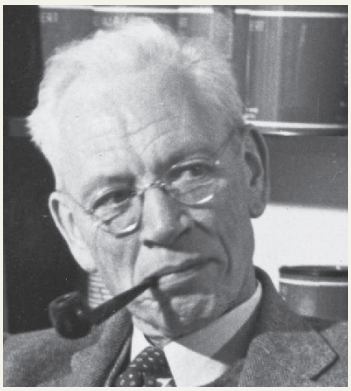
### *The First Chromosomal Map*

The first chromosomal map ever published included just five loci on the X chromosome of *Drosophila melanogaster* (fig. 1). It was published in 1913 by Alfred H. Sturtevant, who began working in Thomas Hunt Morgan's "fly lab" while an undergraduate student at Columbia University. The fly lab included H. J. Muller, later to win a Nobel Prize, and Calvin B. Bridges, whose work on sex determination in *Drosophila* we discussed in the last chapter.

Sturtevant worked with six mutants: yellow body (*y*); white (*w*), eosin ($w^e$), and vermilion eyes (*v*); and miniature (*m*) and rudimentary wings (*r*). (White and eosin are actually allelic; Sturtevant found no crossing over between the two "loci.") Using crosses similar to the ones we outline in this chapter, he constructed the map shown in figure 1. The map distances we accept today are very similar to the ones he obtained.

Sturtevant's work was especially important at this point because his data supported several basic concepts, including the linear arrangement of genes, which argued for the placement of genes on chromosomes as the only linear structures in the nucleus. Sturtevant also pointed out crossover interference. His summary is clear and succinct:

> It has been found possible to arrange six sex-linked factors in *Drosophila* in a linear series, using the number of crossovers per one hundred cases as an index of the distance between any two factors. This scheme gives consistent results, in the main.
>
> A source of error in predicting the strength of association between untried factors is found in double crossing over. The occurrence of this phenomenon is demonstrated, and it is shown not to occur as often as would be expected from a purely mathematical point of view, but the conditions governing its frequency are as yet not worked out.
>
> These results . . . form a new argument in favor of the chromosome view of inheritance, since they strongly indicate that the factors investigated are arranged in a linear series, at least mathematically.



Alfred H. Sturtevant (1891–1970). (Courtesy of the Archives, California Institute of Technology.)

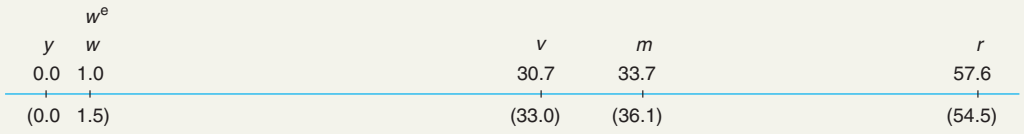



**Figure 1** The first chromosomal linkage map. Five loci in *Drosophila melanogaster* are mapped to the X chromosome. The numbers in parentheses are the more accurately mapped distances recognized today. We also show today's allelic designations rather than Sturtevant's original nomenclature. (Data from Sturtevant. "The linear arrangement of six sex-linked factors in *Drosophila,* as shown by their mode of association," *Journal of Experimental Zoology,* 14:43–59, 1913.)

Of twenty-eight offspring examined, all were consistent with the predictions of the Punnett square in figure 6.14. Those of class 8 (lower right box) with the colored, waxy phenotype all had a knobbed interchange chromosome as well as a normal homologue. Those with the colorless, waxy phenotype (class 4) had a knobless interchange chromosome. All of the colored, nonwaxy phenotypes (classes 5, 6, and 7) had a knobbed, normal chromosome, which indicated that only classes 5 and 6 were in the sample. Of the two that were tested, both were *WxWx,* indicating that they were of class 5. The remaining classes (1, 2, and 3) were of the colorless, nonwaxy phenotype. All were knobless. Of those that contained only normal chromosomes, some were

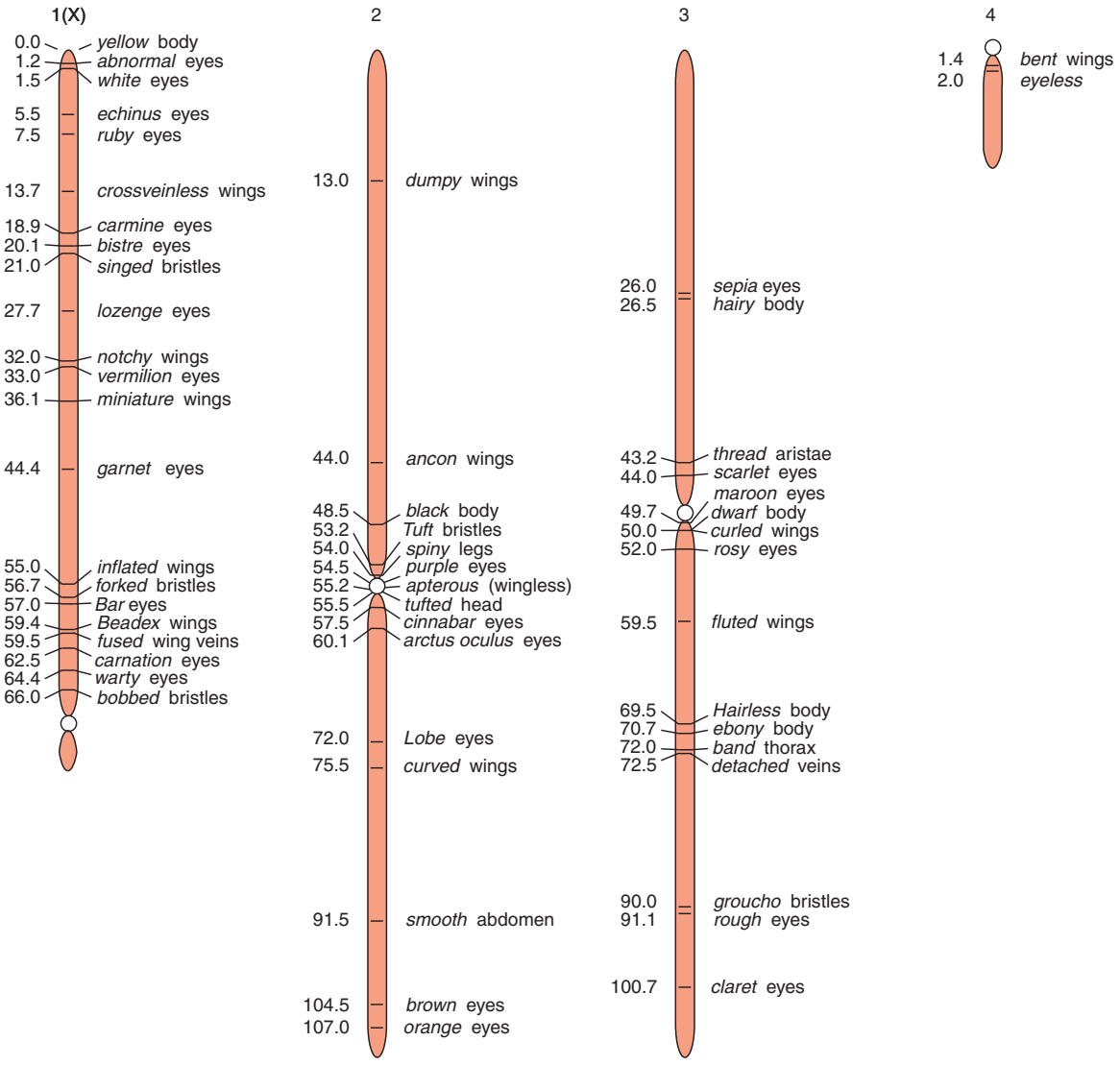



**Figure 6.13** Partial map of the chromosomes of *Drosophila melanogaster.* The centromere is marked by an open circle.
(From C. Bridges, "Salavary Chromosome Maps," *Journal of Heredity,* 26:60–64, 1935. Reprinted with permission of Oxford University Press.)

*WxWx* (class 1) and some were heterozygotes (*Wxwx*, class 2). Of those containing interchange chromosomes, two were heterozygous, representing class 3. Two were homozygous, *WxWx*, yet interchange-normal heteromorphs. These represent a crossover in the region between the waxy locus and the extra piece of chromatin, producing a knobless-*c*-*Wx*-extra-piece chromosome. When combined with a *c*-*Wx*-normal chromosome, these would give these anomalous genotypes. The sample size was not large enough to pick up the reciprocal event. Creighton and McClintock concluded: "Pairing chromosomes, heteromorphic in two regions, have been shown to exchange parts at the same time they exchange genes assigned to these regions."

## HAPLOID MAPPING (TETRAD ANALYSIS)

For *Drosophila* and other diploid eukaryotes, the genetic analysis considered earlier in this chapter is referred to as **random strand analysis.** Sperm cells, each of which carry only one chromatid of a meiotic tetrad, unite with

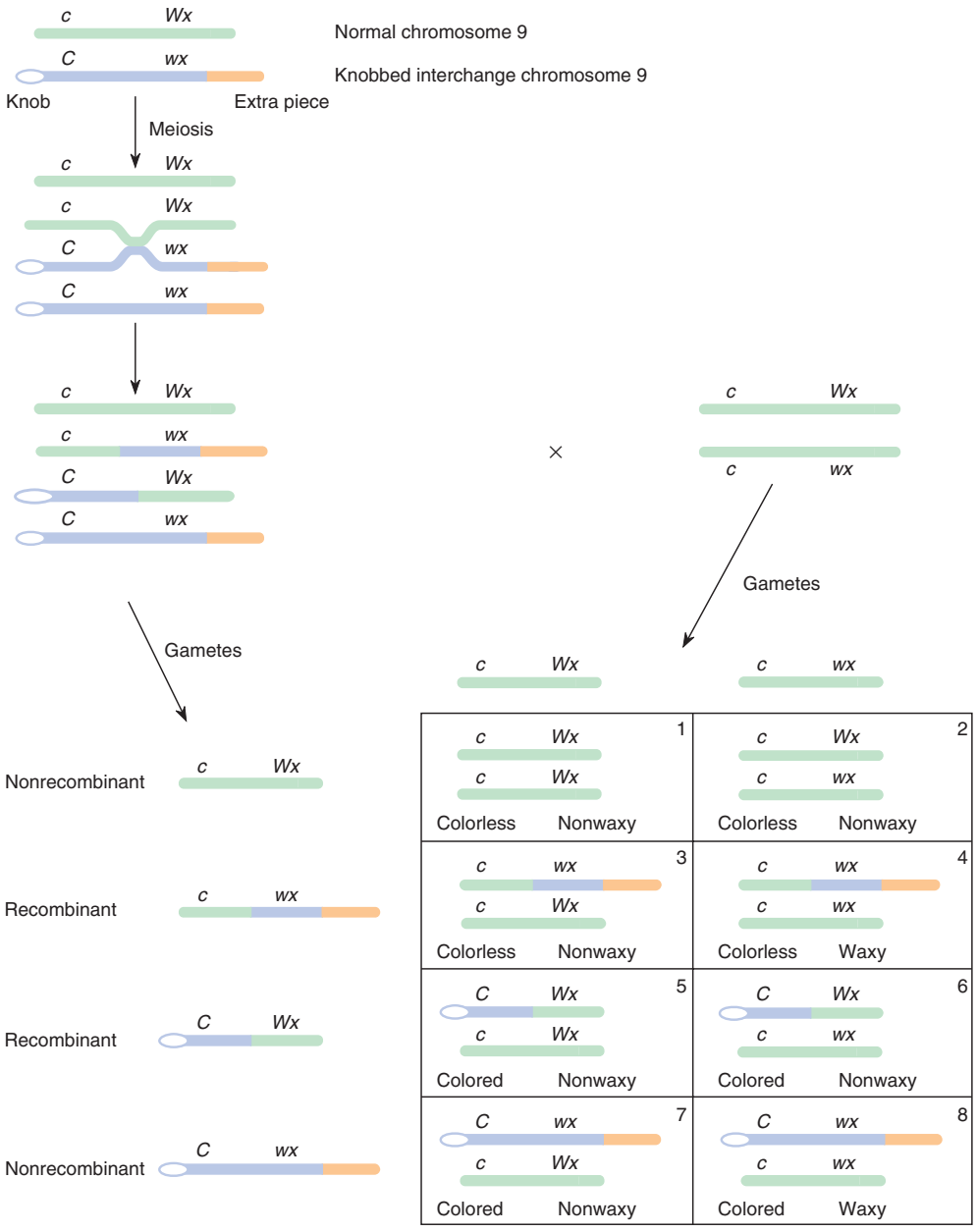



**Figure 6.14** Creighton and McClintock's experiment in maize demonstrated that genetic crossover correlates with cytological crossing over.

eggs, which also carry only one chromatid from a tetrad. Thus, zygotes are the result of the random uniting of chromatids.

Fungi of the class Ascomycetes retain the four haploid products of meiosis in a sac called an **ascus.** These organisms provide a unique opportunity to look at the total products of meiosis in a tetrad. Having the four products of meiosis allowed geneticists to determine such basics as the reciprocity of crossing over and the fact that DNA replication occurs before crossing over. Different techniques are used for these analyses. We will look at two fungi, the common baker's yeast, *Saccharomyces cerevisiae,* and pink bread mold, *Neurospora crassa,* both of which retain the products of meiosis as **ascospores.**

## *Phenotypes of Fungi*

At this point, you might wonder what phenotypes fungi such as yeast and *Neurospora* express. In general, microorganisms have phenotypes that fall into three broad categories: colony morphology, drug resistance, and nutritional requirements. Many microorganisms can be cultured in petri plates or test tubes that contain a supporting medium such as agar, to which various substances can be added (fig. 6.15). Wild-type *Neurospora,* the familiar pink bread mold, generally grows in a filamentous form, whereas yeast tends to form colonies. Various mutations exist that change colony morphology. In yeast, the *ade* gene causes the colonies to be red. In *Neurospora,* fluffy (*fl*), tuft (*tu*), dirty (*dir*), and colonial (*col4*) are all mutants of the basic growth form. In addition, wild-type *Neurospora* is sensitive to the sulfa drug sulfonamide, whereas one of its mutants (*Sfo*) actually requires sulfonamide in order to survive and grow. Yeast shows similar sensitivities to antifungal agents.

Nutritional-requirement phenotypes provide great insight not only into genetic analysis but also into the biochemical pathways of metabolism, as mentioned in chapter 2. Wild-type *Neurospora* can grow on a medium containing only sugar, a nitrogen source, some organic acids and salts, and the vitamin biotin. This is referred to as **minimal medium.** However, several different mutant types, or strains, of **Neurospora** cannot grow on this minimal medium until some essential nutrient is added. For example, one mutant strain will not grow on minimal medium, but will grow if one of the amino acids, arginine, is added (fig. 6.16). From this we can infer that the wild-type has a normal, functional enzyme in the synthetic pathway of arginine. The arginine-requiring mutant has an allele that specifies an enzyme that is incapable of converting one of the intermediates in the pathway directly into arginine or into one of the precursors to arginine. We can see that if the synthetic pathway is long, many different loci may have alleles that cause the strain to require arginine (fig. 6.17). This, in fact, happens, and the different loci are usually named $arg_1$, $arg_2$, and so on. There are numerous biosynthetic pathways in yeast and *Neurospora,* and mutants exhibit many different nutritional requirements. Mutants can be induced experimentally by radiation or by chemicals and other treatments. These, then, are the tools we use to analyze and map the chromosomes of microorganisms, including yeast and *Neurospora.* These techniques are expanded on in the next chapter.

## *Unordered Spores (Yeast)*

Baker's, or budding, yeast, *Saccharomyces cerevisiae,* exists in both a haploid and diploid form (fig. 6.18). The haploid form usually forms under nutritional stress (starvation). When better conditions return, haploid cells of the two sexes, called **a** and α **mating types,** fuse to form the diploid. (Mating types are generally the result of a one-locus, two-allele genetic system that determines that only opposite mating types can fuse. We discuss this system in more detail in chapter 16.) The haploid is again established by meiosis under starvation conditions. In yeast, all the products of meiosis are contained in the ascus. Let us look at a mapping problem, using the *a* and *b* loci for convenience.

When an *ab* spore (or gamete) fuses with an $a^+b^+$ spore (or gamete), and the diploid then undergoes meiosis, the spores can be isolated and grown as haploid colonies, which are then observed for the phenotypes the two loci control. Only three patterns can occur (table 6.4).

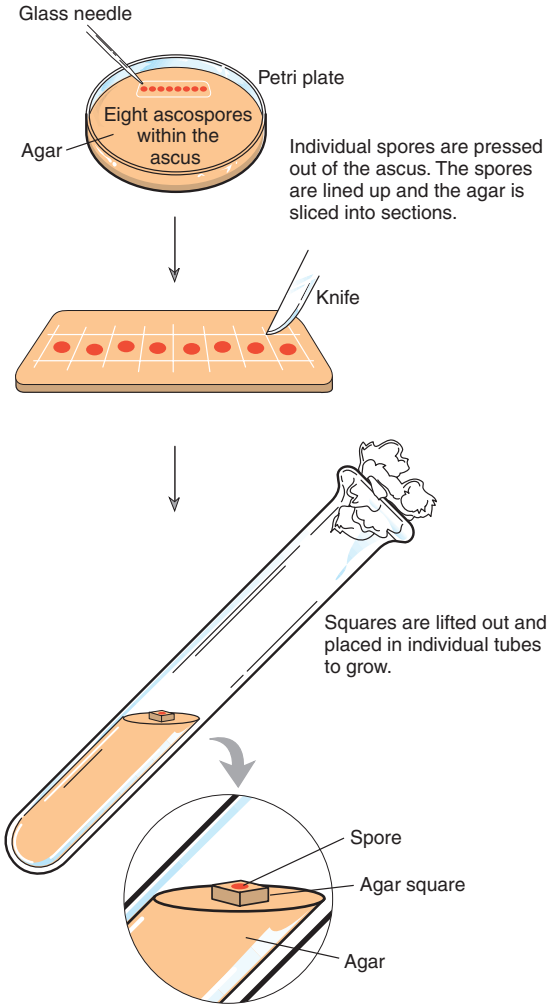



**Figure 6.15** Spore isolation technique in *Neurospora.*

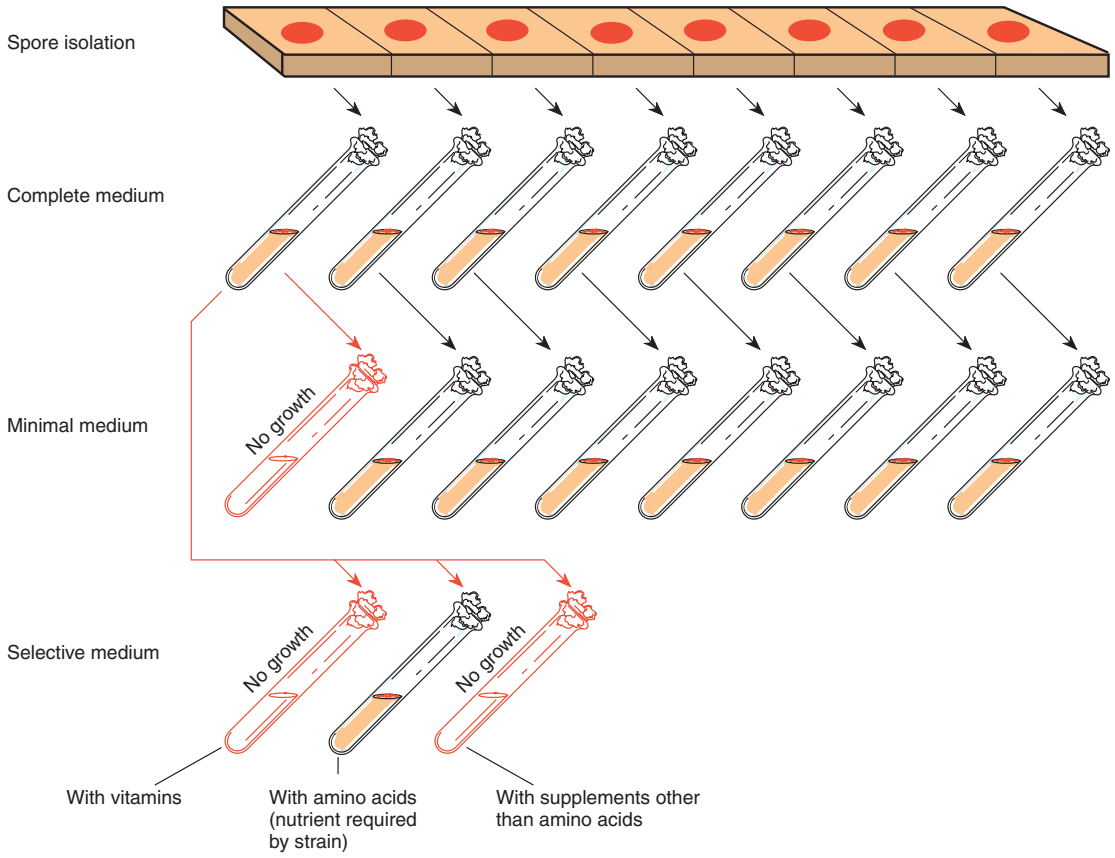



**Figure 6.16** Isolation of nutritional-requirement mutants in *Neurospora*.

Class 1 has two types of spores, which are identical to the parental haploid spores. This ascus type is, therefore, referred to as a **parental ditype (PD).** The second class also has only two spore types, but they are recombinants. This ascus type is referred to as a **nonparental ditype (NPD).** The third class has all four possible spore types and is referred to as a **tetratype (TT).**

All three ascus types can be generated whether or not the two loci are linked. As figure 6.19 shows, if the loci are linked, parental ditypes come from the lack of a crossover, whereas nonparental ditypes come about from four-strand double crossovers (double crossovers involving all four chromatids). We should thus expect parental ditypes to be more numerous than nonparental ditypes for linked loci. However, if the loci are not linked, both parental and nonparental ditypes come about through independent assortment—they should occur in equal frequencies. We can therefore determine whether the loci are linked by comparing parental ditypes and nonparental ditypes. In table 6.4, the parental ditypes greatly outnumber the nonparental ditypes; the two loci are, therefore, linked. What is the map distance between the loci?

A return to figure 6.19 shows that in a nonparental ditype, all four chromatids are recombinant, whereas in a tetratype, only half the chromatids are recombinant. Remembering that 1% recombinant offspring equals 1 map unit, we can use the following formula:

$$\text{map units} = \frac{(1/2)\text{ the number of TT asci} + \text{the number of NPD asci}}{\text{total number of asci}} \times 100$$

Thus, for the data of table 6.4,

$$\text{map units} = \frac{(1/2)20 + 5}{100} \times 100 = \frac{10 + 5}{100} \times 100 = 15$$

## *Ordered Spores* (Neurospora)

Unlike yeast, *Neurospora* has ordered spores; *Neurospora's* life cycle is shown in figure 6.20. Fertilization takes place within an immature fruiting body after a

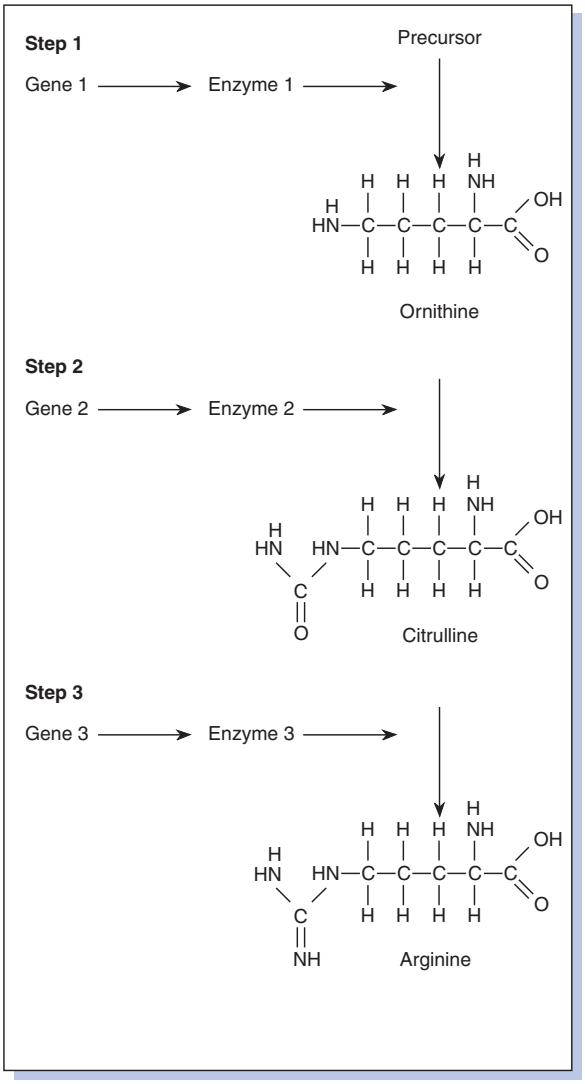



**Figure 6.17** Arginine biosynthetic pathway of *Neurospora.*

**Table 6.4** **The Three Ascus Types in Yeast Resulting from Meiosis in a Dihybrid, $aa^+bb^+$**

| 1 (PD) | 2 (NPD) | 3 (TT) |
|---|---|---|
| *ab* | $ab^+$ | *ab* |
| *ab* | $ab^+$ | $ab^+$ |
| $a^+b^+$ | $a^+b$ | $a^+b$ |
| $a^+b^+$ | $a^+b$ | $a^+b^+$ |
| 75 | 5 | 20 |

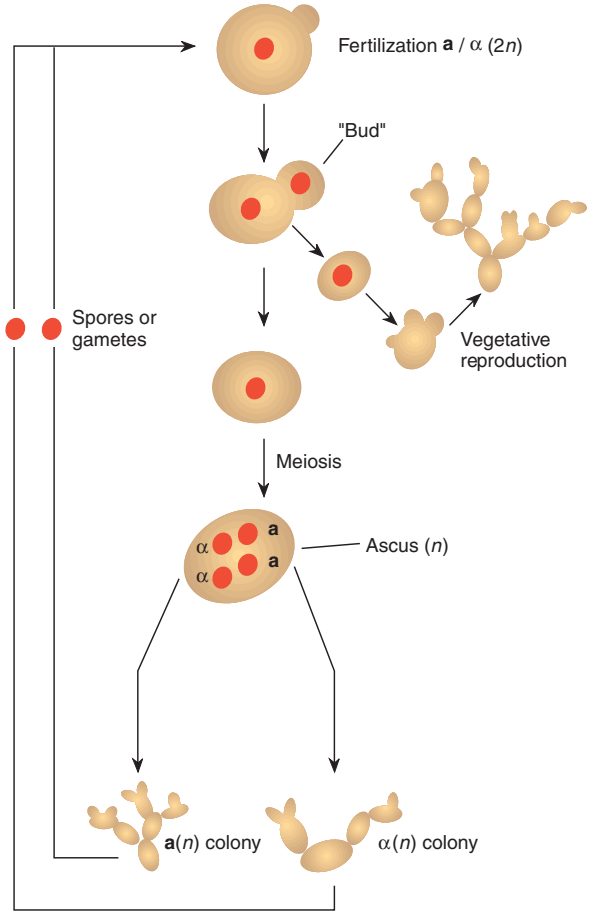



**Figure 6.18** Life cycle of yeast. Mature cells are mating types **a** or α; *n* is the haploid stage; 2*n* is diploid.

spore or filament of one mating type contacts a special filament extending from the fruiting body of the opposite mating type (mating types are referred to as *A* and *a*). The zygote's nucleus undergoes meiosis without any intervening mitosis. Unlike yeast, *Neurospora* does not have a diploid phase in its life cycle. Rather, it undergoes meiosis immediately after the diploid nuclei form.

Since the *Neurospora* ascus is narrow, the meiotic spindle is forced to lie along the cell's long axis. The two nuclei then undergo the second meiotic division, which is also oriented along the long axis of the ascus. The result is that the spores are ordered according to their centromeres (fig. 6.21). That is, if we label one centromere *A* and the other *a,* for the two mating types, a tetrad at meiosis I will consist of one *A* and one *a* centromere. At the end of meiosis in *Neurospora,* the four ascospores are in the order *A A a a* or *a a A A* in regard to centromeres. (We talk more simply of centromeres rather than chromosomes or chromatids because of the complications that

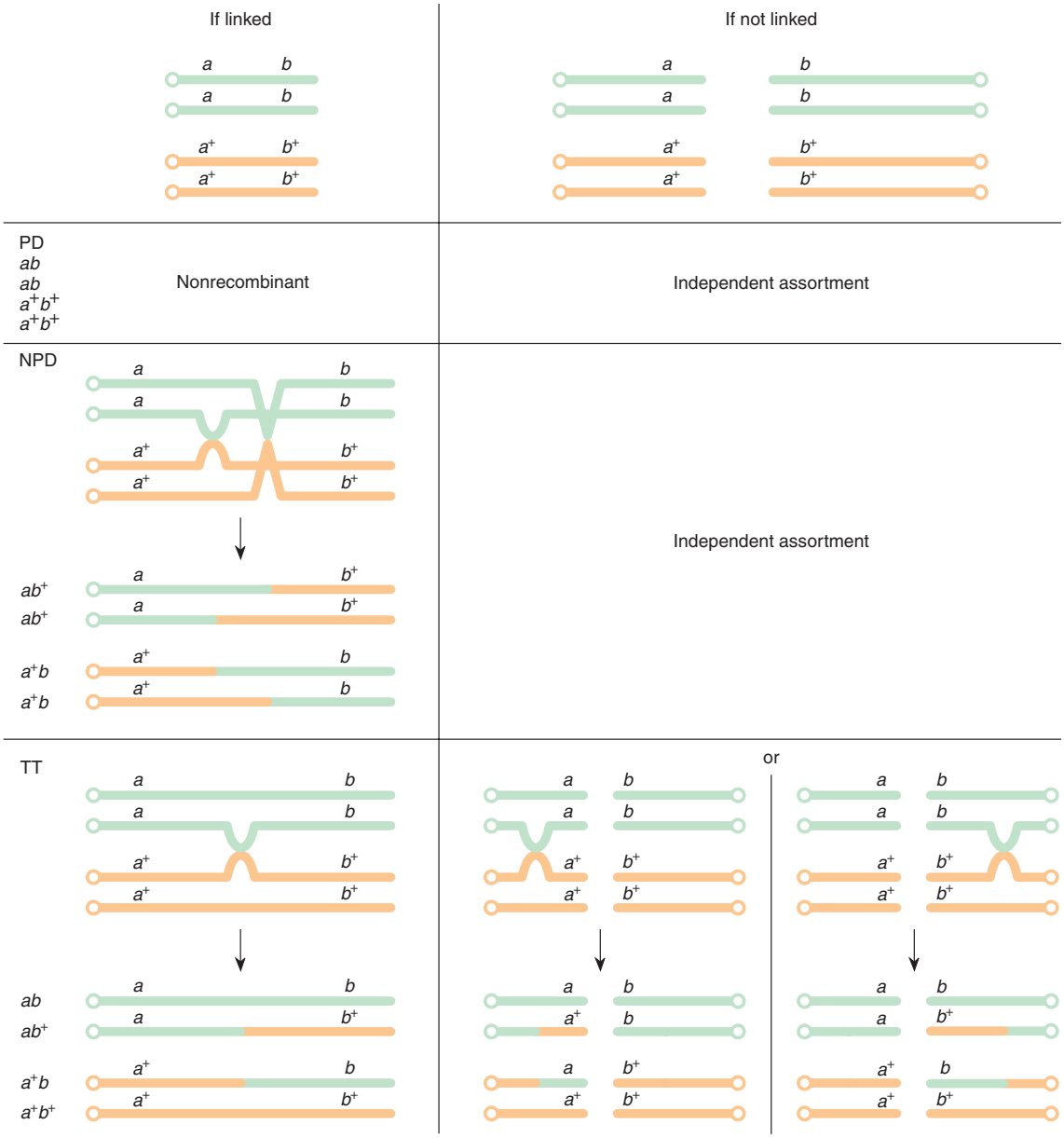



**Figure 6.19** Formation of parental ditype (PD), nonparental ditype (NPD), and tetratype (TT) asci in a dihybrid yeast by linkage or independent assortment at meiosis. Open circles are centromeres.

crossing over adds. A type *A* centromere is always a type *A* centromere, whereas, due to crossing over, a chromosome attached to that centromere may be partly from the type *A* parent and partly from the type *a* parent.)

Before the ascospores mature in *Neurospora,* a mitosis takes place in each nucleus so that four pairs rather than just four spores are formed. In the absence of phenomena such as mutation or gene conversion, to be discussed later in the book, pairs are always identical (fig. 6.21). As we will see in a moment, because of the ordered spores, we can map loci in *Neurospora* in relation to their centromeres.

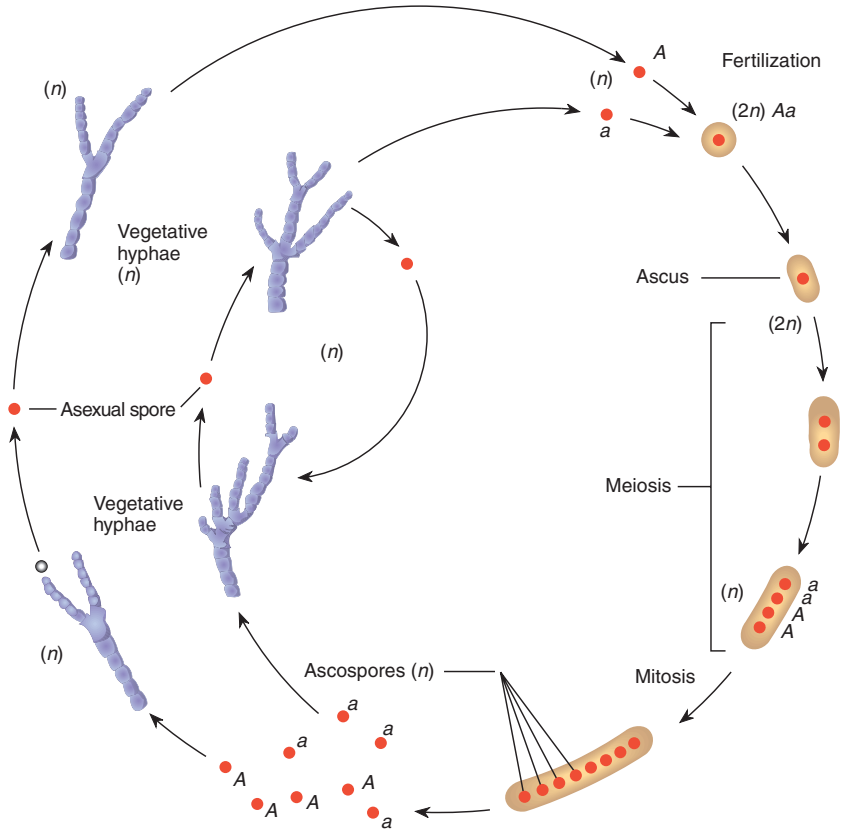



**Figure 6.20** Life cycle of *Neurospora*. *A* and *a* are mating types; *n* is a haploid stage; 2*n* is diploid.

### *First and Second Division Segregation*

Recall that there is a 4:4 segregation of the centromeres in the ascus of *Neurospora*. Two kinds of patterns appear among the loci on these chromosomes. These patterns depend on whether there was a crossover between the locus and its centromere (fig. 6.22). If there was no crossover between the locus and its centromere, the allelic pattern is the same as the centromeric pattern, which is referred to as **first-division segregation (FDS),** because the alleles separate from each other at meiosis I. If, however, a crossover has occurred between the locus and its centromere, patterns of a different type emerge (2:4:2 or 2:2:2:2), each of which is referred to as **second-division segregation (SDS).** Because the spores are ordered, the centromeres always follow a first-division segregation pattern. Hence, we should be able to map the distance of a locus to its centromere. Under the simplest circumstances (fig. 6.22), every second-division segregation configuration has four recombinant and four nonrecombinant chromatids (spores). Thus, half of the chromatids (spores) in a second-division segregation ascus are recombinant. Therefore, since 1% recombinant chromatids equal 1 map unit,

$$\text{map distance} = \frac{(1/2)\text{ the number of SDS asci}}{\text{total number of asci}} \times 100$$

An example using this calculation appears in table 6.5.

Three-point crosses in *Neurospora* can also be examined. Let us map two loci and their centromere. For simplicity, we will use the *a* and *b* loci. Dihybrids are formed from fused mycelia ($ab \times a^+b^+$), which then undergo meiosis. One thousand asci are analyzed, keeping the spore order intact. Before presenting the data, we should consider how to group them. Since each locus can show six different patterns (fig. 6.22), two loci scored together should give thirty-six possible spore arrangements ($6 \times 6$). Some thought, however, tells us that many of these patterns are really random variants of each other. The tetrad in meiosis is a three-dimensional entity rather than a flat, four-rod object, as it is usually drawn. At the first meiotic division, either centromere can go to the left *or* the right, and when centromeres split at the second mei-

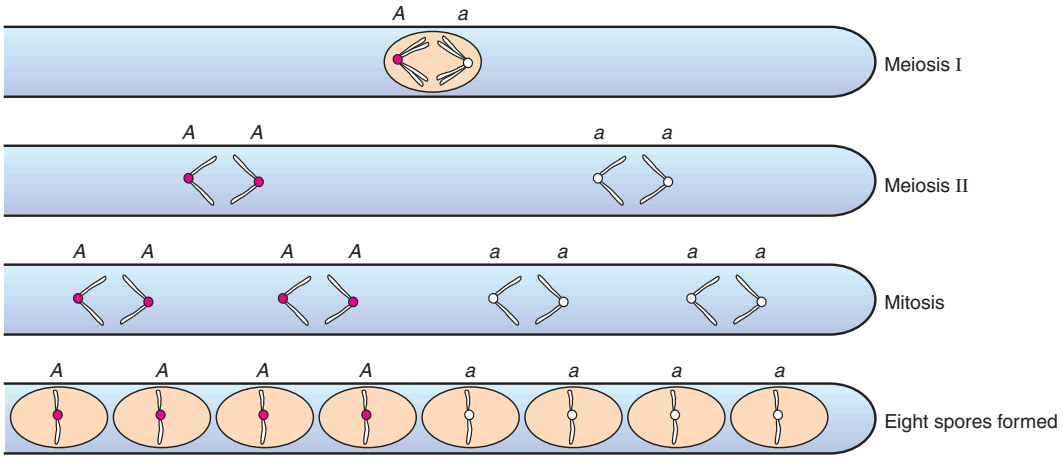



**Figure 6.21** Meiosis in *Neurospora.* Although *Neurospora* has seven pairs of chromosomes at meiosis, only one pair is shown. *A* and *a,* the two mating types, represent the two centromeres of the tetrad.

otic division, movement within the future half-ascus (the four spores to the left or the four spores to the right) is also random. Thus, one genetic event can produce up to eight "different" patterns. For example, consider the arrangements figure 6.23 shows, in which a crossover occurs between the *a* and *b* loci. All eight arrangements, producing the ascus patterns of table 6.6, are equally likely. The thirty-six possible patterns then reduce to only the seven unique patterns shown in table 6.7. Note also that these asci can be grouped into the three types of asci found in yeast with unordered spores: parental ditypes, nonparental ditypes, and tetratypes. Had we not had the order of the spores from the asci, that would, in fact, be the only way we could score the asci (see the bottom of table 6.7).

### *Gene Order*

We can now determine the distance from each locus to its centromere and the linkage arrangement of the loci if they are both linked to the same centromere. We can establish by inspection that the two loci are linked to each

**Table 6.5** **Genetic Patterns Following Meiosis in an $a^+a$ Heterozygous *Neurospora* (Ten Asci Examined)**

| Spore Number | Ascus Number | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| 1 | $a$ | $a$ | $a^+$ | $a$ | $a$ | $a^+$ | $a$ | $a^+$ | $a^+$ | $a^+$ |
| 2 | $a$ | $a$ | $a^+$ | $a$ | $a$ | $a^+$ | $a$ | $a^+$ | $a^+$ | $a^+$ |
| 3 | $a$ | $a$ | $a^+$ | $a^+$ | $a^+$ | $a^+$ | $a$ | $a$ | $a$ | $a^+$ |
| 4 | $a$ | $a$ | $a^+$ | $a^+$ | $a^+$ | $a^+$ | $a$ | $a$ | $a$ | $a^+$ |
| 5 | $a^+$ | $a^+$ | $a$ | $a^+$ | $a$ | $a$ | $a^+$ | $a$ | $a^+$ | $a$ |
| 6 | $a^+$ | $a^+$ | $a$ | $a^+$ | $a$ | $a$ | $a^+$ | $a$ | $a^+$ | $a$ |
| 7 | $a^+$ | $a^+$ | $a$ | $a$ | $a^+$ | $a$ | $a^+$ | $a^+$ | $a$ | $a$ |
| 8 | $a^+$ | $a^+$ | $a$ | $a$ | $a^+$ | $a$ | $a^+$ | $a^+$ | $a$ | $a$ |
| | FDS | FDS | FDS | SDS | SDS | FDS | FDS | SDS | SDS | FDS |

*Note:* Map distance (*a* locus to centromere) = (1/2)% SDS
= (1/2) 40%
= 20 map units

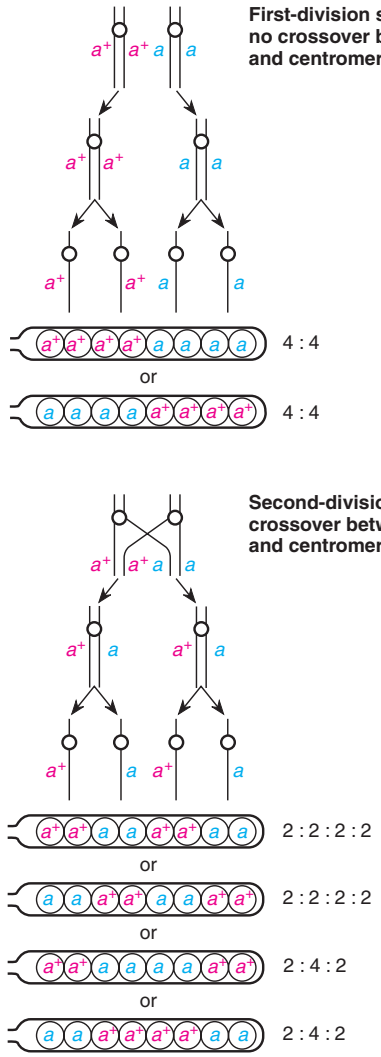



**Figure 6.22** The six possible *Neurospora* ascospore patterns in respect to one locus.

other—and therefore to the same centromere—by examining classes 1 (parental ditype) and 2 (nonparental ditype) in table 6.7. If the two loci are unlinked, these two categories would represent two equally likely alternative events when no crossover takes place. Since category 1 represents almost 75% of all the asci, we can be sure the two loci are linked.

To determine the distance of each locus to the centromere, we calculate one-half the percentage of second-division segregation patterns for each locus. For the *a* locus, classes 4, 5, 6, and 7 are second-division segregation patterns. For the *b* locus, classes 3, 5, 6, and 7 are second-division segregation patterns. Therefore,

**Table 6.6** **Eight of the Thirty-Six Possible Spore Patterns in *Neurospora* Scored for Two Loci, *a* and *b* (All Random Variants of the Same Genetic Event)**

| Spore Number | Ascus Number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| 1 | $ab$ | $ab^+$ | $ab$ | $ab^+$ | $a^+b^+$ | $a^+b^+$ | $a^+b$ | $a^+b$ |
| 2 | $ab$ | $ab^+$ | $ab$ | $ab^+$ | $a^+b^+$ | $a^+b^+$ | $a^+b$ | $a^+b$ |
| 3 | $ab^+$ | $ab$ | $ab^+$ | $ab$ | $a^+b$ | $a^+b$ | $a^+b^+$ | $a^+b^+$ |
| 4 | $ab^+$ | $ab$ | $ab^+$ | $ab$ | $a^+b$ | $a^+b$ | $a^+b^+$ | $a^+b^+$ |
| 5 | $a^+b$ | $a^+b$ | $a^+b^+$ | $a^+b^+$ | $ab^+$ | $ab$ | $ab^+$ | $ab$ |
| 6 | $a^+b$ | $a^+b$ | $a^+b^+$ | $a^+b^+$ | $ab^+$ | $ab$ | $ab^+$ | $ab$ |
| 7 | $a^+b^+$ | $a^+b^+$ | $a^+b$ | $a^+b$ | $ab$ | $ab^+$ | $ab$ | $ab^+$ |
| 8 | $a^+b^+$ | $a^+b^+$ | $a^+b$ | $a^+b$ | $ab$ | $ab^+$ | $ab$ | $ab^+$ |

**Table 6.7** **The Seven Unique Classes of Asci Resulting from Meiosis in a Dihybrid *Neurospora*, $ab/a^+b^+$**

| Spore Number | Ascus Number 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| 1 | $ab$ | $ab^+$ | $ab$ | $ab$ | $ab$ | $ab^+$ | $ab$ |
| 2 | $ab$ | $ab^+$ | $ab$ | $ab$ | $ab$ | $ab^+$ | $ab$ |
| 3 | $ab$ | $ab^+$ | $ab^+$ | $a^+b$ | $a^+b^+$ | $a^+b$ | $a^+b^+$ |
| 4 | $ab$ | $ab^+$ | $ab^+$ | $a^+b$ | $a^+b^+$ | $a^+b$ | $a^+b^+$ |
| 5 | $a^+b^+$ | $a^+b$ | $a^+b^+$ | $a^+b^+$ | $a^+b^+$ | $a^+b$ | $a^+b$ |
| 6 | $a^+b^+$ | $a^+b$ | $a^+b^+$ | $a^+b^+$ | $a^+b^+$ | $a^+b$ | $a^+b$ |
| 7 | $a^+b^+$ | $a^+b$ | $a^+b$ | $ab^+$ | $ab$ | $ab^+$ | $ab^+$ |
| 8 | $a^+b^+$ | $a^+b$ | $a^+b$ | $ab^+$ | $ab$ | $ab^+$ | $ab^+$ |
| | 729 | 2 | 101 | 9 | 150 | 1 | 8 |
| SDS for *a* locus | | | | 9 | 150 | 1 | 8 |
| SDS for *b* locus | | | 101 | | 150 | 1 | 8 |
| Unordered: | PD | NPD | TT | TT | PD | NPD | TT |

the distances to the centromere, in map units, for each locus are

$$\text{for locus } a\text{: } (1/2)\frac{9 + 150 + 1 + 8}{1{,}000} \times 100$$

$$= 8.4 \text{ centimorgans}$$

$$\text{for locus } b\text{: } (1/2)\frac{101 + 150 + 1 + 8}{1{,}000} \times 100$$

$$= 13.0 \text{ centimorgans}$$

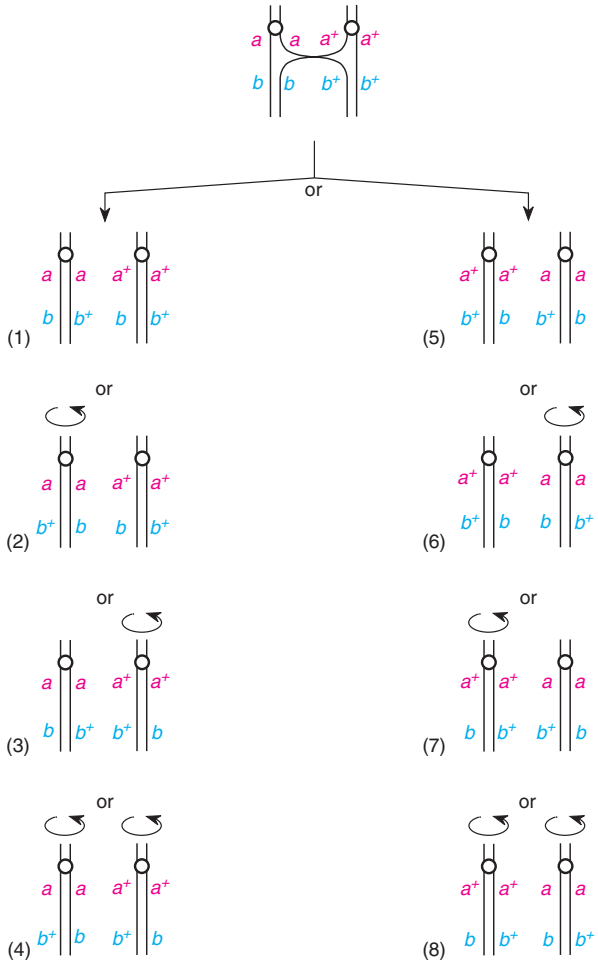



**Figure 6.23** The eight random arrangements possible when a single crossover occurs between the *a* and *b* loci in *Neurospora* (see table 6.6). *Circular arrows* represent the rotation of a centromere from its position in the original configuration.

It should now be possible to describe exactly what type of crossover event produced each of the seven classes in table 6.7.

Unfortunately, these two distances do not provide a unique determination of gene order. In figure 6.24, we see that two alternatives are possible: one has a map distance between the loci of 21.4 map units; the other has 4.6 map units between loci. How do we determine which of these is correct? The simplest way is to calculate the *a*–*b* distance using the unordered spore information. That is, the map distance is

$$\text{map units} = \frac{(1/2)\text{ the number of TT asci} + \text{the number of NPD asci}}{\text{total number of asci}} \times 100$$

$$= \frac{(1/2)118 + 3}{1{,}000} \times 100 = 6.2$$

Since 6.2 map units is much closer to the *a*–*b* distance expected if both loci are on the same side of the centromere, we accept alternative 2 in figure 6.24.

A second way to choose between the alternatives in figure 6.24 is to find out what happens to the *b* locus when a crossover occurs between the *a* locus and its centromere. If the order in alternative 1 is correct, crossovers between the *a* locus and its centromere should have no effect on the *b* locus; if 2 is correct, most of the crossovers that move the *a* locus in relation to its centromere should also move the *b* locus.

Asci classes 4, 5, 6, and 7 include all the SDS patterns for the *a* locus. Of 168 asci, 150 (class 5) have similar SDS patterns for the *b* locus. Thus, 89% of the time, a crossover between the *a* locus and its centromere is also a crossover between the *b* locus and its centromere—compelling evidence in favor of alternative 2. (What form would the data take if alternative 1 were correct?)

In summary, mapping by tetrad analysis proceeds as follows. For both ordered and unordered spores, linkage is indicated by an excess of parental ditypes over nonparental ditypes. For unordered spores (yeast), the distance between two loci is one-half the number of

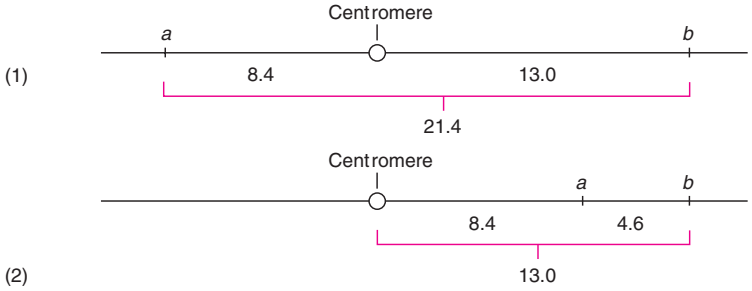



**Figure 6.24** Two possible arrangements of the *a* and *b* loci and their centromere. Distances are in map units.

tetratypes plus the number of nonparental ditypes, all divided by the total number of asci, expressed as a percentage. For ordered spores (*Neurospora*), the distance from a locus to its centromere is one-half the percentage of second-division segregants. Mapping the distance between two loci is similar to the process in unordered spores.

## SOMATIC (MITOTIC) CROSSING OVER

Crossing over is known to occur in somatic cells as well as during meiosis. It apparently occurs when two homologous chromatids come to lie next to each other and breakage and reunion follow, most likely as a consequence of DNA repair (see chapter 12). Unlike in meiosis, no synaptonemal complex forms. The occurrence of mitotic crossing over is relatively rare. In the fungus *Aspergillus nidulans,* mitotic crossing over occurs about once in every one hundred cell divisions.

Mitotic recombination was discovered in 1936 by Curt Stern, who noticed the occurrence of *twin spots* in fruit flies that were dihybrid for the yellow allele for body color (*y*) and the singed allele (*sn*) for bristle morphology (fig. 6.25). A twin spot could be explained by mitotic crossing over between the *sn* locus and its centromere (fig. 6.26). A crossover in the *sn–y* region would produce only a yellow spot, whereas a double crossover, one between *y* and *sn* and the other between *sn* and the centromere, would produce only a singed spot. (Verify this for yourself.) These three phenotypes were found in the relative frequencies expected. That is, given that the gene locations are drawn to scale in figure 6.26, we would expect double spots to be most common, followed by yellow spots, with singed spots rarest of all because they require a double crossover. This in fact occurred, and no other obvious explanation was consistent with these facts. Mitotic crossing over has been used in fungal genetics as a supplemental, or even a primary, method for determining linkage relations. Although gene orders are consistent between mitotic and meiotic mapping, relative distances are usually not, which is not totally unexpected. We know that neither meiotic nor mitotic crossing over is uniform along a chromosome. Apparently, the factors that cause deviation from uniformity differ in the two processes.

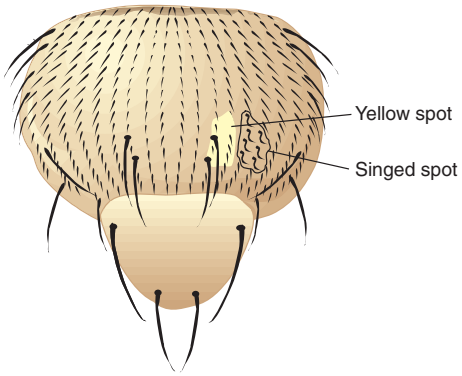
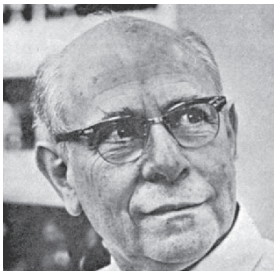



**Figure 6.25** Yellow and singed twin spots on the thorax of a female *Drosophila*.



Curt Stern (1902–1981)
(Courtesy of the Science Council of Japan.)

## HUMAN CHROMOSOMAL MAPS

In theory, we can map human chromosomes as we would those of any other organism. Realistically, the problems mentioned earlier (the inability to make specific crosses coupled with the relatively small number of offspring) make these techniques of human chromosome mapping very difficult. However, some progress has been made based on pedigrees, especially in assigning genes to the X chromosome. As the pedigree analysis in the previous chapter has shown, X chromosomal traits have unique patterns of inheritance, and loci on the X chromosome are easy to identify. Currently over four hundred loci are known to be on the X chromosome. It has been estimated, by several different methods, that between fifty and one hundred thousand loci exist on human chromosomes. In later chapters, we will discuss several additional methods of human chromosomal mapping that use molecular genetic techniques.

### *X Linkage*

After determining that a human gene is X linked, the next problem is to determine the position of the locus on the X chromosome and the map units between loci. Sometimes we can do this with the proper pedigrees, if crossing over can be ascertained. An example of this "grand-

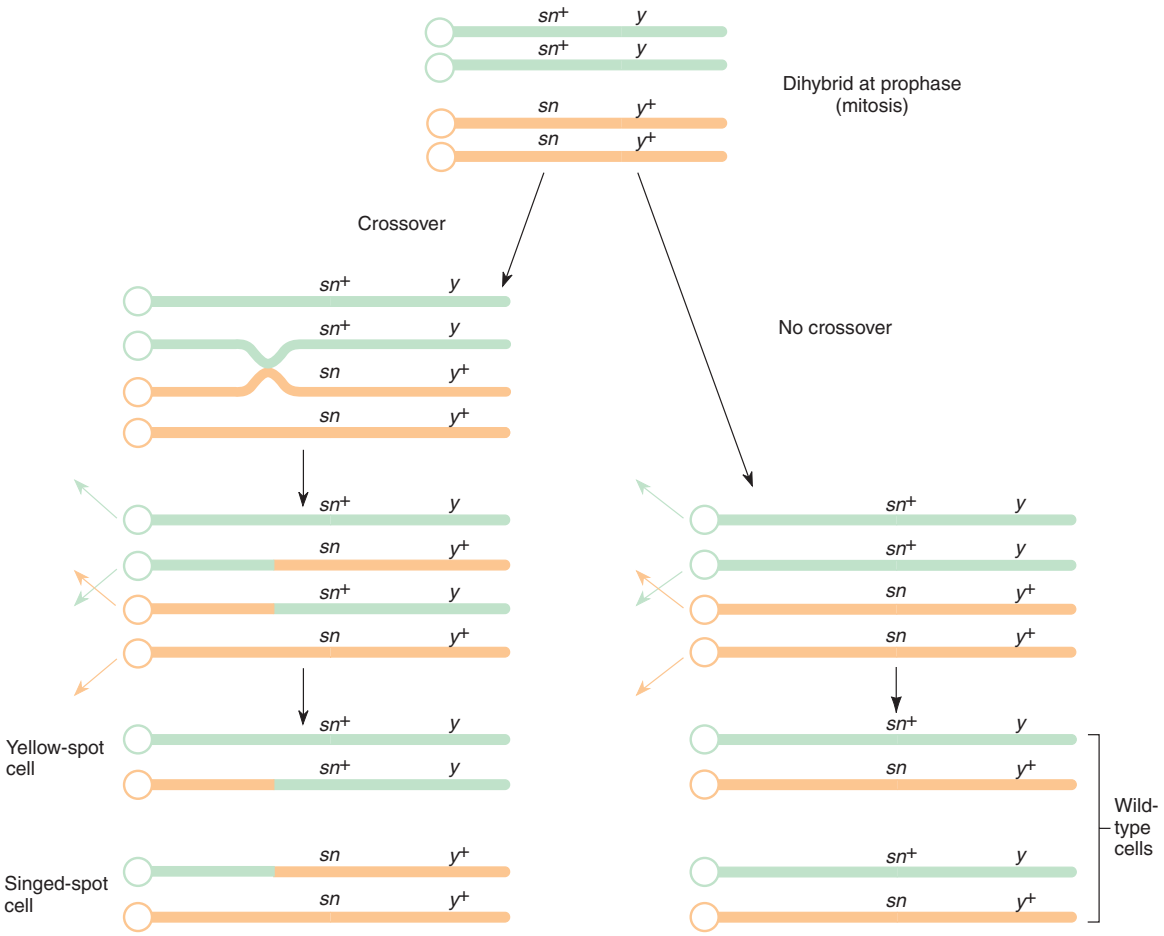



**Figure 6.26** Formation of twin spots by somatic crossing over.

father method" appears in figure 6.27. In this example, a grandfather has one of the traits in question (here, color blindness). We then find that he has a grandson who is glucose-6-phosphate dehydrogenase (G-6-PD) deficient. From this we can infer that the mother (of the grandson) was dihybrid for the two alleles in the *trans* configuration. That is, she received her color-blindness allele on one of her X chromosomes from her father, and she must have received the G-6-PD-deficiency allele on the other X chromosome from her mother (why?). Thus, the two sons on the left in figure 6.27 are nonrecombinant, and the two on the right are recombinant. Theoretically, we can determine map distance by simply totaling the recombinant grandsons and dividing by the total number of grandsons. Of course, the methodology would be the same if the grandfather were both color-blind and G-6-PD deficient. The mother would then be dihybrid in the *cis* configuration, and the sons would be tabulated in the reverse manner. The point is that the grandfather's phenotype gives us information that allows us to infer that the mother was dihybrid, as well as telling us the *cis-trans* arrangement of her alleles. We can then score her sons as either recombinant or nonrecombinant.

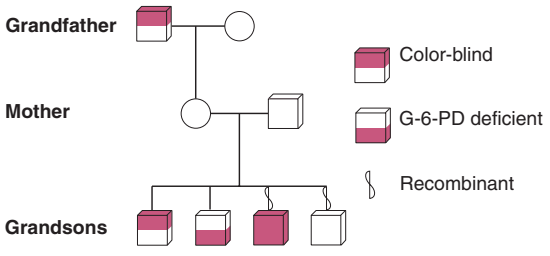



**Figure 6.27** "Grandfather method" of determining crossing over between loci on the human X chromosome. G-6-PD is glucose-6-phosphate dehydrogenase.

## *Autosomal Linkage*

From this we can see that it is relatively easy to map the X chromosome. The autosomes are another story. Since there are twenty-two autosomal linkage groups (twenty-two pairs of nonsex chromosomes), it is virtually impossible to determine from simple pedigrees which chromosome two loci are located on. Pedigrees can tell us if two loci are linked to each other, but not on which chromosome. In figure 6.28, the nail-patella syndrome includes, among other things, abnormal nail growth coupled with the absence or underdevelopment of kneecaps. It is a dominant trait. The male in generation II is dihybrid, with the *A* allele of the ABO blood type system associated with the nail-patella allele (*NPS1*) and the *B* allele with the normal nail-patella allele (*nps1*). Thus only one child in eight (III-5) is recombinant. Actually, the map distance is about 10%. In general, map distances appear greater in females than in males because more crossing over occurs in females (box 6.3).

We now turn our attention to the localization of loci to particular human chromosomes. The first locus that was definitely established to be on a particular autosome was the Duffy blood group on chromosome 1. This was ascertained in 1968 from a family that had a morphologically odd, or "uncoiled," chromosome 1. Inheritance in the Duffy blood group system followed the pattern of inheritance of the "uncoiled" chromosome. Real strides have been made since then. Two techniques, chromosomal banding and somatic-cell hybridization, have been crucial to autosomal mapping.

### *Chromosomal Banding*

Techniques were developed around 1970 that make use of certain histochemical stains that produce repeatable banding patterns on the chromosomes. For example, Giemsa staining is one such technique; the resulting bands are called **G-bands.** More detail on these techniques is presented in chapter 15. Before these techniques, human and other mammalian chromosomes were grouped into general size categories because of the difficulty of differentiating many of them. With banding techniques came the ability to identify each human chromosome in a karyotype (see fig. 5.1).

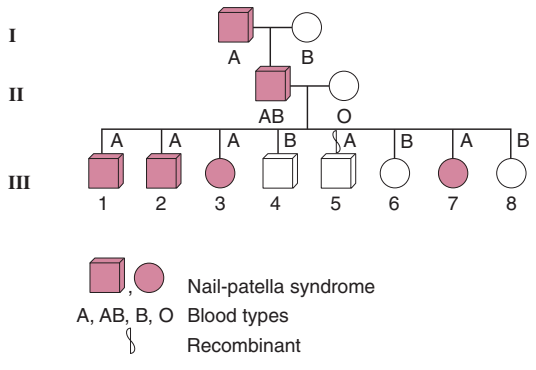



**Figure 6.28** Linkage of the nail-patella syndrome and ABO loci.

### *Somatic-Cell Hybridization*

The ability to distinguish each human chromosome is required to perform somatic-cell hybridization, in which human and mouse (or hamster) cells are fused in culture to form a hybrid. The fusion is usually mediated chemically with polyethylene glycol, which affects cell membranes; or with an inactivated virus, for example the Sendai virus, that is able to fuse to more than one cell at the same time. (The virus is able to do this because it has a lipid membrane derived from its host cells that easily fuses with new host cells. Because of this property, the virus can fuse to two cells close together, forming a cytoplasmic bridge between them that facilitates their fusion.) When two cells fuse, their nuclei are at first separate, forming a **heterokaryon,** a cell with nuclei from different sources. When the nuclei fuse, a hybrid cell is formed, and this hybrid tends to lose human chromosomes preferentially through succeeding generations. Upon stabilization, the result is a cell with one or more human chromosomes in addition to the original mouse or hamster chromosomal complement. Banding techniques allow the observer to recognize the human chromosomes. A geneticist looks for specific human phenotypes, such as enzyme products, and can then assign the phenotype to one of the human chromosomes in the cell line.

When cells are mixed together for hybridization, some cells do not hybridize. It is thus necessary to be able to select for study just those cells that are hybrids. One technique, originally devised by J. W. Littlefield in 1964, makes use of genetic differences in the way the cell lines synthesize DNA. Normally, in mammalian cells, aminopterin acts as an inhibitor of enzymes involved in DNA metabolism. Two enzymes, hypoxanthine phosphoribosyl transferase (HPRT) and thymidine kinase (TK), can bypass aminopterin inhibition by making use of secondary, or salvage, pathways in the cell. If hypoxanthine is provided, HPRT converts it to a purine, and if thymidine is provided, TK converts it to the nucleotide thymidylate. (Purines are converted to nucleotides and nucleotides are the subunits of DNA—see chapter 9.) Thus, normal cells in the absence of aminopterin synthesize DNA even if they lack HPRT activity ($HPRT^-$) or TK activity ($TK^-$). In the presence of aminopterin, $HPRT^-$ or $TK^-$ cells die. However, in the presence of aminopterin, $HPRT^+$ $TK^+$ cells can synthesize DNA and survive. Using this information, the following selection system was developed.

Mouse cells that have the phenotype of $HPRT^+$ $TK^-$ are mixed with human cells that have the phenotype of

**BOX 6.3**

## Experimental Methods

### Lod *Scores*

Human population geneticists can increase the accuracy of their linkage analysis by using a probability technique, developed by Newton Morton, called the ***lod* score method** (*Lo*g *Od*ds). The geneticist asks what the probability is of getting a particular pedigree assuming a particular recombination frequency (Θ), as compared with getting the same pedigree assuming independent assortment (Θ = 0.50). In other words, he or she calculates the ratio of the probability of genotypes in a family given a certain crossover frequency compared with the probability of those genotypes if the loci are unlinked. Logarithms are used for ease of calculation, and the parameter is called $z$, the *lod* score. Using this method, a researcher can try different crossover frequencies until the one giving the highest *lod* score is found.

For example, take the pedigree in figure 6.28. The father in generation II can have one of two allelic arrangements: *A NPS1/B nps1* or *A/B NPS1/nps1*. The former assumes linkage, whereas the latter does not. Our initial estimate of recombination, assuming linkage, was (1/8) × 100, or 12.5 map units. We now need to calculate the ratio of two probabilities:

$$z = \log \frac{\text{probability of birth sequence assuming 12.5 map units}}{\text{probability of birth sequence assuming independent assortment}}$$

Newton E. Morton (1929– ).
(Courtesy of Dr. Newton E. Morton.)

Assuming 12.5 map units (or a probability of 0.125 of a crossover; Θ = 0.125), the probability of child III-1 is 0.4375. This child would be a nonrecombinant, so his probability of having the nail-patella syndrome and type A blood is half the probability of no crossover during meiosis, or (1 − 0.125)/2. We divide by two because there are two nonrecombinant types. This is the same probability for all children except III-5, whose probability of occurrence is 0.125/2 = 0.0625, since he is a recombinant. Thus, the numerator of the previous equation is $(0.4375)^7(0.0625)$.

If the two loci are not linked, then any genotype has a probability of 1/4, or 0.25. Thus, the sequence of the eight children has the probability of $(0.25)^8$. This is the denominator of the equation. Thus,

$$z = \log \frac{(0.4375)^7(0.0625)}{(0.25)^8}$$

$$z = \log [12.566] = 1.099$$

Any *lod* score greater than zero favors linkage. A *lod* score less than zero suggests that Θ has been underestimated. A *lod* of 3.0 or greater ($10^3$ or one thousand times more likely than independent assortment) is considered a strong likelihood of linkage. Thus, in our example, we have an indication of linkage with a recombination frequency of 0.125. Now we can calculate *lod* scores assuming other values of recombination, as table 1 does. You can see that the recombination frequency as calculated, 0.125 (12.5 map units), gives the highest *lod* score.

**Table 1** ***Lod* Scores for the Cross in Figure 6.28**

| Recombination Frequency (Θ) | *Lod* Score |
|---|---|
| 0.05 | 0.951 |
| 0.10 | 1.088 |
| 0.125 | 1.099 |
| 0.15 | 1.090 |
| 0.20 | 1.031 |
| 0.25 | 0.932 |
| 0.30 | 0.801 |
| 0.35 | 0.643 |
| 0.40 | 0.457 |
| 0.45 | 0.244 |
| 0.50 | 0.000 |

$HPRT^-$ $TK^+$ in the presence of Sendai virus or polyethylene glycol. Fusion takes place in some of the cells, and the mixture is grown in a medium containing hypoxanthine, aminopterin, and thymidine (called **HAT medium**). In the presence of aminopterin, unfused mouse cells ($TK^-$) and unfused human cells ($HPRT^-$) die. Hybrid cells, however, survive because they are $HPRT^+$ $TK^+$. Eventually, the hybrid cells end up with random numbers of human chromosomes. There is one restriction: All cell lines selected are $TK^+$. This HAT method (using the HAT medium) not only selects for hybrid clones, but also localizes the *TK* gene to human chromosome 17, the one human chromosome found in every successful cell line.

**Table 6.8** **Assignment of the Gene for Blood Coagulating Factor III to Human Chromosome 1 Using Human-Mouse Hybrid Cell Lines**

| Hybrid Cell Line Designation | Tissue/ Factor Score | Human Chromosome Present | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | X |
| *WIL1* | − | − | − | − | − | − | − | − | + | − | − | − | − | − | + | − | − | + | − | − | − | + | − | + |
| *WIL6* | − | − | + | − | + | + | + | + | + | − | + | + | − | − | + | − | − | + | − | + | + | + | − | + |
| *WIL7* | − | − | + | + | − | + | + | − | + | − | + | + | − | + | + | − | − | + | + | − | − | + | − | + |
| *WIL14* | + | + | − | + | − | − | − | + | + | − | + | − | + | − | + | + | − | + | − | − | − | − | − | + |
| *SIR3* | + | + | + | + | + | + | + | + | − | + | + | + | + | + | − | − | + | + | + | + | + | + | + | + |
| *SIR8* | + | + | + | + | + | + | − | + | + | + | + | + | + | + | + | + | + | + | + | − | − | + | + | + |
| *SIR11* | − | − | − | − | − | − | − | + | − | − | − | − | − | + | − | − | − | − | − | − | − | + | + | + |
| *REW7* | + | + | + | + | + | + | + | + | + | − | + | + | + | + | + | + | − | + | + | + | + | + | + | + |
| *REW15* | + | + | + | + | + | + | + | + | + | − | + | − | + | + | + | + | − | + | + | + | + | + | + | + |
| *DUA1A* | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | * | − | − | − | − | − | − | − | * |
| *DUA1CsAzF* | − | − | − | − | − | − | − | + | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| *DUA1CsAzH* | − | − | − | − | − | − | − | + | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| *TSL1* | − | − | − | + | + | − | − | − | − | − | + | + | − | + | + | − | + | + | + | − | + | − | − | − |
| *TSL2* | − | − | + | * | − | + | + | − | − | − | + | − | + | − | − | − | − | * | + | − | + | + | − | + |
| *TSL2CsBF* | − | − | − | − | − | + | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| *XTR1* | + | + | − | * | − | + | + | + | + | + | + | + | + | + | + | − | − | + | + | + | + | + | + | + |
| *XTR2* | − | − | − | * | − | + | − | − | + | − | + | − | + | + | − | − | − | − | + | − | + | + | − | * |
| *XTR3BsAgE* | + | + | − | * | − | + | + | + | + | + | + | − | − | + | + | − | − | + | + | + | − | + | − | * |
| *XTR22* | − | − | + | * | + | + | + | − | + | − | + | + | − | − | − | + | − | − | + | + | + | + | + | * |
| *XER9* | − | − | + | − | + | − | − | − | + | − | + | * | + | − | + | − | − | + | + | − | − | + | − | * |
| *XER11* | + | + | − | + | + | − | + | + | + | − | + | * | + | + | − | + | + | + | + | + | + | + | + | * |
| *REX12* | − | − | − | + | − | − | − | + | − | − | − | + | − | − | + | − | − | − | − | − | − | − | + | * |
| *JSR29* | + | + | + | + | + | + | + | * | + | * | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| *JVR22* | + | + | + | + | + | + | + | + | + | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| *JWR22H* | + | * | * | − | + | − | + | − | − | − | + | + | + | − | + | + | − | + | + | − | + | + | − | − |
| *ALR2* | + | + | + | + | + | + | + | + | − | + | + | + | + | + | + | + | + | + | − | + | + | + | + | + |
| *ICL15* | − | − | − | − | − | − | − | − | − | − | − | − | + | − | − | − | − | + | − | − | + | + | − | − |
| *ICL15CsBF* | − | − | − | − | − | − | − | − | − | − | − | − | + | − | − | − | − | − | − | − | + | + | − | − |
| *MH21* | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | + | − | − |
| % Discord† | | 0 | 32 | 17 | 24 | 31 | 21 | 21 | 31 | 21 | 24 | 30 | 21 | 21 | 28 | 14 | 24 | 21 | 28 | 17 | 34 | 41 | 21 | 27 |

*Source:* Reprinted with permission from S.D. Carson, et al., "Tissue Factor Gene Localized to Human Chromosome 1 (after 1p21)," *Science*, 229:229–291. Copyright © 1985 American Association for the Advancement of Science.

* A translocation in which only part of the chromosome is present.

† Discord refers to cases in which the tissue factor score is plus, and the human chromosome is absent, or in which the score is minus and the chromosome is present.

After successful cell hybrids are formed, two particular tests are used to map human genes. A **synteny test** (same linkage group) determines whether two loci are in the same linkage group if the phenotypes of the two loci are either always together or always absent in various hybrid cell lines. An **assignment test** determines which chromosome a particular locus is on by the concordant appearance of the phenotype whenever that particular chromosome is in a cell line, or by the lack of the particular phenotype when a particular chromosome is absent from a cell line. The first autosomal synteny test, performed in 1970, demonstrated that the *B* locus of lactate dehydrogenase ($LDH_B$) was linked to the *B* locus of peptidase ($PEP_B$). (Both enzymes are formed from subunits

controlled by two loci each. In addition to the *B* locus, each protein has subunits controlled by an *A* locus.) Later, these loci were shown to reside on chromosome 12.

In another example, a blood-coagulating glycoprotein (a protein-polysaccharide complex) called tissue factor III was localized by assignment tests to chromosome 1. Table 6.8 shows twenty-nine human-mouse hybrid cell lines, or **clones,** the human chromosomes they contain, and their tissue factor score, the results of an assay for the presence of the coagulating factor. (Clones are cells arising from a single ancestor.) It is obvious from table 6.8 that the gene for tissue factor III is on human chromosome 1. Every time human chromosome 1 is present in a cell line, so is tissue factor III. Every time human chromosome 1 is absent, so is the tissue factor (zero discordance or 100% concordance). No other chromosome showed that pattern.

The human map as we know it now (compiled by Victor McKusick at Johns Hopkins University), containing over six thousand assigned loci of over twelve thousand known to exist, is shown in table 6.9 and figure 6.29. At

Victor A. McKusick (1921– ). (Courtesy of Victor A. McKusick.)

**Table 6.9** **Definition of Selected Loci of the Human Chromosome Map (figure 6.29)**

| Locus | Protein Product | Chromosome | Locus | Protein Product | Chromosome |
|---|---|---|---|---|---|
| *ABO* | ABO blood group | 9 | *IGH* | Immunoglobulin heavy-chain gene family | 14 |
| *AG* | Alpha globin gene family | 16 | *IGK* | Immunoglobulin kappa-chain gene family | 2 |
| *ALB* | Albumin | 4 | *INS* | Insulin | 11 |
| *AMY1* | Amylase, salivary | 1 | *LDHA* | Lactate dehydrogenase A | 11 |
| *AMY2* | Amylase, pancreatic | 1 | *MDI* | Manic depressive illness | 6 |
| *BCS* | Breast cancer susceptibility | 16 | *MHC* | Major histocompatibility complex | 6 |
| *C2* | Complement component-2 | 6 | *MN* | MN blood group | 4 |
| *CAT* | Catalase | 11 | *MYB* | Avian myeloblastosis virus oncogene | 6 |
| *CBD* | Color blindness, deutan | X | *NHCP1* | Nonhistone chromosomal protein-1 | 7 |
| *CBP* | Color blindness, protan | X | *NPS1* | Nail-patella syndrome | 9 |
| *CML* | Chronic myeloid leukemia | 22 | *PEPA* | Peptidase A | 18 |
| *DMD* | Duchenne muscular dystrophy | X | *PVS* | Polio virus sensitivity | 19 |
| *FES* | Feline sarcoma virus oncogene | 15 | *Rb* | Rhesus blood group | 1 |
| *FY* | Duffy blood group | 1 | *RN5S* | 5S RNA gene(s) | 1 |
| *GLB1* | Beta-galactosidase-1 | 3 | *RNTMI* | Initiator methionine tRNA | 6 |
| *H1* | Histone-1 | 7 | *RWS* | Ragweed sensitivity | 6 |
| *HBB* | Hemoglobin beta chain | 11 | *S1* | Surface antigen 1 | 11 |
| *HEMA* | Classic hemophilia | X | *SIS* | Simian sarcoma virus oncogene | 22 |
| *HEXA* | Hexosaminidase A | 15 | *STA* | Stature | Y |
| *HLA* | Human leukocyte antigens | 6 | *TF* | Transferrin | 3 |
| *HP* | Haptoglobin | 16 | *XG* | Xg blood group | X |
| *HYA* | Y histocompatibility antigen, locus A | Y | *XRS* | X-ray sensitivity | 13 |
| *IDDM* | Insulin-dependent diabetes mellitus | 6 | | | |
| *IFF* | Interferon, fibroblast | 9 | | | |

*Note:* A more complete list appears in V. A. McKusick, *Mendelian Inheritance in Man: A Catalog of Human Genes* (Baltimore: Johns Hopkins University Press, 1994).

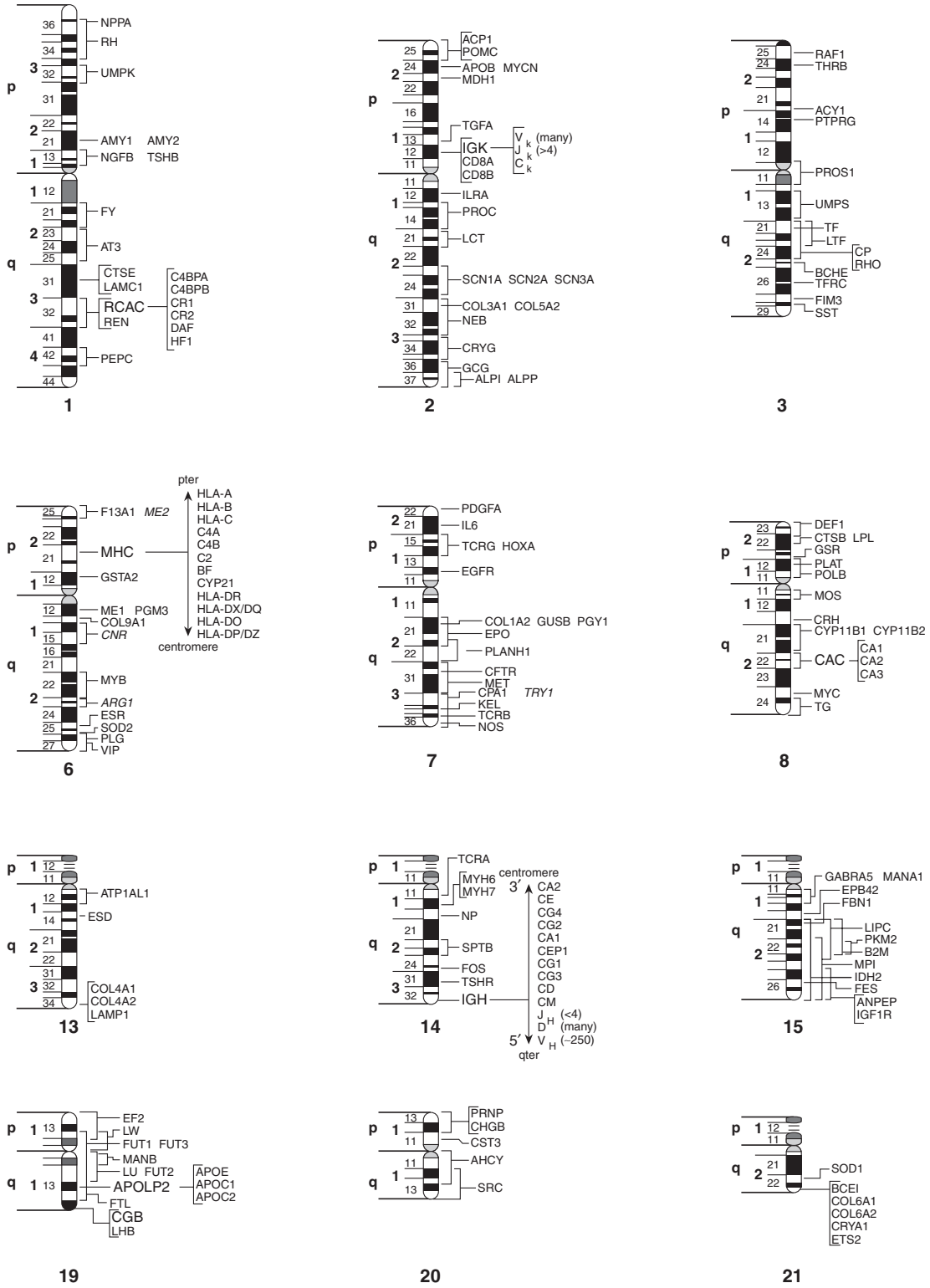
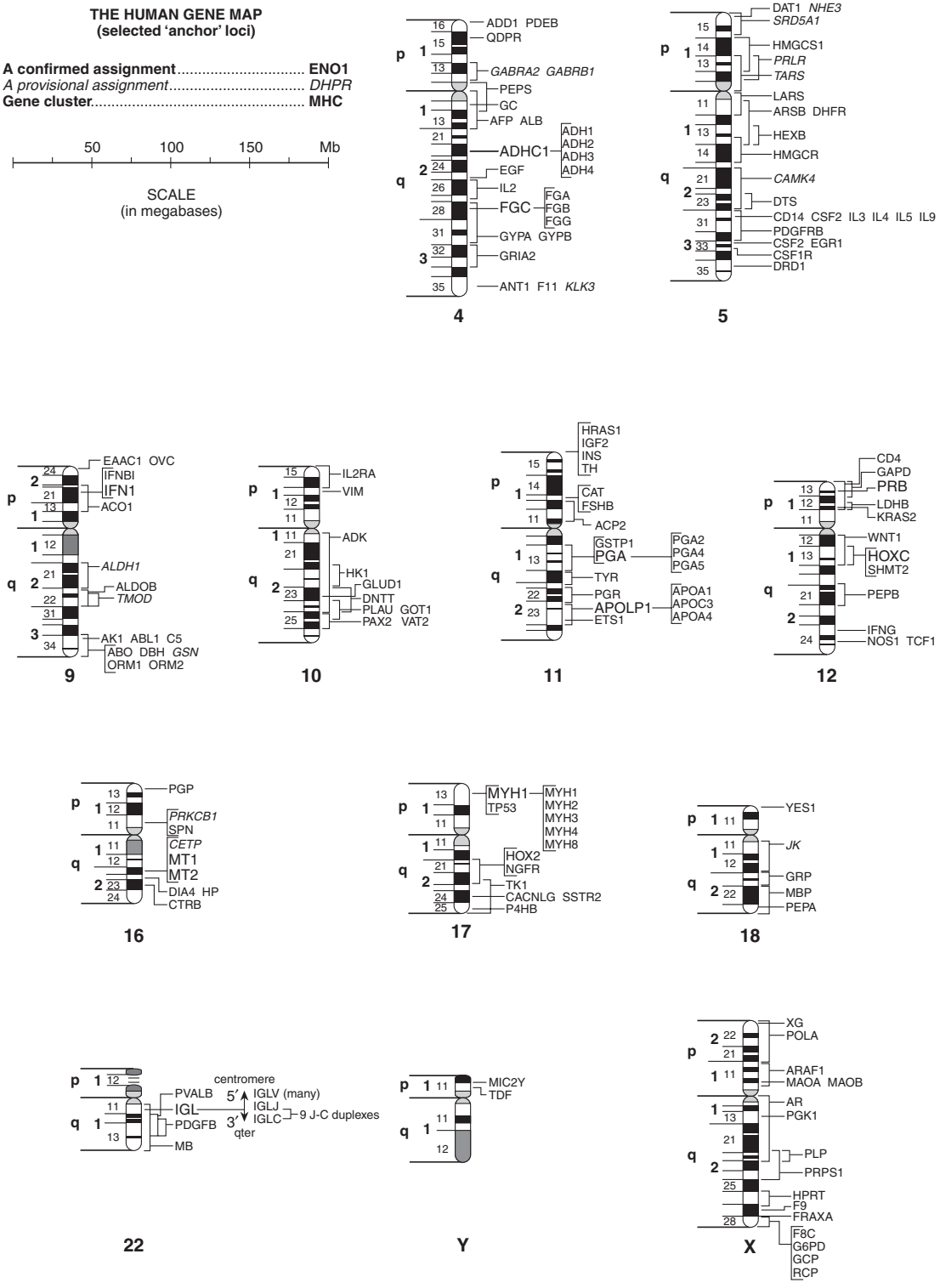



**Figure 6.29** Human G-banded chromosomes with their accompanying assigned loci. The *p* and *q* refer to the short and long arms of the chromosomes, respectively. A key to the loci is given in McKusick (1994). (From Victor A. McKusick, *Mendelian inheritance in man,* 11th edition, 1994. Reprinted by permission of Johns Hopkins University Press, Baltimore, MD.)


THE HUMAN GENE MAP
(selected 'anchor' loci)
A confirmed assignment ..... ENO1
A provisional assignment ..... DHPR
Gene cluster ..... MHC
50
100
150
Mb
SCALE
(in megabases)
ADD1 PDEB
QDPR
GABRA2 GABRB1
PEPS
GC
AFP ALB
ADH1
ADH2
ADH3
ADH4
ADHC1
EGF
IL2
FGA
FGB
FGG
FGC
GYPA GYPB
GRIA2
ANT1 F11 KLK3
4
DAT1 NHE3
SRD5A1
HMGCS1
PRLR
TARS
LARS
ARSB DHFR
HEXB
HMGCR
CAMK4
DTS
CD14 CSF2 IL3 IL4 IL5 IL9
PDGFRB
CSF2 EGR1
CSF1R
DRD1
5
EAAC1 OVC
IFNBI
IFN1
ACO1
ALDH1
ALDOB
TMOD
AK1 ABL1 C5
ABO DBH GSN
ORM1 ORM2
9
IL2RA
VIM
ADK
HK1
GLUD1
DNTT
PLAU GOT1
PAX2 VAT2
10
HRAS1
IGF2
INS
TH
CAT
FSHB
ACP2
GSTP1
PGA
PGA2
PGA4
PGA5
TYR
PGR
APOLP1
APOA1
APOC3
APOA4
ETS1
11
CD4
GAPD
PRB
LDHB
KRAS2
WNT1
HOXC
SHMT2
PEPB
IFNG
NOS1 TCF1
12
PGP
PRKCB1
SPN
CETP
MT1
MT2
DIA4 HP
CTRB
16
MYH1
TP53
MYH1
MYH2
MYH3
MYH4
MYH8
HOX2
NGFR
TK1
CACNLG SSTR2
P4HB
17
YES1
JK
GRP
MBP
PEPA
18
centromere
PVALB
IGL
PDGFB
MB
5′
3′
IGLV (many)
IGLJ
IGLC
9 J-C duplexes
qter
22
MIC2Y
TDF
Y
XG
POLA
ARAF1
MAOA MAOB
AR
PGK1
PLP
PRPS1
HPRT
F9
FRAXA
F8C
G6PD
GCP
RCP
X

present, geneticists studying human chromosomes are hampered not by a lack of techniques but by a lack of marker loci. When a new locus is discovered, it is now relatively easy to assign it to its proper chromosome.

The problem still exists of determining exactly where a particular locus belongs on a chromosome. This is facilitated by developing particular cell lines with broken chromosomes, so that parts are either missing or have moved to other chromosomes. These processes reveal new linkage arrangements and make it possible to determine the region in which a locus is situated on a particular chromosome. In chapter 13, we describe additional techniques used to locate genes on human chromosomes, including a description of the Human Genome Project, the program that sequenced the entire human genome as well as the genomes of other model organisms.

## SUMMARY

**STUDY OBJECTIVE 1:** To learn about analytical techniques for locating the relative positions of genes on chromosomes in diploid eukaryotic organisms 110–122

The principle of independent assortment is violated when loci lie near each other on the same chromosome. Recombination between these loci results from the crossing over of chromosomes during meiosis. The amount of recombination provides a measure of the distance between these loci. One map unit (centimorgan) equals 1% recombinant gametes. Map units can be determined by testcrossing a dihybrid and recording the percentage of recombinant offspring. If three loci are used (a three-point cross), double crossovers will be revealed. A coefficient of coincidence, the ratio of observed to expected double crossovers, can be calculated to determine if one crossover changes the probability that a second one will occur nearby.

A chiasma seen during prophase I of meiosis represents both a physical and a genetic crossing over. This can be demonstrated by using homologous chromosomes with morphological distinctions.

Because of multiple crossovers, the measured percentage recombination underestimates the true map distance, especially for loci relatively far apart; the best map estimates come from summing the distances between closely linked loci. A mapping function can be used to translate observed map distances into more accurate ones.

**STUDY OBJECTIVE 2:** To learn about analytical techniques for locating the relative positions of genes on chromosomes in ascomycete fungi 122–132

Organisms that retain all the products of meiosis lend themselves to chromosome mapping by haploid mapping (tetrad analysis). With unordered spores, such as in yeast, we use

$$\text{map units} = \frac{(1/2)\text{ the number of TT asci} + \text{the number of NPD asci}}{\text{total number or asci}} \times 100$$

Map units between a locus and its centromere in organisms with ordered spores, such as *Neurospora,* can be calculated as

$$\text{map units} = \frac{(1/2)\text{ the number of SDS asci}}{\text{total number of asci}} \times 100$$

Crossing over also occurs during mitosis, but at a much reduced rate. Somatic (mitotic) crossing over can be used to map loci.

**STUDY OBJECTIVE 3:** To learn about analytical techniques for locating the relative positions of genes on human chromosomes 132–140

Human chromosomes can be mapped. Recombination distances can be established by pedigrees, and loci can be attributed to specific chromosomes by synteny and assignment tests in hybrid cell lines.

## SOLVED PROBLEMS

**PROBLEM 1:** A homozygous claret (*ca,* claret eye color), curled (*cu,* upcurved wings), fluted ( *fl,* creased wings) fruit fly is crossed with a pure-breeding wild-type fly. The $F_1$ females are testcrossed with the following results:

| | |
|---|---|
| fluted | 4 |
| claret | 173 |
| curled | 26 |
| fluted, claret | 24 |
| fluted, curled | 167 |
| claret, curled | 6 |
| fluted, claret, curled | 298 |
| wild-type | 302 |

**a.** Are the loci linked?
**b.** If so, give the gene order, map distances, and coefficient of coincidence.

***Answer:*** The pattern of numbers among the eight offspring classes is the pattern we are used to seeing for linkage of three loci. We can tell from the two groups in largest numbers (the nonrecombinants—fluted, claret, curled and wild-type) that the alleles are in the coupling (*cis*) arrangement. If we compare either of the nonrecombinant classes with either of the double crossover classes (fluted and claret, curled), we see that the fluted locus is in the center. For example, compare fluted, a double crossover offspring, with the wild-type, a nonrecombinant; clearly, fluted has the odd pattern. Thus the trihybrid female parent had the following arrangement of alleles:

$$\frac{ca\ fl\ cu}{ca^+\ fl^+\ cu^+}$$

A crossover in the *ca–fl* region produces claret and fluted, curled offspring, and a crossover in the *fl–cu* region produces fluted, claret and curled offspring. Counting the crossovers in each region, including the double crossovers in each, and then converting to percentages, yields a claret-to-fluted distance of 35.0 map units (173 + 167 + 6 + 4) and a fluted-to-curled distance of 6.0 map units (26 + 24 + 6 + 4). We expect $0.35 \times 0.06 \times 1{,}000 = 21$ double crossovers, but we observed only 6 + 4 = 10. Thus, the coefficient of coincidence is 10/21 = 0.48.

**PROBLEM 2:** The *ad5* locus in *Neurospora* is a gene for an enzyme in the synthesis pathway for the DNA base adenine. A wild-type strain ($ad5^+$) is crossed with an adenine-requiring strain, $ad5^-$. The diploid undergoes meiosis, and one hundred asci are scored for their segregation patterns with the following results:

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| $ad5^+$ | $ad5^+$ | $ad5^+$ | $ad5^+$ | $ad5^-$ | $ad5^-$ | $ad5^-$ | $ad5^-$ | 40 |
| $ad5^-$ | $ad5^-$ | $ad5^-$ | $ad5^-$ | $ad5^+$ | $ad5^+$ | $ad5^+$ | $ad5^+$ | 46 |
| $ad5^+$ | $ad5^+$ | $ad5^-$ | $ad5^-$ | $ad5^-$ | $ad5^-$ | $ad5^+$ | $ad5^+$ | 5 |
| $ad5^-$ | $ad5^-$ | $ad5^+$ | $ad5^+$ | $ad5^+$ | $ad5^+$ | $ad5^-$ | $ad5^-$ | 3 |
| $ad5^-$ | $ad5^-$ | $ad5^+$ | $ad5^+$ | $ad5^-$ | $ad5^-$ | $ad5^+$ | $ad5^+$ | 4 |
| $ad5^+$ | $ad5^+$ | $ad5^-$ | $ad5^-$ | $ad5^+$ | $ad5^+$ | $ad5^-$ | $ad5^-$ | 2 |

What can you say about the linkage arrangements at this locus?

***Answer:*** You can see that 14 (5 + 3 + 4 + 2) asci are of the second-division segregation type (SDS) and 86 (40 + 46) are of the first-division segregation type (FDS). To map the distance of the locus to its centromere, we divide the percentage of SDS types by 2: 14/100 = 14%; divided by 2 is 7%. Thus, the *ad5* locus is 7 map units from its centromere.

**PROBLEM 3:** In yeast, the *his5* locus is a gene for an enzyme in the synthesis pathway for the amino acid histidine, and the *lys11* locus is a gene for an enzyme in the synthesis pathway for the amino acid lysine. A haploid wild-type strain ($his5^+\ lys11^+$) is crossed with the double mutant ($his5^-\ lys11^-$). The diploid is allowed to undergo meiosis, and 100 asci are scored with the following results:

| | | |
|---|---|---|
| $his5^+\ lys11^+$ | $his5^+\ lys11^+$ | $his5^+\ lys11^-$ |
| $his5^+\ lys11^+$ | $his5^-\ lys11^-$ | $his5^+\ lys11^-$ |
| $his5^-\ lys11^-$ | $his5^-\ lys11^+$ | $his5^-\ lys11^+$ |
| $his5^-\ lys11^-$ | $his5^+\ lys11^-$ | $his5^-\ lys11^+$ |
| 62 | 30 | 8 |

What is the linkage arrangement of these loci?

***Answer:*** Of the 100 asci analyzed, 62 were parental ditypes (PD), 30 were tetratypes (TT), and 8 were nonparental ditypes (NPD). To map the distance between the two loci, we take the percentage of NPD (8%) plus half the percentage of TT (1/2 of 30 = 15%) = 23% or 23 centimorgans between loci.

**PROBLEM 4:** A particular human enzyme is present only in clone B. The human chromosomes present in clones A, B, and C appear as pluses in the following table. Determine the probable chromosomal location of the gene for the enzyme.

| | **Human Chromosome** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Clone | *1* | *2* | *3* | *4* | *5* | *6* | *7* | *8* |
| A | + | + | + | + | − | − | − | − |
| B | + | + | − | − | + | + | − | − |
| C | + | − | + | − | + | − | + | − |

***Answer:*** If a gene is located on a chromosome, the gene must be present in the clones with the chromosome (+). Chromosomes 1, 2, 5, 6 are present in B. If the gene in question were located on chromosome 1, the enzyme should have been present in all three clones. A similar argument holds for chromosome 2, in which the enzyme should have been present in clones A and B, and so on for the rest of the chromosomes. The only chromosome that is unique to clone B is 6. Therefore, the gene is located on chromosome 6.

# EXERCISES AND PROBLEMS*

**DIPLOID MAPPING**

**1.** A homozygous groucho fly (*gro,* bristles clumped above the eyes) is crossed with a homozygous rough fly (*ro,* eye abnormality). The $F_1$ females are testcrossed, producing these offspring:

| | |
|---|---|
| groucho | 518 |
| rough | 471 |
| groucho, rough | 6 |
| wild-type | 5 |
| | 1,000 |

**a.** What is the linkage arrangement of these loci?
**b.** What offspring would result if the $F_1$ dihybrids were crossed among themselves instead of being testcrossed?

**2.** A female fruit fly with abnormal eyes (*abe*) of a brown color (*bis,* bistre) is crossed with a wild-type male. Her sons have abnormal, brown eyes; her daughters are of the wild-type. When these $F_1$ flies are crossed among themselves, the following offspring are produced:

| | Sons | Daughters |
|---|---|---|
| abnormal, brown | 219 | 197 |
| abnormal | 43 | 45 |
| brown | 37 | 35 |
| wild-type | 201 | 223 |

What is the linkage arrangement of these loci?

**3.** In *Drosophila,* the loci inflated (*if*, small, inflated wings) and warty *(wa*, abnormal eyes) are about 10 map units apart on the X chromosome. Construct a data set that would allow you to determine this linkage arrangement. What differences would be involved if the loci were located on an autosome?

**4.** A geneticist crossed female fruit flies that were heterozygous at three electrophoretic loci, each with fast and slow alleles, with males homozygous for the slow alleles. The three loci were *got1* (glutamate oxaloacetate transaminase-1), *amy* (alpha-amylase), and *sdh* (succinate dehydrogenase). The first 1,000 offspring isolated had the following genotypes:

| | | |
|---|---|---|
| Class 1 | $got^s\ got^s\ amy^s\ amy^s\ sdh^s\ sdh^s$ | 441 |
| Class 2 | $got^f\ got^s\ amy^f\ amy^s\ sdh^f\ sdh^s$ | 421 |
| Class 3 | $got^f\ got^s\ amy^s\ amy^s\ sdh^s\ sdh^s$ | 11 |
| Class 4 | $got^s\ got^s\ amy^f\ amy^s\ sdh^f\ sdh^s$ | 14 |
| Class 5 | $got^f\ got^s\ amy^f\ amy^s\ sdh^s\ sdh^s$ | 58 |
| Class 6 | $got^s\ got^s\ amy^s\ amy^s\ sdh^f\ sdh^s$ | 53 |
| Class 7 | $got^f\ got^s\ amy^s\ amy^s\ sdh^f\ sdh^s$ | 1 |
| Class 8 | $got^s\ got^s\ amy^f\ amy^s\ sdh^s\ sdh^s$ | 1 |

What are the linkage arrangements of these three loci, including map units? If the three loci are linked, what is the coefficient of coincidence?

**5.** The following three recessive markers are known in lab mice: *h*, hotfoot; *o*, obese; and *wa*, waved. A trihybrid of unknown origin is testcrossed, producing the following offspring:

| | |
|---|---|
| hotfoot, obese, waved | 357 |
| hotfoot, obese | 74 |
| waved | 66 |
| obese | 79 |
| wild-type | 343 |
| hotfoot, waved | 61 |
| obese, waved | 11 |
| hotfoot | 9 |
| | 1,000 |

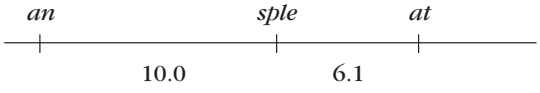
**a.** If the genes are linked, determine the relative order and the map distances between them.
**b.** What was the *cis-trans* allele arrangement in the trihybrid parent?
**c.** Is there any crossover interference? If yes, how much?

**6.** The following three recessive genes are found in corn: *bt1*, brittle endosperm; *gl17*, glossy leaf; *rgdl,* ragged seedling. A trihybrid of unknown origin is testcrossed, producing the following offspring:

| | |
|---|---|
| brittle, glossy, ragged | 236 |
| brittle, glossy | 241 |
| ragged | 219 |
| glossy | 23 |
| wild-type | 224 |
| brittle, ragged | 17 |
| glossy, ragged | 21 |
| brittle | 19 |
| | 1,000 |

**a.** If the genes are linked, determine the relative order and map distances.
**b.** Reconstruct the chromosomes of the trihybrid.
**c.** Is there any crossover interference? If yes, how much?

**7.** In *Drosophila,* ancon (*an*, legs and wings short), spiny legs (*sple*, irregular leg hairs), and arctus oculus (*at*, small narrow eyes) have the following linkage arrangement on chromosome 3:




*Answers to selected exercises and problems are on page A-6.

**a.** Devise a data set with no crossover interference that would yield these map units.
**b.** What data would yield the same map units but with a coefficient of coincidence of 0.60?

**8.** Ancon (*an*) and spiny legs (*sple*), from problem 7, are 10 map units apart on chromosome 3. Notchy (*ny,* wing tips nicked) is on the X chromosome (chromosome 1). Create a data set that would result if you were making crosses to determine the linkage arrangement of these three loci. How would you know that the notchy locus is on the X chromosome?

**9.** In the house mouse, the autosomal alleles Trembling and Rex (short hair) are dominant to not trembling (normal) and long hair, respectively. Heterozygous Trembling, Rex females were crossed with normal, long-haired males and yielded the following offspring:

| | |
|---|---|
| Trembling, Rex | 42 |
| Trembling, long-haired | 105 |
| normal, Rex | 109 |
| normal, long-haired | 44 |

**a.** Are the two genes linked? How do you know?
**b.** In the heterozygous females, were Trembling and Rex in *cis* or *trans* position? Explain.
**c.** Calculate the percent recombination between the two genes.

**10.** In corn, a trihybrid Tunicate (*T*), Glossy (*G*), Liguled (*L*) plant was crossed with a nontunicate, nonglossy, liguleless plant, producing the following offspring:

| | |
|---|---|
| Tunicate, liguleless, Glossy | 58 |
| Tunicate, liguleless, nonglossy | 15 |
| Tunicate, Liguled, Glossy | 55 |
| Tunicate, Liguled, nonglossy | 13 |
| nontunicate, Liguled, Glossy | 16 |
| nontunicate, Liguled, nonglossy | 53 |
| nontunicate, liguleless, Glossy | 14 |
| nontunicate, liguleless, nonglossy | 59 |

**a.** Determine which genes are linked.
**b.** Determine the genotype of the heterozygote; be sure to indicate which alleles are on which chromosome.
**c.** Calculate the map distances between the linked genes.

**11.** In *Drosophila*, kidney-shaped eye (*k*), cardinal eye (*cd*), and ebony body (*e*) are three recessive genes. If homozygous kidney, cardinal females are crossed with homozygous ebony males, the $F_1$ offspring are all wild-type. If heterozygous $F_1$ females are mated with kidney, cardinal, ebony males, the following 2,000 progeny appear:

| | |
|---|---|
| 880 | kidney, cardinal |
| 887 | ebony |
| 64 | kidney, ebony |
| 67 | cardinal |
| 49 | kidney |
| 46 | ebony, cardinal |
| 3 | kidney, ebony, cardinal |
| 4 | wild-type |

**a.** Determine the chromosomal composition of the $F_1$ females.
**b.** Derive a map of the three genes.

**12.** Following is a partial map of the third chromosome in *Drosophila.*

19.2 javelin bristles (*jv*)
43.2 thread arista (*th*)
66.2 Delta veins (*Dl*)
70.7 ebony body (*e*)

**a.** If flies heterozygous in *cis* position for javelin and ebony are mated among themselves, what phenotypic ratio do you expect in the progeny?
**b.** A true-breeding thread, ebony fly is crossed with a true-breeding Delta fly. An $F_1$ female is test-crossed to a thread, ebony male. Predict the expected progeny and their frequencies for this cross. Assume no interference.
**c.** Repeat *b,* but assume a coefficient of coincidence of 0.4.

**13.** Suppose that you have determined the order of three genes to be *a*, *c*, *b*, and that by doing two-point crosses you have determined map distances as $a$–$c = 10$ and $c$–$b = 5$. If interference is −1.5, and the three-point cross is

$$\frac{A\ C\ B}{a\ c\ b} \times \frac{a\ c\ b}{a\ c\ b}$$

what frequency of double crossovers do you expect?

**HAPLOID MAPPING (TETRAD ANALYSIS)**

**14.** Given the following cross in *Neurospora: ab* × $a^+b^+$, construct results showing that crossing over occurs in two of the four chromatids of a tetrad at meiosis. What would the results be if crossing over occurred during interphase before each chromosome became two chromatids? if each crossover event involved three or four chromatids?

**15.** A strain of yeast requiring both tyrosine ($tyr^-$) and arginine ($arg^-$) is crossed to the wild-type. After meiosis, the following ten asci are dissected. Classify each ascus as to segregational type (PD, NPD, TT). What is the linkage relationship between these two loci?

| | | | | |
|---|---|---|---|---|
| 1 | $arg^-\ tyr^-$ | $arg^+\ tyr^+$ | $arg^+\ tyr^+$ | $arg^-\ tyr^-$ |
| 2 | $arg^+\ tyr^+$ | $arg^+\ tyr^+$ | $arg^-\ tyr^-$ | $arg^-\ tyr^-$ |
| 3 | $arg^-\ tyr^+$ | $arg^-\ tyr^+$ | $arg^+\ tyr^-$ | $arg^+\ tyr^-$ |
| 4 | $arg^-\ tyr^-$ | $arg^-\ tyr^-$ | $arg^+\ tyr^+$ | $arg^+\ tyr^+$ |
| 5 | $arg^-\ tyr^-$ | $arg^-\ tyr^+$ | $arg^+\ tyr^-$ | $arg^+\ tyr^+$ |
| 6 | $arg^+\ tyr^+$ | $arg^+\ tyr^+$ | $arg^-\ tyr^-$ | $arg^-\ tyr^-$ |
| 7 | $arg^-\ tyr^-$ | $arg^+\ tyr^+$ | $arg^-\ tyr^+$ | $arg^+\ tyr^-$ |
| 8 | $arg^+\ tyr^+$ | $arg^+\ tyr^+$ | $arg^-\ tyr^-$ | $arg^-\ tyr^-$ |
| 9 | $arg^+\ tyr^+$ | $arg^-\ tyr^-$ | $arg^-\ tyr^-$ | $arg^+\ tyr^+$ |
| 10 | $arg^-\ tyr^-$ | $arg^+\ tyr^+$ | $arg^+\ tyr^+$ | $arg^-\ tyr^-$ |

**16.** A certain haploid strain of yeast was deficient for the synthesis of the amino acids tryptophan ($try^-$) and methionine ($met^-$). It was crossed to the wild-type, and meiosis occurred. One dozen asci were analyzed for their tryptophan and methionine requirements. The following results, with the inevitable lost spores, were obtained:

| | | | | |
|---|---|---|---|---|
| 1 | $try^-\ met^-$ | ? | ? | $try^-\ met^-$ |
| 2 | ? | $try^-\ met^-$ | $try^+\ met^+$ | $try^+\ met^+$ |
| 3 | $try^-\ met^+$ | $try^-\ met^-$ | $try^+\ met^-$ | $try^+\ met^+$ |
| 4 | $try^-\ met^-$ | $try^+\ met^+$ | ? | $try^+\ met^-$ |
| 5 | $try^-\ met^+$ | ? | ? | $try^+\ met^-$ |
| 6 | $try^+\ met^+$ | $try^+\ met^+$ | $try^-\ met^-$ | $try^-\ met^-$ |
| 7 | $try^+\ met^+$ | $try^+\ met^-$ | ? | $try^-\ met^-$ |
| 8 | $try^+\ met^+$ | $try^-\ met^-$ | ? | $try^+\ met^+$ |
| 9 | $try^-\ met^+$ | $try^+\ met^-$ | $try^-\ met^+$ | $try^+\ met^-$ |
| 10 | $try^-\ met^-$ | $try^+\ met^+$ | $try^-\ met^-$ | $try^+\ met^+$ |
| 11 | $try^+\ met^+$ | $try^+\ met^+$ | ? | ? |
| 12 | ? | $try^+\ met^-$ | ? | $try^-\ met^+$ |

**a.** Classify each ascus as to segregational type (note that some asci may not be classifiable).
**b.** Are the genes linked?
**c.** If so, how far apart are they?

**17.** In *Neurospora*, a haploid strain requiring arginine ($arg^-$) is crossed with the wild-type ($arg^+$). Meiosis occurs, and ten asci are dissected with the following results. Map the *arg* locus.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 1 | $arg^+$ | $arg^+$ | $arg^-$ | $arg^-$ | $arg^+$ | $arg^+$ | $arg^-$ | $arg^-$ |
| 2 | $arg^-$ | $arg^-$ | $arg^+$ | $arg^+$ | $arg^-$ | $arg^-$ | $arg^+$ | $arg^+$ |
| 3 | $arg^+$ | $arg^+$ | $arg^+$ | $arg^+$ | $arg^-$ | $arg^-$ | $arg^-$ | $arg^-$ |
| 4 | $arg^+$ | $arg^+$ | $arg^+$ | $arg^+$ | $arg^-$ | $arg^-$ | $arg^-$ | $arg^-$ |
| 5 | $arg^-$ | $arg^-$ | $arg^-$ | $arg^-$ | $arg^+$ | $arg^+$ | $arg^+$ | $arg^+$ |
| 6 | $arg^+$ | $arg^+$ | $arg^-$ | $arg^-$ | $arg^-$ | $arg^-$ | $arg^+$ | $arg^+$ |
| 7 | $arg^-$ | $arg^-$ | $arg^+$ | $arg^+$ | $arg^+$ | $arg^+$ | $arg^-$ | $arg^-$ |
| 8 | $arg^+$ | $arg^+$ | $arg^+$ | $arg^+$ | $arg^-$ | $arg^-$ | $arg^-$ | $arg^-$ |
| 9 | $arg^-$ | $arg^-$ | $arg^+$ | $arg^+$ | $arg^+$ | $arg^+$ | $arg^-$ | $arg^-$ |
| 10 | $arg^-$ | $arg^-$ | $arg^-$ | $arg^-$ | $arg^+$ | $arg^+$ | $arg^+$ | $arg^+$ |

**18.** A haploid strain of *Neurospora* with fuzzy colony morphology ($f$) was crossed with the wild-type ($f^+$). Twelve asci were scored. The following results, with the inevitable lost spores were obtained:

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 1 | ? | $f$ | $f$ | ? | ? | $f^+$ | $f^+$ | $f^+$ |
| 2 | $f$ | $f$ | $f^+$ | $f^+$ | $f^+$ | $f^+$ | $f$ | $f$ |
| 3 | $f$ | ? | ? | ? | $f^+$ | ? | ? | ? |
| 4 | $f^+$ | ? | ? | ? | $f$ | $f$ | $f$ | $f$ |
| 5 | $f$ | $f$ | ? | ? | ? | $f^+$ | ? | $f^+$ |
| 6 | ? | $f$ | $f$ | ? | ? | ? | ? | ? |
| 7 | $f^+$ | $f^+$ | $f$ | $f$ | $f$ | $f$ | $f^+$ | $f^+$ |
| 8 | $f$ | $f$ | $f$ | ? | ? | $f^+$ | $f^+$ | $f^+$ |
| 9 | $f^+$ | ? | ? | ? | ? | $f$ | $f$ | ? |
| 10 | $f$ | $f$ | $f^+$ | $f^+$ | $f$ | $f$ | $f^+$ | $f^+$ |
| 11 | $f$ | $f$ | $f$ | $f$ | $f^+$ | $f^+$ | $f^+$ | $f^+$ |
| 12 | $f$ | $f$ | ? | ? | ? | ? | $f^+$ | $f^+$ |

**a.** Classify each ascus as to segregational type, and note which asci cannot be classified.
**b.** Map the chromosome containing the $f$ locus with all the relevant measurements.

**19.** Draw ten of the remaining twenty-eight ascus patterns not included in table 6.6. To which of the seven major categories of table 6.7 does each belong?

**20.** In yeast, the *a* and *b* loci are 12 map units apart. Construct a data set to demonstrate this.

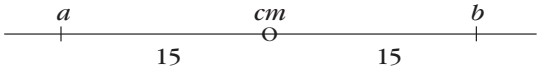
**21.** In *Neurospora*, the *a* locus is 12 map units from its centromere. Construct a data set to show this.

**22.** An *ab Neurospora* was crossed with an $a^+b^+$ form. Meiosis occurred, and 1,000 asci were dissected. Using the classes of table 6.7, the following data resulted:

| | | | |
|---|---|---|---|
| Class 1 | 700 | Class 5 | 5 |
| Class 2 | 0 | Class 6 | 5 |
| Class 3 | 190 | Class 7 | 10 |
| Class 4 | 90 | | |

What is the linkage arrangement of these loci?

**23.** Given the following linkage arrangement in *Neurospora*, construct a data set similar to that in table 6.7 that is consistent with it (*cm* is centromere).




**24.** Determine crossover events that led to each of the seven classes in table 6.7.

**25.** In *Neurospora,* a cross is made between $ab^+$ and $a^+b$ individuals. The following one hundred ordered tetrads are obtained:

| Spores | I | II | III | IV | V | VI | VII | VIII |
|---|---|---|---|---|---|---|---|---|
| 1, 2 | $a^+b$ | $a^+b$ | $a^+b$ | $a^+b^+$ | $a^+b^+$ | $a^+b$ | $a^+b$ | $ab^+$ |
| 3, 4 | $a^+b$ | $a^+b^+$ | $a^+b^+$ | $a^+b$ | $a^+b$ | $ab^+$ | $ab^+$ | $a^+b$ |
| 5, 6 | $ab^+$ | $ab$ | $ab^+$ | $ab$ | $ab^+$ | $a^+b$ | $ab^+$ | $a^+b$ |
| 7, 8 | $ab^+$ | $ab^+$ | $ab$ | $ab^+$ | $ab$ | $ab^+$ | $a^+b$ | $ab^+$ |
| | 85 | 2 | 3 | 2 | 3 | 3 | 1 | 1 |

**a.** Are genes *a* and *b* linked? How do you know?
**b.** Calculate the gene-to-centromere distances for *a* and *b*.

**26.** *Neurospora* has four genes—*a*, *b*, *c*, and *d*—that control four different phenotypes. Your job is to map these genes by performing pairwise crosses. You obtain the following ordered tetrads:

| | $ab^+ \times a^+b$ | | | | $bc^+ \times b^+c$ | | |
|---|---|---|---|---|---|---|---|
| **Spores** | **I** | **II** | **III** | **Spores** | **I** | **II** | **III** |
| 1, 2 | $ab^+$ | $ab$ | $ab^+$ | 1, 2 | $bc^+$ | $b^+c^+$ | $b^+c$ |
| 3, 4 | $ab^+$ | $ab$ | $a^+b^+$ | 3, 4 | $bc^+$ | $b^+c^+$ | $b^+c^+$ |
| 5, 6 | $a^+b$ | $a^+b^+$ | $a^+b$ | 5, 6 | $b^+c$ | $bc$ | $bc$ |
| 7, 8 | $a^+b$ | $a^+b^+$ | $ab$ | 7, 8 | $b^+c$ | $bc$ | $bc^+$ |
| | 45 | 43 | 12 | | 70 | 4 | 26 |

| | $cd^+ \times c^+d$ | | | | | | |
|---|---|---|---|---|---|---|---|
| **Spores** | **I** | **II** | **III** | **IV** | **V** | **VI** | **VII** |
| 1, 2 | $cd^+$ | $cd$ | $cd$ | $cd$ | $cd^+$ | $cd$ | $cd^+$ |
| 3, 4 | $cd^+$ | $cd$ | $cd^+$ | $c^+d$ | $c^+d$ | $c^+d^+$ | $c^+d$ |
| 5, 6 | $c^+d$ | $c^+d^+$ | $c^+d^+$ | $c^+d^+$ | $c^+d$ | $c^+d^+$ | $c^+d^+$ |
| 7, 8 | $c^+d$ | $c^+d^+$ | $c^+d$ | $cd^+$ | $cd^+$ | $cd$ | $cd$ |
| | 42 | 2 | 30 | 15 | 5 | 1 | 5 |

**a.** Calculate the gene-to-centromere distances.
**b.** Which genes are linked? Explain.
**c.** Derive a complete map for all four genes.

**27.** You have isolated a new fungus and have obtained a strain that requires both arginine ($arg^-$) and adenine ($ad^-$). You cross these two strains and collect four hundred random spores that you plate on minimal medium. If twenty-five spores grow, what is the distance between these two genes?

**28.** Three distinct genes, *pab*, *pk*, and *ad*, were scored in a cross of *Neurospora.* From the cross $pab\ pk^+\ ad^+ \times pab^+\ pk\ ad$, the following ordered tetrads were recovered:

| **Spores** | **I** | **II** | **III** | **IV** | **V** | **VI** | **VII** | **VIII** |
|---|---|---|---|---|---|---|---|---|
| 1, 2 | $pab\ pk^+\ ad^+$ | $pab\ pk^+\ ad^+$ | $pab\ pk^+\ ad^+$ | $pab\ pk^+\ ad^+$ | $pab\ pk^+\ ad^+$ | $pab\ pk^+\ ad^+$ | $pab\ pk^+\ ad$ | $pab\ pk^+\ ad$ |
| 3, 4 | $pab\ pk^+\ ad^+$ | $pab^+pk\ ad$ | $pab\ pk\ ad$ | $pab\ pk^+ad$ | $pab^+\ pk\ ad$ | $pab^+\ pk\ ad$ | $pab^+\ pk\ ad$ | $pab^+pk\ ad^+$ |
| 5, 6 | $pab^+pk\ ad$ | $pab\ pk^+ad^+$ | $pab^+pk^+ad^+$ | $pab^+pk\ ad^+$ | $pab\ pk\ ad$ | $pab\ pk^+ad$ | $pab^+pk\ ad^+$ | $pab\ pk^+ad^+$ |
| 7, 8 | $pab^+pk\ ad$ | $pab^+pk\ ad$ | $pab^+pk\ ad$ | $pab^+pk\ ad$ | $pab^+pk^+\ ad^+$ | $pab^+pk\ ad^+$ | $pab\ pk^+ad^+$ | $pab^+pk\ ad$ |
| | 34 | 35 | 9 | 7 | 2 | 2 | 1 | 3 |

Based on the data, construct a map of the three genes. Be sure to indicate centromeres.

**HUMAN CHROMOSOMAL MAPS**

**29.** The Duffy blood group with alleles $FY^a$ and $FY^b$ was localized to chromosome 1 in human beings when an "uncoiled" chromosome was associated with it. Construct a pedigree that would verify this.

**30.** What pattern of scores would you expect to get, using the hybrid clones in table 6.8, for a locus on human chromosome 6? 14? X?

**31.** A man with X-linked color blindness and X-linked Fabry disease (alpha-galactosidase-A deficiency) mates with a normal woman and has a normal daughter. This daughter then mates with a normal man and produces ten sons (as well as eight normal daughters). Of the sons, five are normal, three are like their grandfather, one is only color-blind, and one has Fabry disease. From these data, what can you say about the relationship of these two X-linked loci?

**32.** In people, the ABO system ($I^A$, $I^B$, $i$ alleles) is linked to the aldolase-B locus (*ALDOB*), a gene that functions in the liver. Deficiency, which is recessive, results in fructose intolerance. A man with blood type AB has a fructose-intolerant, type B father and a normal, type AB mother. He and a woman with blood type O and fructose intolerance have ten children. Five are type A and normal, three are fructose intolerant and type B, and two are type A and intolerant to fructose. Draw a pedigree of this family and determine the map distances involved. (Calculate a *lod* score to determine the most likely recombination frequency between the loci.)

**33.** Hemophilia and color-blindness are X-linked recessive traits. A normal woman whose mother was color-blind and whose father was a hemophiliac mates with a normal man whose father was color-blind. They have the following children:

4 normal daughters
1 normal son
2 color-blind sons
2 hemophiliac sons
1 color-blind, hemophiliac son

Estimate the distance between the two genes.

**34.** The results of an analysis of five human-mouse hybrids for five enzymes are given in table along with the human chromosomal content of each clone (+ = enzyme or chromosome present; − = absent). Deduce which chromosome carries which gene.

| | Clone | | | | |
|---|---|---|---|---|---|
| **Human Enzyme** | **A** | **B** | **C** | **D** | **E** |
| glutathione reductase | + | + | − | − | − |
| malate dehydrogenase | − | + | − | − | − |
| adenosine deaminase | − | + | − | + | + |
| galactokinase | − | + | + | − | − |
| hexosaminidase | + | − | − | + | − |

| | Human Chromosome | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** |
| Clone A | − | − | − | − | + | + | + | + | − | + | − | − | − | − | + | + | − | − | − | − | + | + |
| Clone B | + | + | − | + | − | − | − | + | − | − | + | − | − | + | − | − | + | − | − | + | − | − |
| Clone C | − | − | − | + | − | − | + | − | − | + | − | + | + | + | + | − | + | − | + | − | − | + |
| Clone D | + | − | + | − | + | − | − | − | − | + | − | − | − | + | + | − | − | + | + | + | + | − |
| Clone E | − | − | − | + | − | − | − | − | + | + | + | + | − | + | − | + | − | + | − | + | + | + |

**35.** You have selected three mouse-human hybrid clones and analyzed them for the presence of human chromosomes. You then analyze each clone for the presence or absence of particular human enzymes (+ = presence of human chromosome or enzyme activity). Based on the following results indicate the probable chromosomal location for each enzyme.

| | Human Chromosomes | | | | | | |
|---|---|---|---|---|---|---|---|
| **Clone** | **3** | **7** | **9** | **11** | **15** | **18** | **20** |
| X | − | + | − | + | + | − | + |
| Y | + | + | − | + | − | + | − |
| Z | − | + | + | − | − | + | + |

| | Enzyme | | | | |
|---|---|---|---|---|---|
| **Clone** | **A** | **B** | **C** | **D** | **E** |
| X | + | + | − | − | + |
| Y | + | − | + | + | + |
| Z | − | − | + | − | + |

**36.** Three mouse-human cell lines were scored for the presence (+) or absence (−) of human chromosomes, with the results as follows:

| | Human Chromosomes | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Clone** | **1** | **2** | **3** | **4** | **5** | **14** | **15** | **18** |
| A | + | + | + | + | − | − | − | − |
| B | + | + | − | − | + | + | − | − |
| C | + | − | + | − | + | − | + | − |

If a particular gene is located on chromosome 3, which clones should be positive for the enzyme from that gene?

## CRITICAL THINKING QUESTIONS

1. Do three-point crosses in fruit flies capture all the multiple crossovers in a region?
2. If 4% of all tetrads have a single crossover between two loci: (a) What is the map distance between these loci if these are fruit flies? (b) What is the proportion of second-division segregants if these are *Neurospora?* (c) What is the proportion of nonparental ditypes if these are yeast?

*Suggested Readings for chapter 6 are on page B-3.*

# 7

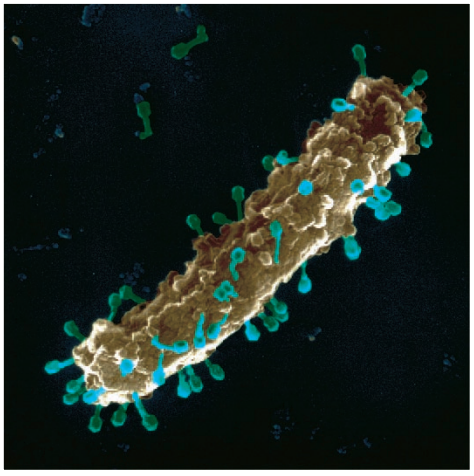
# LINKAGE AND MAPPING IN PROKARYOTES AND BACTERIAL VIRUSES

## 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.

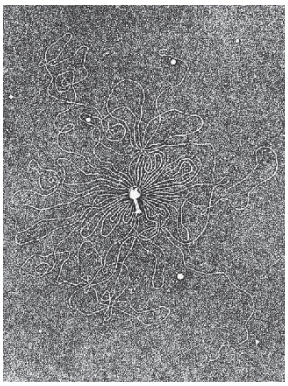
The true bacteria can be classified according to shape: a spherical bacterium is called a **coccus;** a rod-shaped bacterium is called a **bacillus;** and a spiral bacterium is called a **spirillum.** Prokaryotes do not undergo mitosis or meiosis but simply divide in two after their chromosome (usually 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

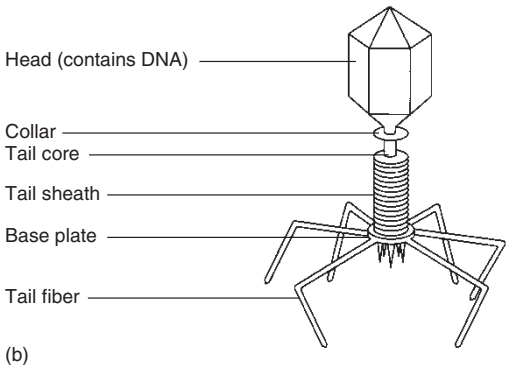
Several properties of bacteria and viruses make them especially suitable for genetic research. First, bacteria and their viruses generally have a short generation time. Some viruses increase three-hundredfold in about a half hour; an *Escherichia coli* cell divides every twenty minutes. In contrast, 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.

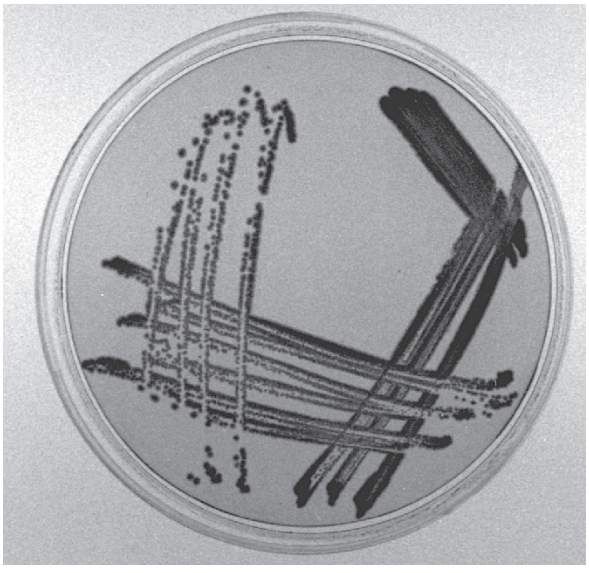


**Figure 7.2** Confluent growth of bacterial colonies on a petri plate. Bacteria were streaked on the petri plate with an inoculation loop—a metal wire with a looped end—covered with bacteria. Streaks began at the upper right and continued around clockwise. With a heavy inoculation on the loop, bacterial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

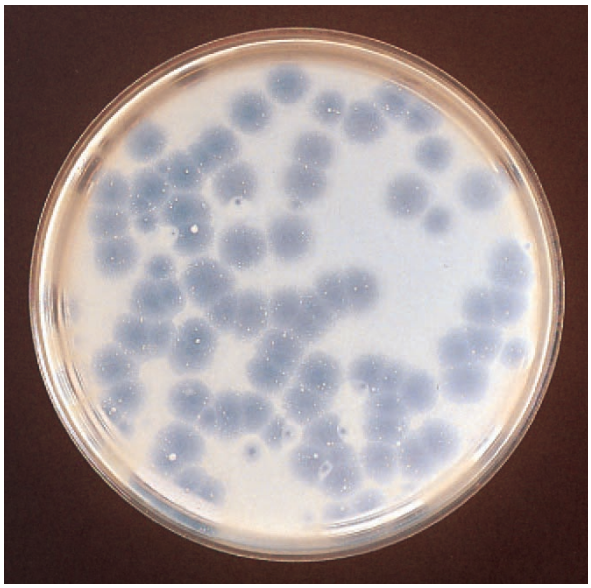


**Figure 7.3** Viral plaques (phage T1) on a bacterial lawn of *E. coli.* (© Bruce Iverson, BSc.)

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

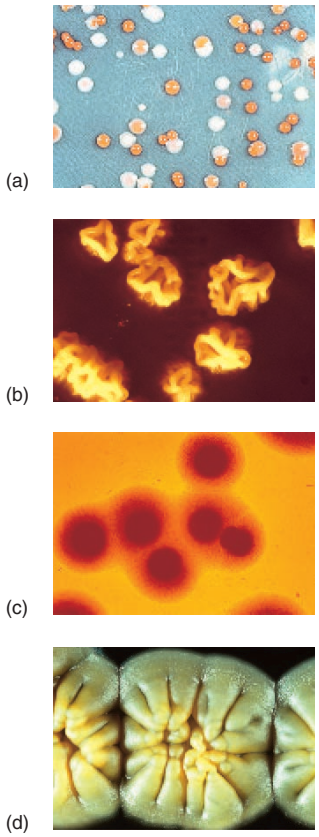



**Figure 7.4** Various bacterial colony forms on agar petri plates. (*a*) Red and white colonies of *Serratia marcescens.* (*b*) Irregular raised folds of *Streptomyces griseus*. (*c*) Round colonies with concentrated centers and diffuse edges of *Mycoplasma.* (*d*) Irregularly folded raised colonies 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.)

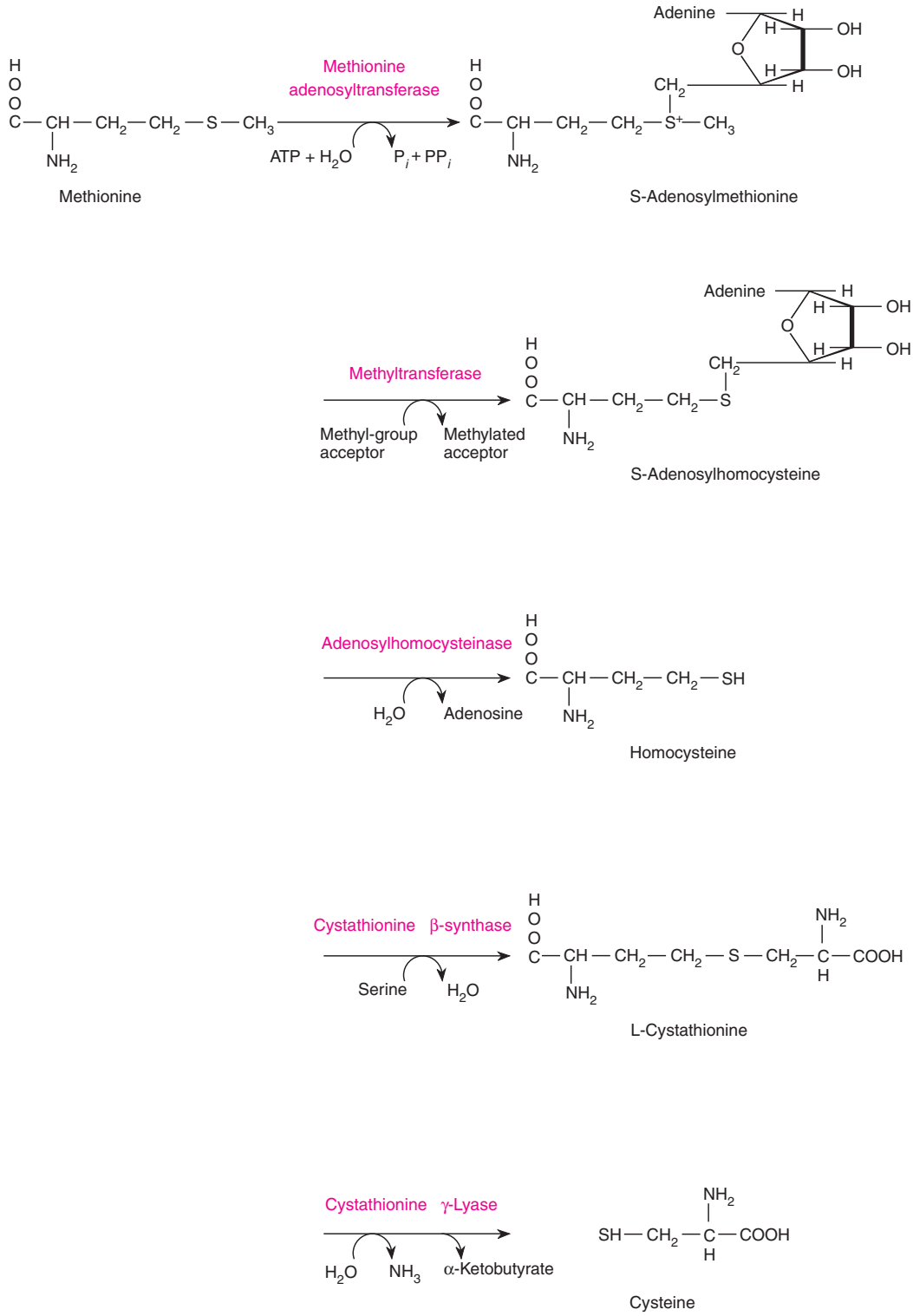
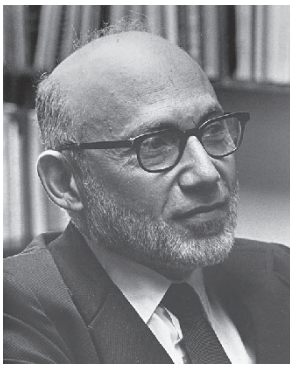



**Figure 7.5** Five-step conversion of methionine to cysteine. Each step is controlled by a different enzyme (*red*).



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

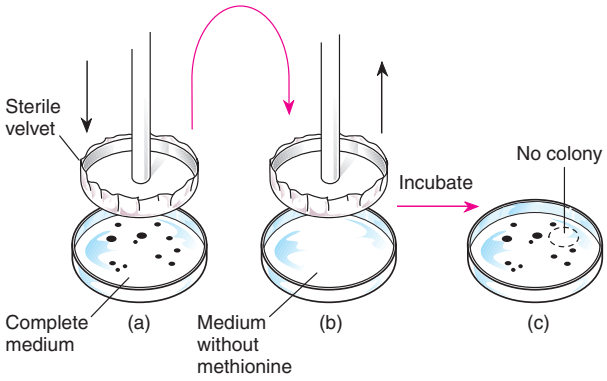



**Figure 7.6** Replica-plating technique. (*a*) A pattern of colonies from a plate of complete medium is transferred (*b*) to a second plate of medium that lacks methionine. (*c*) In the locations where colonies fail to grow on the second plate, we can infer that the original colony was a methionine-requiring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

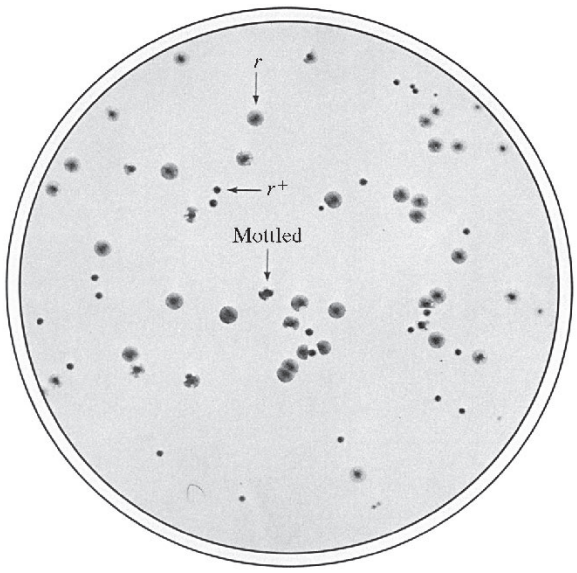



**Figure 7.7** Normal ($r^+$) and rapid-lysis (*r*) mutants of phage T2. Mottled plaques occur when *r* and $r^+$ phages grow together. (From *Molecular Biology of Bacterial Viruses* by Gunther S. Stent. © 1963, 1978 by W. H. Freeman and Company. Used with permission.)

## 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

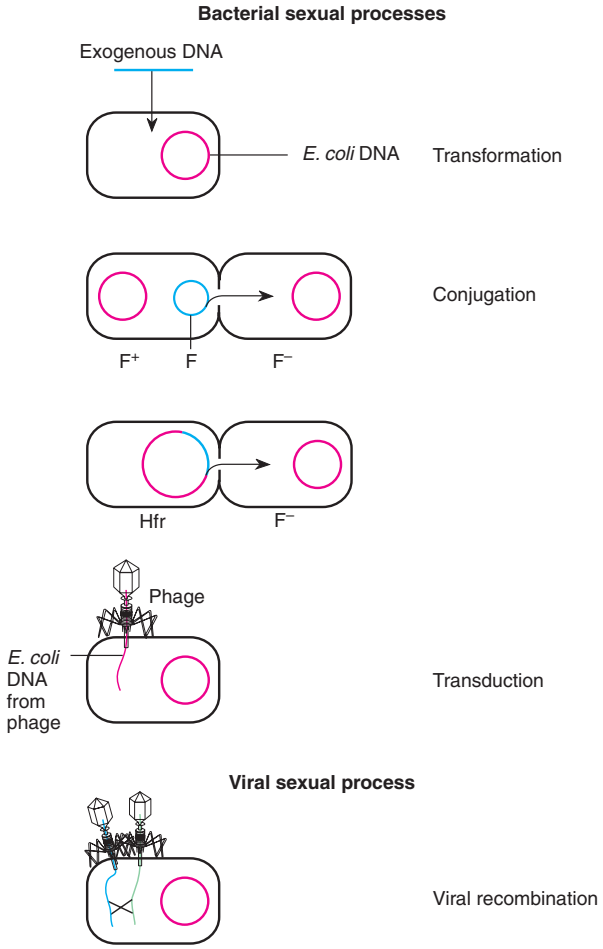



**Figure 7.8** Summary of bacterial and viral sexual processes.

(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

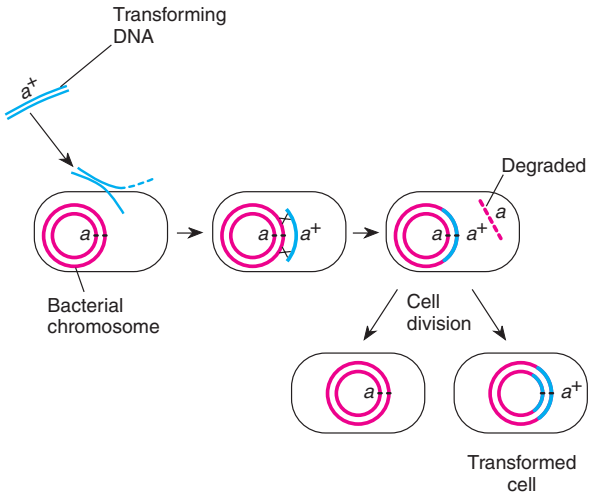


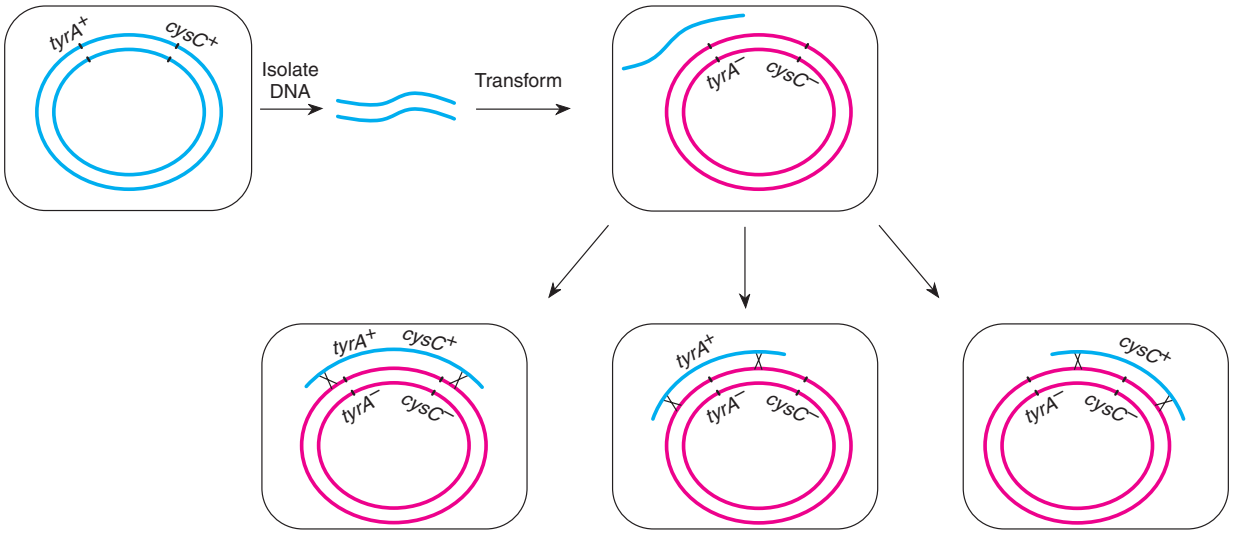

**Figure 7.9** A single strand of transforming DNA (*blue* with $a^+$ allele) enters a bacterial cell (*red* chromosome with *a* allele). Two crossovers bring the foreign DNA into the bacterial chromosome. After DNA replication and cell division, one cell has the *a* allele and the other the $a^+$ allele. 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).

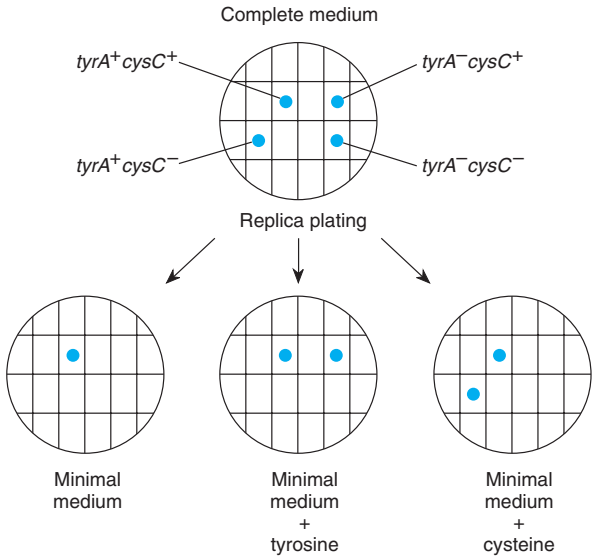



**Figure 7.11** Four patterns of growth on different media reveal the genotypes of transformed and untransformed cells. Only four colonies are shown, and a grid is added for ease of identification. After transformation (see fig. 7.10), cells are plated on complete medium and then replica-plated onto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

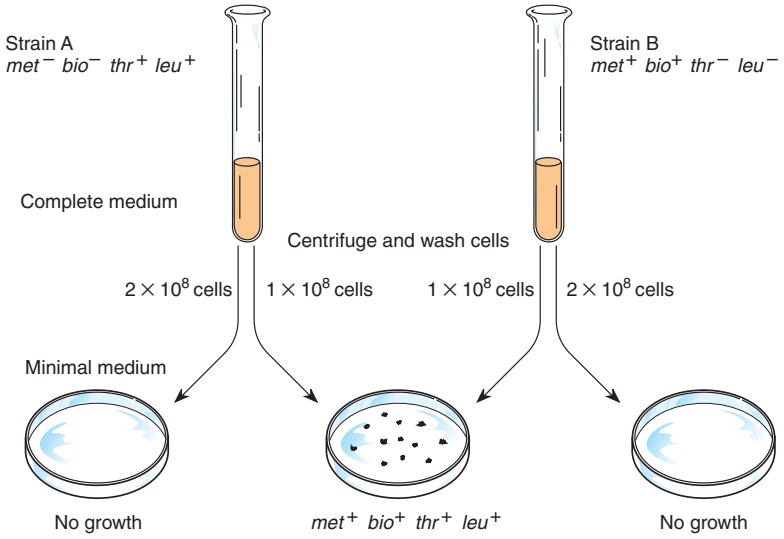



**Figure 7.12** Lederberg and Tatum's cross showing that *E. coli* undergoes genetic recombination.

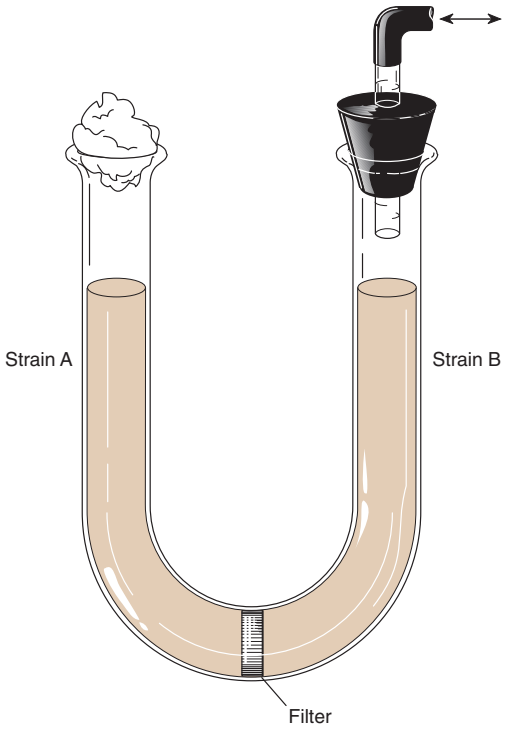



**Figure 7.13** The U-tube experiment. Alternating suction and pressure force liquid and macromolecules back and forth across the filter.

### *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.

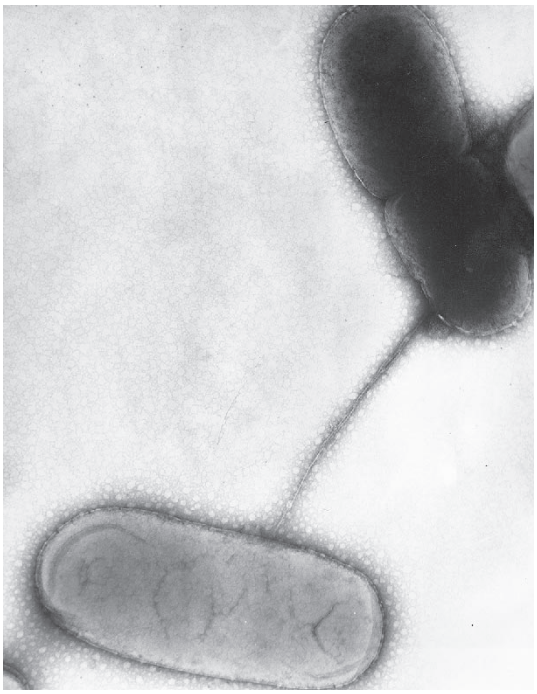


**Figure 7.14** Electron micrograph of conjugation between an $F^+$ (*upper right*) and an $F^-$ (*lower left*) cell with the F-pilus between them. Magnification 3,700×. (Courtesy of Wayne Rosenkrans and Dr. Sonia Guterman.)

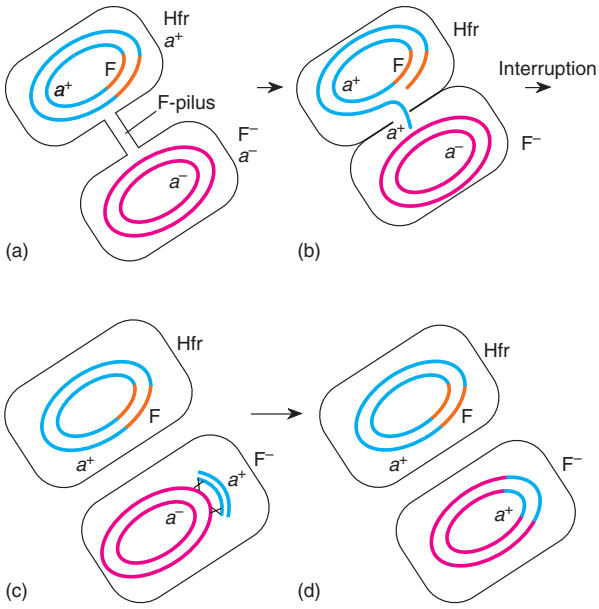



**Figure 7.15** Bacterial conjugation. (*a*) The F-pilus draws an Hfr and an $F^-$ cell close together. (*b*) The Hfr chromosome then begins to pass into the $F^-$ cell, beginning at the F region of the Hfr chromosome but in the direction away from the F factor. Only a single strand passes into the $F^-$ 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.)

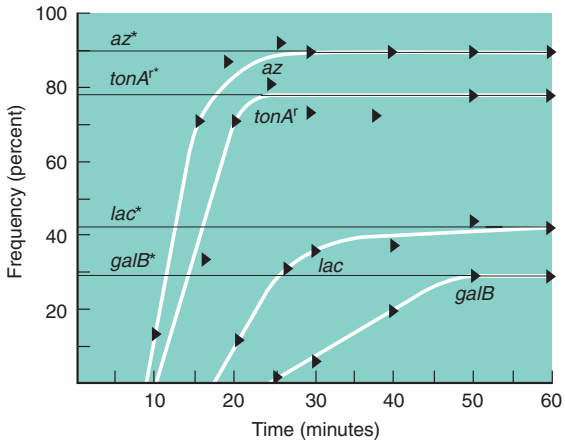
After waiting a specific amount of time, Jacob and Wollman turned the blender on. The spinning motion separated conjugating cells and thereby interrupted their mating. Then the researchers tested the $F^-$ cells for various alleles originally in the Hfr cell. In an experiment like this, the Hfr strain 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.)

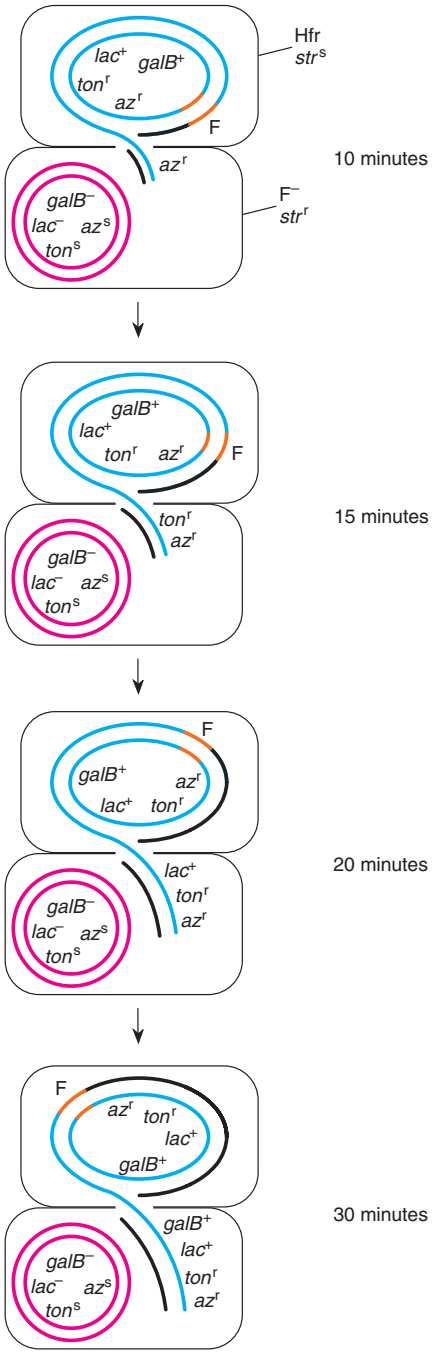



**Figure 7.17** Conjugation in *E. coli*. Hfr chromosome is *blue;* $F^-$ chromosome is *red;* and new DNA replication is *black*. As time proceeds, alleles from the Hfr enter the $F^-$ cell in an orderly, sequential fashion. After the cells separate, two crossovers can bring Hfr alleles into the $F^-$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

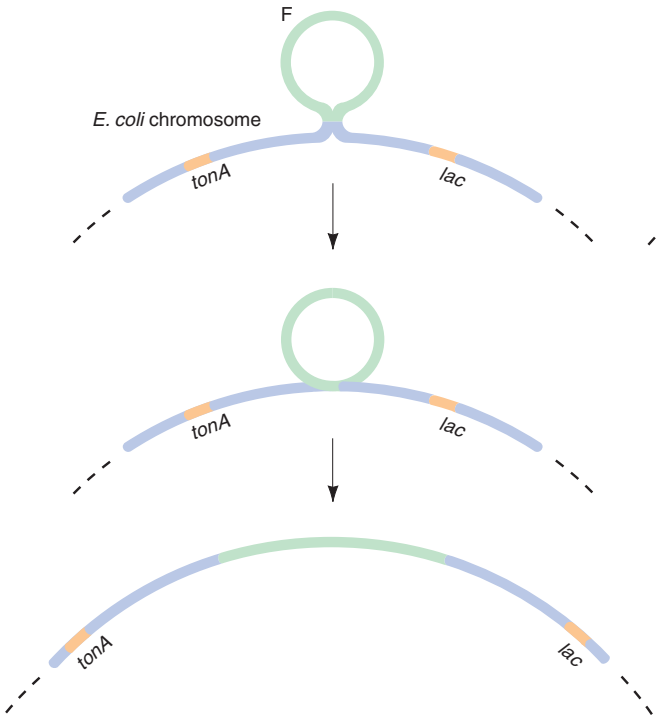



**Figure 7.18** Integration of the F factor by a single crossover. After a simultaneous breakage in both the F factor and the *E. coli* chromosome, the two broken circles reunite to make one large circle, the Hfr chromosome. In this case, the integration occurs between the *tonA* and *lac* loci.

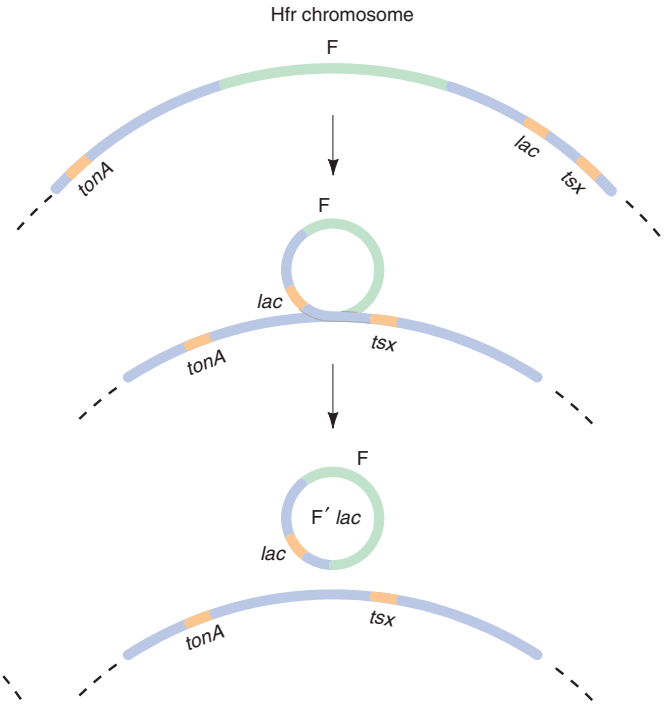



**Figure 7.19** Occasionally, the F factor loops out imprecisely, taking part of the cell's genome in the loop. The circular F factor is freed by a single recombination (crossover) at the loop point.

## 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.

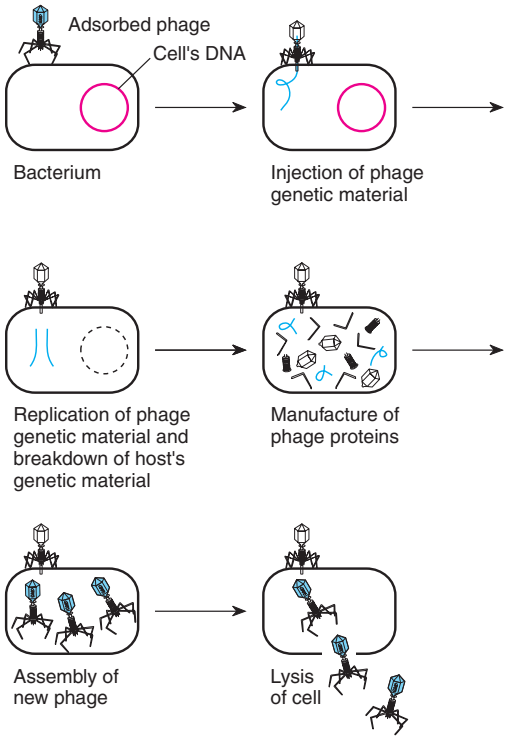



**Figure 7.20** The viral life cycle, using T4 infection of *E. coli* as an example.

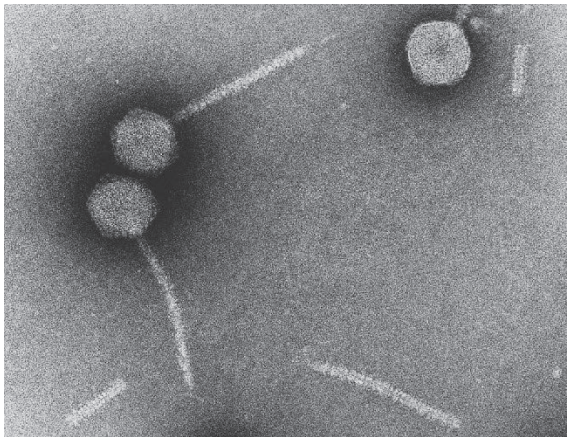


**Figure 7.21** Phage λ. Magnification 167,300×. Note that phage λ lacks the tail fibers and base plate of phage T2 (see fig. 7.1). (Courtesy of Robley Williams.)

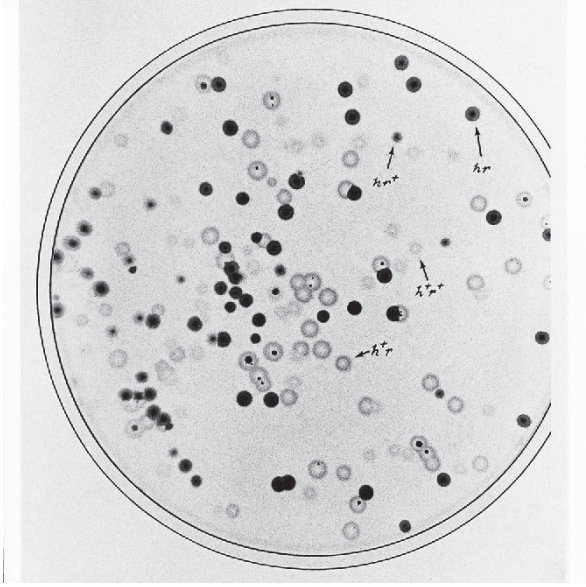


**Figure 7.22** Four types of plaques produced by mixed phage T2 on a mixed lawn of *E. coli*. (From *Molecular Biology of Bacterial Viruses* by Gunther S. Stent, © 1963, 1978 by W. H. Freeman & Company. Used with permission.)

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

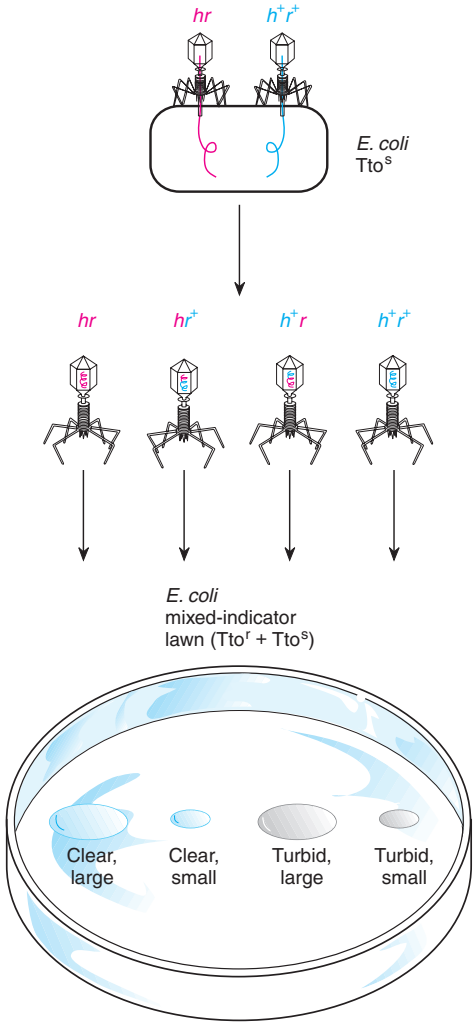



**Figure 7.23** Crossing *hr* and $h^+r^+$ phage. Enough of both types are added to sensitive bacterial cells (Tto$^s$) to ensure multiple infections. The lysate, consisting of four genotypes, is grown on a mixed-indicator bacterial lawn (Tto$^s$ and Tto$^r$). Plaques of four types appear (see fig. 7.22),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

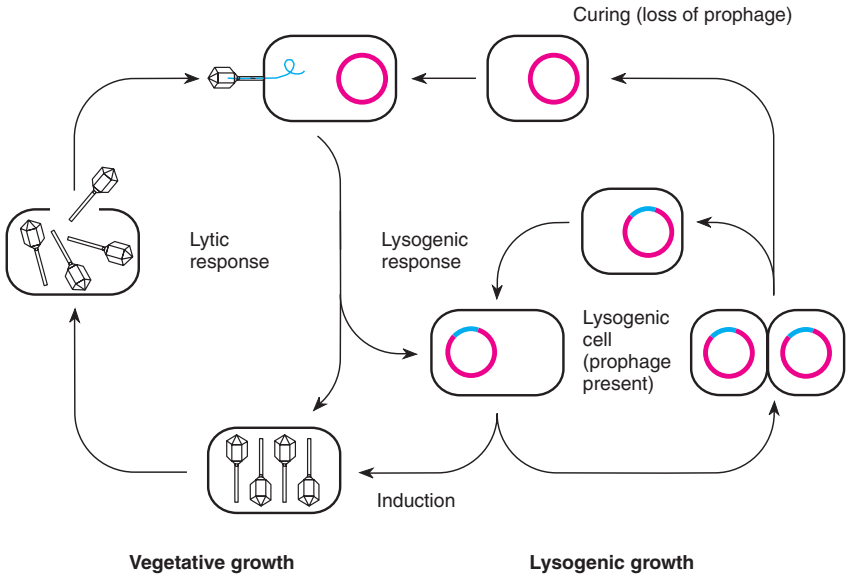



**Figure 7.24** Alternative life-cycle stages of a temperate phage (lysogenic and vegetative growth).

## *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.

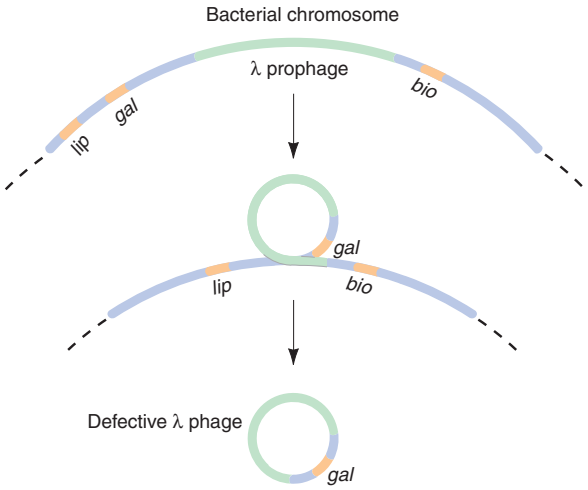



**Figure 7.25** Imprecise excision, or looping out, of the λ prophage, resulting in a defective phage carrying the *gal* locus.

## 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*

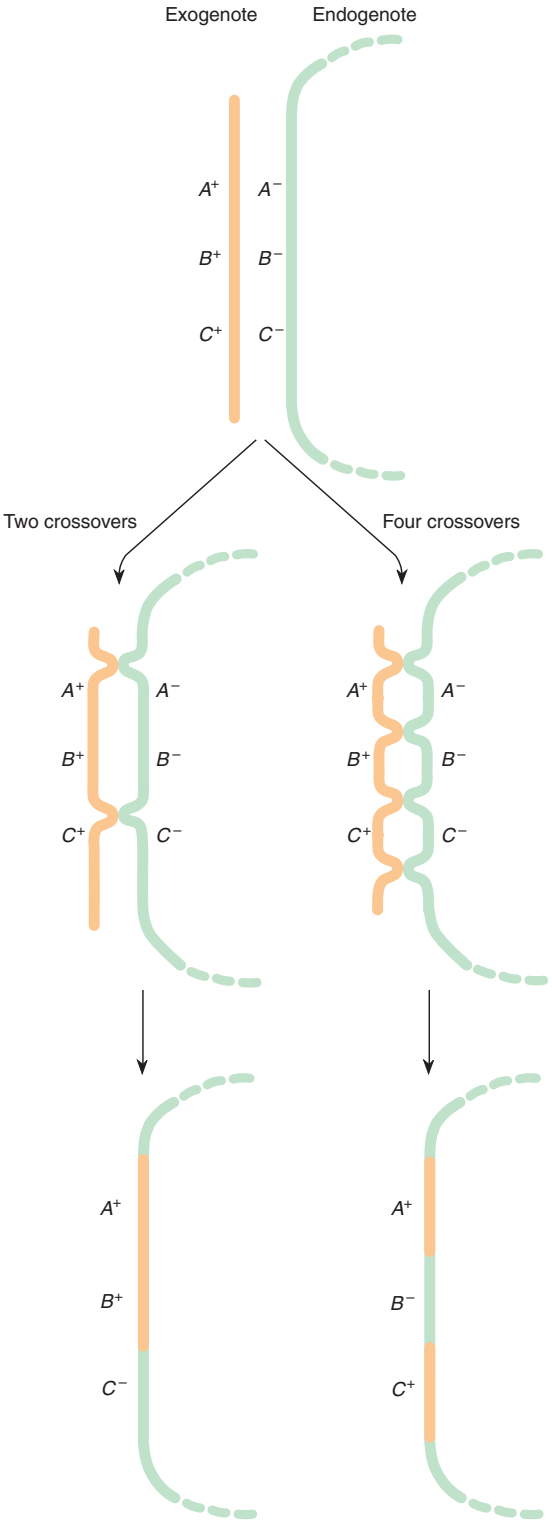



**Figure 7.26** The rarest transductant requires four crossovers.

**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.)

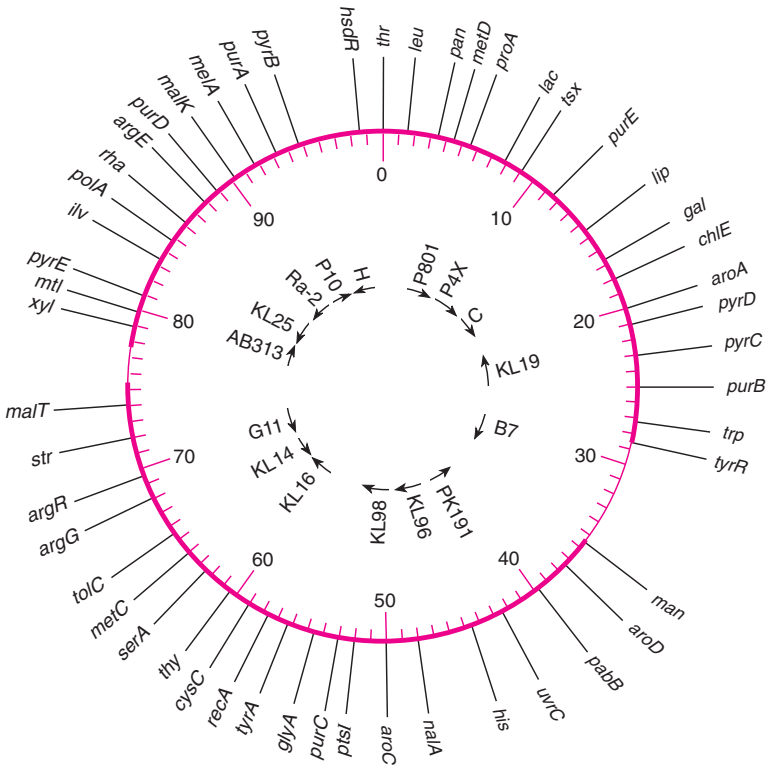



**Figure 7.27** Selected loci on a circular map of *E. coli*. Definitions of loci not found in the text can be found in table 7.8. Units on the map are in minutes. *Arrows* within the circle refer to Hfr-strain transfer starting points, with directions indicated. The two thin regions on the outer circle 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*$\lambda^-$ 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*$\lambda^-$ | 71 |
| Class 2 $gal^+$ $bio^+$ *att*$\lambda^-$ | 0 |
| Class 3 $gal^+$ $bio^-$ *att*$\lambda^+$ | 9 |
| Class 4 $gal^+$ $bio^+$ *att*$\lambda^+$ | 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*$\lambda$ 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*$\lambda$; 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?

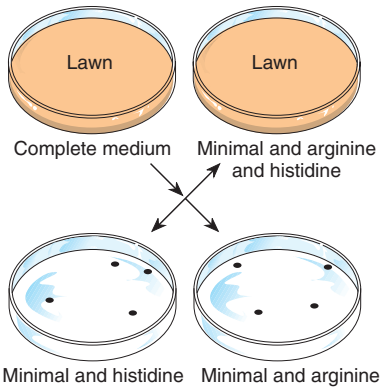
### BACTERIAL PHENOTYPES

**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?

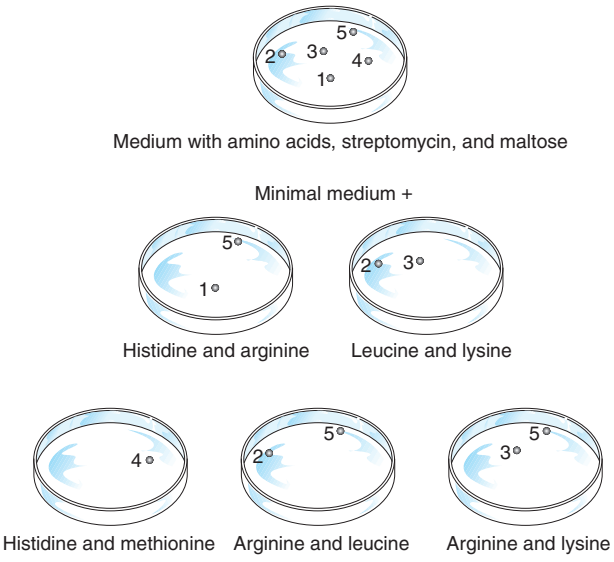
**5.** An *E. coli* cell is placed on a petri plate containing $\lambda$ phages. It produces a colony overnight. By what mechanisms might it have survived?

**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 $250 \times 10^7$ phage. By dilution, approximately 1/10,000 of the progeny are plated on K12 to generate these wild-type recombinants (plaques on K12):

| | |
|---|---|
| $1 \times 2$ | 50 |
| $1 \times 3$ | 25 |
| $2 \times 3$ | 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.*