Figure 7.18
An individual with xeroderma pigmentosum.

Transposable elements fall into two general classes based on how they move from location to location in the genome. One class—found in both prokaryotes and eukaryotes—moves as a DNA segment. Members of the other class—found only in eukaryotes—are related to retroviruses and move via an RNA. First an RNA copy of the element is synthesized; then a DNA copy of that RNA is made, and it integrates at a new site in the genome.

In bacteria, transposable elements can move to new positions on the same chromosome (because there is only one chromosome) or onto plasmids or phage chromosomes; in eukaryotes, transposable elements may move to new positions within the same chromosome or to a different chromosome. In both bacteria and eukaryotes, transposable elements insert into new chromosome locations with which they have no sequence homology; therefore, transposition is a process different from homologous recombination (recombination between matching DNA sequences) and is called *nonhomologous recombination*. Transposable elements are important due to the genetic changes they cause. For example, they can produce mutations by inserting into genes (a process called *insertional mutagenesis*), they can increase or decrease gene expression by inserting into gene regulatory sequences (such as by disrupting promoter function or stimulating a gene's expression through the activity of promoters on the element), and they can produce various kinds of chromosomal mutations through the mechanics of transposition. In fact, transposable elements have made important contributions to the evolution of the genomes of both bacteria and eukaryotes through the chromosome rearrangements they have caused.

The frequency of transposition, though typically low, varies with the particular element. If the frequency were high, the genetic changes caused by the transpositions would likely kill the cell.

Transposable Elements in Bacteria

Two examples of transposable elements in bacteria are insertion sequence (IS) elements and transposons (Tn).

Insertion Sequences. An **insertion sequence (IS)**, or **IS element**, is the simplest transposable element found in bacteria. An IS element contains only genes required to mobilize the element and insert it into a new location in the genome. IS elements are normal constituents of bacterial chromosomes and plasmids.

IS elements were first identified in *E. coli* as a result of their effects on the expression of three genes that control the metabolism of the sugar galactose. Some mutations affecting the expression of these genes did not have properties typical of point mutations or deletions, but rather had an insertion of an approximately 800-bp DNA segment into a gene. This particular DNA segment is now called *insertion sequence 1*, or IS*1* (Figure 7.19), and the insertion of IS*1* into the genome is an example of a *transposition event*.

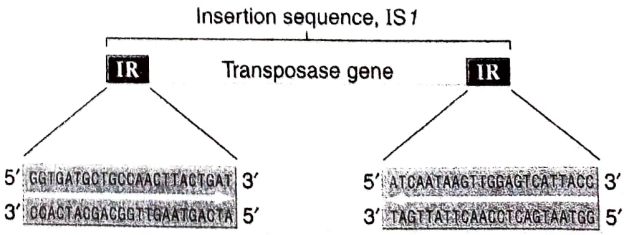
animation **Insertion Sequences in Bacteria**

E. coli contains a number of IS elements (e.g., IS1, IS2, and IS*10R*), each present in up to 30 copies per genome and each with a characteristic length and unique nucleotide sequence. IS*1* (see Figure 7.19), for instance, is 768 bp long and is present in 4 to 19 copies on the *E. coli* chromosome. Among bacteria as a whole, the IS elements range in size from 768 bp to more than 5,000 bp and are found in most cells.

All IS elements end with perfect or nearly perfect terminal inverted repeats (IRs) of 9 to 41 bp. This means that essentially the same sequence is found at each end of an IS, but in opposite orientations. The inverted repeats of IS*1* are 23 bp long (see Figure 7.19).

When IS elements integrate at random points along the chromosome, they often cause mutations by disrupting either the coding sequence of a gene or a gene's regulatory region. Promoters within the IS elements themselves may also have effects by altering the expression of nearby genes. In addition, the presence of an IS element in the chromosome can cause mutations such as deletions and inversions in the adjacent DNA. Finally, deletion and

Figure 7.19
The insertion sequence (IS) transposable element IS*1*. The 768-bp IS element has inverted repeat (IR) sequences at the ends. Shown below the element are the sequences for the 23-bp terminal inverted repeats (IR).



insertion events can also occur as a result of crossing-over between duplicated IS elements in the genome.

The transposition of an IS element requires an enzyme encoded by the IS element called **transposase**. The transposase recognizes the IR sequences of the element to initiate transposition. The frequency of transposition is characteristic of each IS element and ranges from 10^{-5} to 10^{-7} per generation.

Figure 7.20 shows how an IS element inserts into a new location in a chromosome. Insertion takes place at a *target site* with which the element has no sequence homology. First, a staggered cut is made in the target site and the IS element is then inserted, becoming joined to the single-stranded ends. DNA polymerase and DNA ligase fill in the gaps, producing an integrated IS element with two direct repeats of the target-site sequence flanking the IS element. In this case, *direct* means that the two sequences are repeated in the same orientation (see Figure 7.20). The direct repeats are called *target-site duplications*. Their size is specific to the IS element, but they tend to be small (4 to 13 bp).

Transposons. Like an IS element, a **transposon (Tn)** contains genes for the insertion of the DNA segment into the chromosome and mobilization of the element to other locations on the chromosome. A transposon is more complex than an IS element in that it contains additional genes.

There are two types of bacterial transposons: composite transposons and noncomposite transposons (Figure 7.21). *Composite transposons* (Figure 7.21a), exemplified by Tn*10*, are complex transposons with a central region containing genes (for example, genes that confer resistance to antibiotics), flanked on both sides by IS elements (also called *IS modules*). Composite transposons may be thousands of base pairs long. The IS elements are both of the same type and are called IS*L* (for "left") and IS*R* (for "right"). Depending on the transposon, IS*L* and IS*R* may be in the same or inverted orientation relative to each other. Because the ISs themselves have terminal inverted repeats, the composite transposons also have terminal inverted repeats.

Transposition of composite transposons occurs because one or both IS elements supply the transposase, which recognizes the inverted repeats of the IS elements at the two ends of the transposon and initiates transposition (as with the transposition of IS elements). Transposition of Tn*10* is rare, occurring once in 10^7 cell generations. Like IS elements, composite transposons produce target-site

Figure 7.20

Process of integration of an IS element into chromosomal DNA. As a result of the integration event, the target site becomes duplicated, producing direct target repeats. Thus, the integrated IS element is characterized by its inverted repeat (IR) sequences, flanked by direct target-site duplications. Integration involves making staggered cuts in the host target site. After insertion of the IS, the gaps that result are filled in with DNA polymerase and DNA ligase. (*Note*: The base sequences given for the IR are for illustration only and are neither the actual sequences found nor their actual length.)

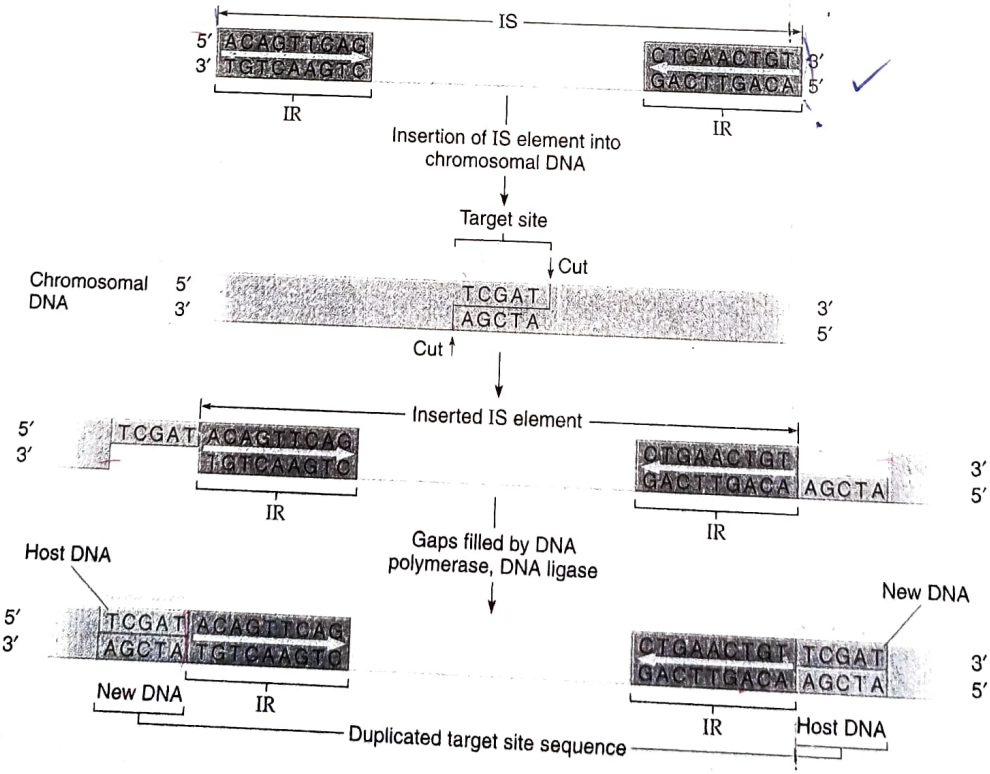
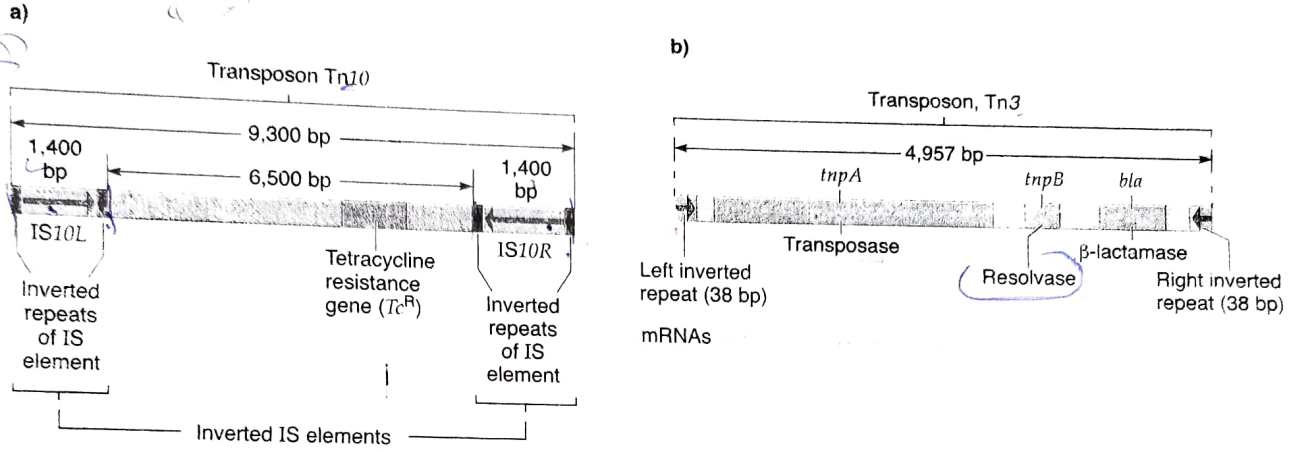


Figure 7.21

Structures of bacterial transposons. (a) The composite transposon Tn*10*. The general features of composite transposons are a central region carrying a gene or genes, such as a gene for drug resistance, flanked by either direct or inverted IS elements. The Tn*10* transposon is 9,300 bp long and consists of 6,500 bp of central, nonrepeating DNA containing the tetracycline resistance gene, flanked at each end with 1,400-bp IS elements IS*10L* and IS*10R* arranged in an inverted orientation. The IS elements themselves have terminal inverted repeats. (b) The noncomposite transposon Tn3. The 4,957-bp Tn3 has genes for three enzymes in its central region: *bla* encodes β-lactamase (destroys antibiotics such as penicillin and ampicillin), *tnpA* encodes transposase, and *tnpB* encodes resolvase. Transposase and resolvase are involved in the transposition process. Tn3 has 38-bp terminal inverted repeats that are unrelated to IS elements.



duplications after transposition. In the case of Tn*10*, the target-site duplications are 9bp long.

Noncomposite transposons (Figure 7.21b), exemplified by Tn3, also contain genes such as those conferring resistance to antibiotics, but they do not terminate with IS elements. However, at their ends they have inverted repeated sequences that are required for transposition. Enzymes for transposition are encoded by genes in the central region of noncomposite transposons. Transposase catalyzes the insertion of a transposon into new sites, and resolvase is an enzyme involved in the particular recombinational events associated with transposition. Like composite transposons, noncomposite transposons cause target-site duplications when they move. For example, Tn3 produces a 5-bp target-site duplication when it inserts into the genome.

Figure 7.22 shows a *cointegration* mechanism for the transposition of a transposon from one DNA to another (e.g., from a plasmid to a bacterial chromosome, or vice versa). Similar events can occur between two locations on the same chromosome. First, the donor DNA containing the transposable element fuses with the recipient DNA to form a *cointegrate*. Because of the way this occurs, the transposable element is duplicated and one copy is located at each junction between donor and recipient DNA. Next, recombination between the duplicated transposable elements resolves the cointegrate into two genomes, each with one copy of the element. Because the transposable element becomes duplicated, the process is called *replicative transposition* (also called *copy-and-paste transposition*). Tn3 and related noncomposite transposons move by replicative transposition.

A second type of transposition mechanism involves the movement of a transposable element from one location to another on the same or different DNA *without* replication of the element. This mechanism is called *conservative (nonreplicative) transposition* (also called *cut-and-paste transposition*). In other words, the element is lost from the original position when it transposes. Tn*10* transposes by conservative transposition.

As with the movement of IS elements, the transposition of transposons can cause mutations. The insertion of a transposon into the reading frame of a gene disrupts it, causing a loss-of-function mutation of that gene. Insertion into a gene's controlling region can cause changes in the level of expression of the gene, depending on the promoter elements in the transposon and how they are oriented with respect to the gene. Deletion and insertion events also result from the activities of the transposons and from crossing-over between duplicated transposons in the genome.

iActivity

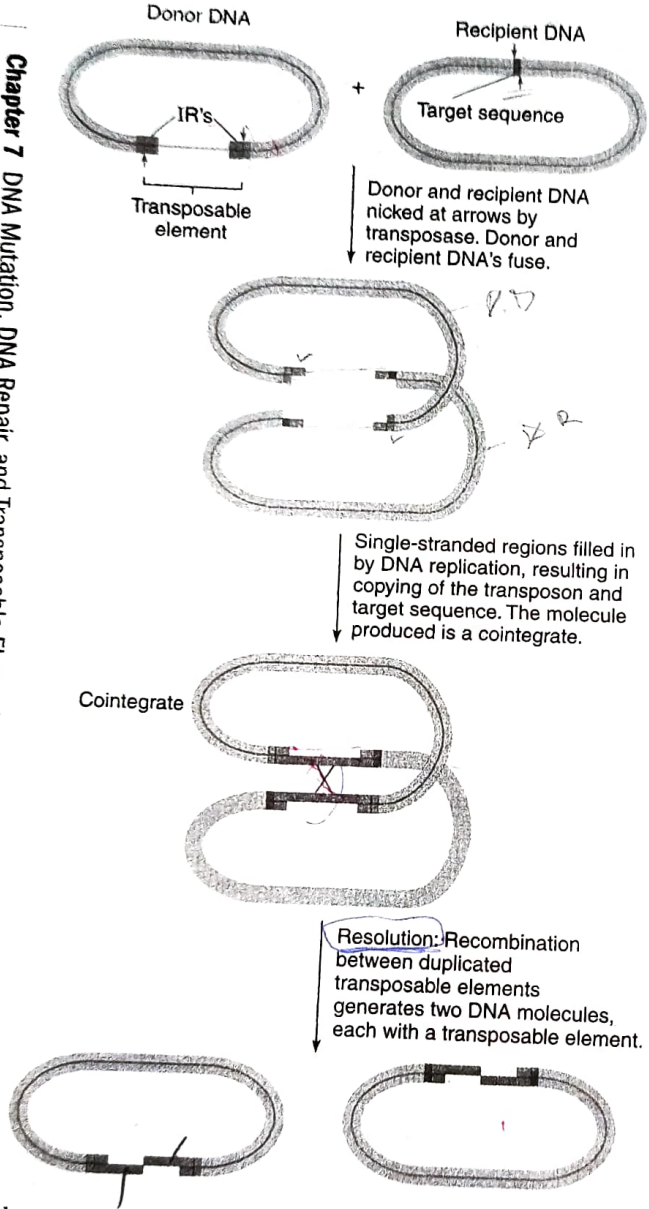
Go to the iActivity *The Genetics Shuffle* on the student website, where you will assume the role of a researcher in a genetics lab investigating how the Tn*10* transposon is transposed.

Transposable Elements in Eukaryotes

Transposable elements have been identified in many eukaryotes. They have been studied extensively, with most research being done with yeast, *Drosophila*, corn, and

Figure 7.22

Cointegration model for the replicative transposition of a transposable element. A donor DNA with a transposable element fuses with a recipient DNA. During the fusion, the transposable element is duplicated, so that the product is a cointegrate molecule with one transposable element at each junction between donor and recipient DNA. The cointegrate is resolved by recombination into two molecules, each with one copy of the transposable element.



humans. In general, their structure and function are similar to those of prokaryotic transposable elements. Functional eukaryotic transposable elements have genes that encode enzymes required for transposition, and they can integrate into chromosomes at a number of sites. Thus, such elements may affect the function of any gene. Typically, the effects range from activation or repression of adjacent genes to chromosome mutations such as duplications, deletions, inversions, translocations, or breakage. That is, as with bacterial IS elements and transposons, the transposition of transposable element into genes generally causes mutations. Disruption of the amino acid-coding region of a gene typically results in a *null mutation*, which is a mutation that reduces the expression of the gene to zero. If a transposable element moves into the promoter of a gene, the efficiency of that promoter can be decreased or obliterated. Alternatively, the transposable element may provide promoter function itself and lead to an *increase* in gene expression.

General Properties of Plant Transposable Elements. Like some of the transposable elements discussed earlier, plant transposable elements have inverted repeated (IR) sequences at their ends and generate short, direct repeats of the target-site DNA when they integrate.

Transposable elements have been particularly well studied in corn. Geneticists have identified several families of transposable elements. Each family consists of a characteristic array of transposable elements with respect to numbers, types, and locations. Each family has two forms of transposable elements: *autonomous elements*, which can transpose by themselves, and *nonautonomous elements*, which cannot transpose by themselves because they lack the gene for transposition. The nonautonomous elements require an autonomous element to supply the missing functions. Often, the nonautonomous element is a defective derivative of the autonomous element in the family. When an autonomous element is inserted into a host gene, the resulting mutant allele is *unstable*, because the element can excise and transpose to a new location. This transposition event results in restoration of function of the gene. The frequency of transposition out of a gene is higher than the spontaneous reversion frequency for a regular point mutation; therefore, the allele produced by an autonomous element is called a *mutable allele*.

Animation
Transposable Elements in Plants

By contrast, mutant alleles resulting from the insertion of a nonautonomous element in a gene are *stable*, because the element is unable to transpose out of the locus by itself. However, if the autonomous element of its family is also either already present in, or introduced into, the same genome, the autonomous element can provide the enzymes needed for transposition, and the nonautonomous element can then transpose.

McClintock's Study of Transposable Elements in Corn. In the 1940s and 1950s, Barbara McClintock did a series of elegant genetic experiments with *Zea mays* (corn) that led her to hypothesize the existence of what she called "controlling elements," which modify or suppress gene activity in corn and are mobile in the genome. Decades later, the controlling elements she studied were shown to be transposable elements. McClintock was awarded the 1983 Nobel Prize in Physiology or Medicine for her "discovery of mobile genetic elements." A fascinating and moving biographical sketch of Barbara McClintock is given in Box 7.1.

Box 7.1 Barbara McClintock (1902–1992)

Barbara McClintock's remarkable life spanned the history of genetics in the twentieth century. She was born in Hartford, Connecticut, to Sara Handy McClintock, an accomplished pianist, poet, and painter, and Thomas Henry McClintock, a physician. Both parents were quite unconventional in their attitudes toward rearing children: They were interested in what their children would and could be rather than what they should be.

During her high school years, Barbara discovered science, and she loved to learn and figure things out. After high school, Barbara attended Cornell University, where she flourished both socially and intellectually. She enjoyed her social life, but her comfort with solitude and the tremendous joy she experienced in knowing, learning, and understanding things were to be the defining themes of her life. The decisions she made during her university years were consistent with her adamant individuality and self-containment. In Barbara's junior year, after a particularly exciting undergraduate course in genetics, her professor invited her to take a graduate course in genetics. After that, she was treated much like a graduate student. By the time she had finished her undergraduate course work, there was no question in her mind: She had to continue her studies of genetics.

At Cornell, genetics was taught in the plant-breeding department, which at the time did not take female graduate students. To circumvent this obstacle, McClintock registered in the botany department with a major in cytology and a minor in genetics and zoology. She began to work as a paid assistant to Lowell Randolph, a cytologist. McClintock and Randolph did not get along well and soon dissolved their working relationship, but as McClintock's colleague and lifelong friend Marcus Rhoades later wrote, "Their brief association was momentous because it led to the birth of maize cytogenetics." McClintock discovered that metaphase or late-prophase chromosomes in the first microspore mitosis were far better for cytological discrimination than were root-tip chromosomes. In a few weeks, she prepared detailed drawings of the maize chromosomes, which she published in *Science*.

This was McClintock's first major contribution to maize genetics, and it laid the groundwork for a veritable explosion of discoveries that connected the behavior of chromosomes to the genetic properties of an organism, defining the new field of cytogenetics. McClintock was awarded a Ph.D. in 1927 and appointed an instructor at Cornell, where she continued to work with maize. The Cornell maize genetics group was small. It included Professor R. A. Emerson, the founder of maize genetics, as well as McClintock, George Beadle, C. R. Burnham, Marcus Rhoades, and Lowell Randolph, together with a few graduate students. By all accounts, McClintock was the intellectual driving force of this talented group.

In 1929, a new graduate student, Harriet Creighton, joined the group and was guided by McClintock. Their work showed, for the first time, that genetic recombination is a reflection of the physical exchange of chromosome segments. A paper on their work, published in 1931, was perhaps McClintock's first seminal contribution to the science of genetics.

Barbara McClintock in 1947.

Although McClintock's fame was growing, she had no permanent position. Cornell had no female professors in fields other than home economics, so her prospects were dismal. She had already attained international recognition, but as a woman, she had little hope of securing a permanent academic position at a major research university. R. A. Emerson obtained a grant from the Rockefeller Foundation to support her work for two years, allowing her to continue to work independently. McClintock was discouraged and resentful of the disparity between her prospects and those of her male counterparts. Her extraordinary talents and accomplishments were widely appreciated, but she was also seen as difficult by many of her colleagues, in large part because of her quick mind and intolerance of second-rate work and thinking.

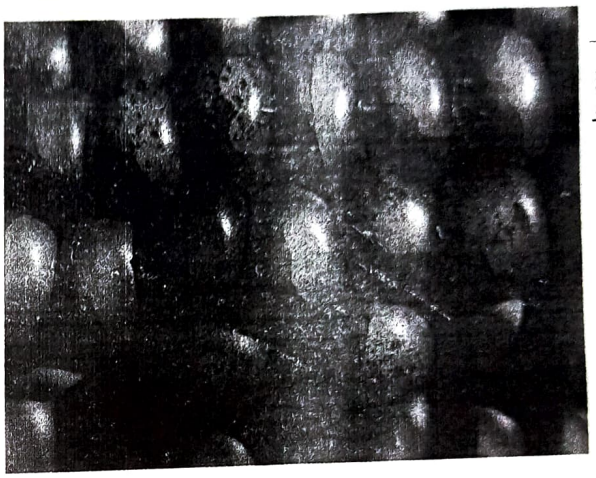
In 1936, Lewis Stadler convinced the University of Missouri to offer McClintock an assistant professorship. She accepted the position and began to follow the behavior of maize chromosomes that had been broken by X irradiation. However, soon after her arrival at Missouri, she understood that hers was a special appointment. She found herself excluded from regular academic activities, including faculty meetings. In 1941, she took a leave of absence from Missouri and departed with no intention of returning. She wrote to her friend Marcus Rhoades, who was planning to go to Cold Spring Harbor, New York, for the summer to grow his corn. An invitation for McClintock was arranged through Milislav Demerec (a member, and later the director, of the genetics department at the Carnegie Institution of Washington, then the dominant research laboratory at Cold Spring Harbor), who offered her a year's research appointment. Though hesitant to commit herself, McClintock accepted. When Demerec later offered

McClintock studied the genetics of corn kernel pigmentation. A number of different genes must function together to synthesize of red anthocyanin pigment, which gives the corn kernel a purple color. Mutation of any one of these genes causes a kernel to be unpigmented. McClintock studied kernels that, rather than being either of a solid color or colorless, had spots of purple pigment on an otherwise colorless kernel (Figure 7.23). She knew that the phenotype was the result of an unstable mutation. From her careful genetic and cytological studies, she concluded that the spotted phenotype was not the result of any conventional kind of mutation (such as a point mutation), but rather the result of a controlling element, which we now know is a transposon.

The explanation for the spotted kernels McClintock studied is as follows: If the corn plant carries a wild-type *C* gene, the kernel is purple; *c* (colorless) mutations are defective in purple pigment production, so the kernel is colorless. During kernel development, revertants of the mutation occur, leading to a spot of purple pigment. The earlier in development the reversion occurs, the larger is the purple spot. McClintock determined that the original *c* (colorless) mutation resulted from a "mobile controlling element" (in modern terms, a transposable element), called *Ds* for "dissociation," being inserted into the *C* gene (Figures 7.24a and 7.24b). We now know *Ds* is a nonautonomous element. Another mobile controlling element, an autonomous element called *Ac* for "activator," is required for transposition of *Ds* into the gene. *Ac* can also result in *Ds* transposing (excising perfectly in this case)

Figure 7.23

Corn kernels, some of which show spots of pigment produced by cells in which a transposable element had transposed out of a pigment-producing gene, thereby allowing the gene's function to be restored. The cells in the white areas of the kernel lack pigment because a pigment-producing gene continues to be inactivated by the presence of a transposable element within that gene.



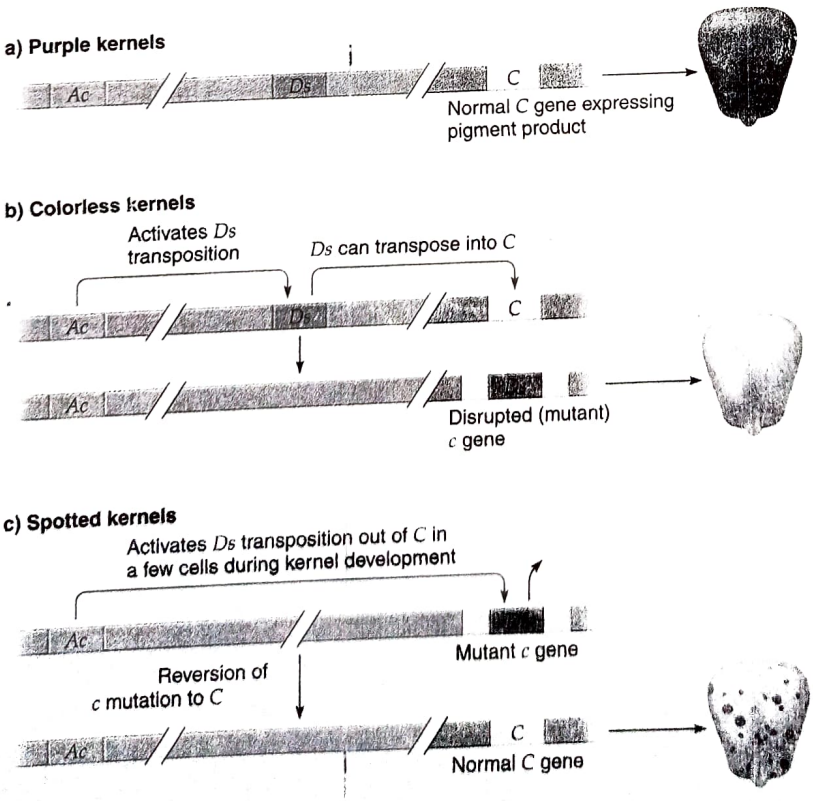


Figure 7.24

Kernel color and transposable element effects in corn. (a) Purple kernels result from the active *C* gene. (b) Colorless kernels can result when the *Ac* transposable element activates *Ds* transposition and *Ds* inserts into *C*, producing a mutation. (c) Spotted kernels result from reversion of the *c* mutation during kernel development when *Ac* activates *Ds* transposition out of the *C* gene.

out of the c gene, giving a wild-type revertant with a purple spot (Figure 7.24c).

The remarkable fact of McClintock's conclusion was that, at the time, there was no precedent for the existence of transposable genetic elements. Rather, the genome was thought to be static with regard to gene locations. Only much more recently have transposable genetic elements been widely identified and studied, and only in 1983 was direct evidence obtained for the movable genetic elements proposed by McClintock.

The Ac-Ds Transposable Elements in Corn. The *Ac-Ds* family of controlling elements has been studied in detail. The autonomous *Ac* element is 4,563 bp long, with short terminal inverted repeats and a single gene encoding the transposase. Upon insertion into the genome, it generates an 8-bp direct duplication of the target site. *Ds* elements are heterogeneous in length and sequence, but all have the same terminal IRs as *Ac* elements, because most have been generated from *Ac* by the deletion of segments or by more complex sequence rearrangements. As a result, *Ds* elements have no complete transposase gene; hence, these elements cannot transpose on their own.

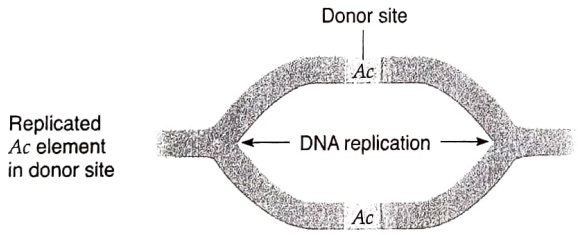
Transposition of the *Ac* element occurs only during chromosome replication and is a result of the cut-and-paste (conservative) transposition mechanism (Figure 7.25). Consider a chromosome with one copy of *Ac* at a site called the *donor site*. When the chromosome region containing *Ac* replicates, two copies of *Ac* result, one on each progeny chromatid! There are two possible results of *Ac* transposition, depending on whether it occurs to a replicated or an unreplicated chromosome site.

If one of the two *Ac* elements transposes to a replicated chromosome site (Figure 7.25a), an empty donor site is left on one chromatid, and an *Ac* element remains in the homologous donor site on the other chromatid. The transposing *Ac* element inserts into a new, already replicated recipient site, which is often on the same chromosome. In Figure 7.25a, the site is shown on the same chromatid as the parental *Ac* element. Thus, in the case of transposition to an already replicated site, there is no net increase in the number of *Ac* elements.

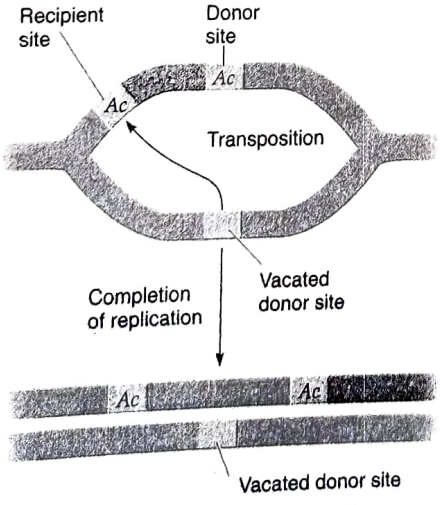
Figure 7.25b shows the transposition of one *Ac* element to an unreplicated chromosome site. As in the first case, one of the two *Ac* elements transposes, leaving an empty donor site on one chromatid and an *Ac* element in

Figure 7.25

The *Ac* transposition mechanism. (a) Transposition to an already replicated recipient site results in no net increase in the number of *Ac* elements in the genome. (b) Transposition to an unreplicated recipient site results in a net increase in the number of *Ac* elements when the region of the chromosome containing the transposed element is replicated.

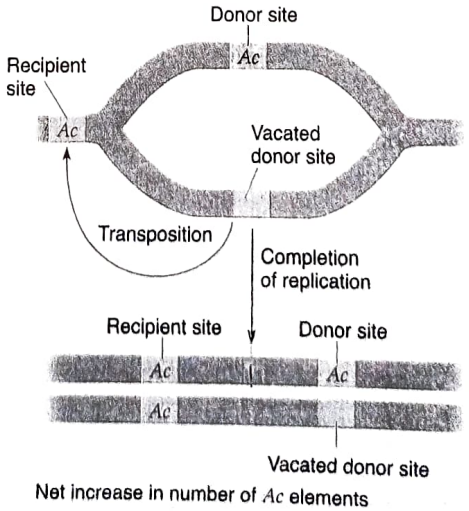


a) Transposition to an already replicated recipient site



No net increase in number of *Ac* elements

b) Transposition to an unreplicated recipient site



Net increase in number of *Ac* elements

the homologous donor site on the other chromatid. But now the transposing element inserts into a nearby recipient site that has yet to be replicated. When that region of the chromosome replicates, the result will be a copy of the transposed *Ac* element on both chromatids, in addition to the one original copy of the *Ac* element at the donor site on one chromatid. Thus, in the case of transposition to an unreplicated recipient site, there is a net increase in the number of *Ac* elements.

The transposition of most *Ds* elements occurs in the same way as *Ac* transposition, using transposase supplied by an *Ac* element in the genome.

Keynote

The transposition mechanism of plant transposable elements is similar to that of bacterial IS elements or transposons. Transposable elements integrate at a target site by a precise mechanism, so that the integrated elements are flanked at the insertion site by a short duplication of target-site DNA of a characteristic length. Many plant transposable elements occur in families, the autonomous elements of which are able to direct their own transposition and the nonautonomous elements of which are able to transpose only when activated by an autonomous element in the same genome. Most nonautonomous elements are derived from autonomous elements by internal deletions or complex sequence rearrangements.

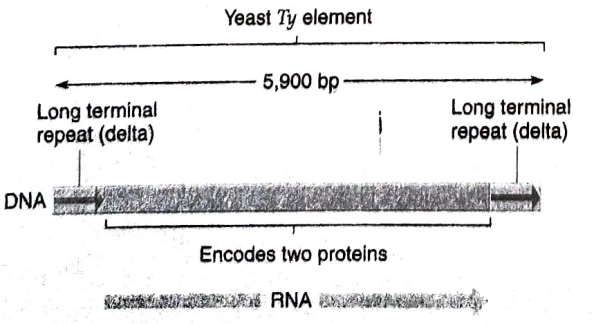
***Ty* Transposable Elements in Yeast.** A *Ty* transposable element is about 5.9 kb long and includes two directly repeated terminal sequences called *long terminal repeats* (LTR) or deltas (δ) (Figure 7.26). Each delta contains a promoter and sequences recognized by transposing enzymes. The *Ty* elements encode a single, 5,700-nucleotide mRNA that begins at the promoter in the delta at the left end of the element (see Figure 7.26). The mRNA transcript contains two open reading frames (ORFs), designated *TyA* and *TyB*, that encode two different proteins required for transposition. On average, a strain contains about 35 *Ty* elements.

Ty elements are similar to **retroviruses**—single-stranded RNA viruses that replicate via double-stranded DNA intermediates. That is, when a retrovirus infects a cell, its RNA genome is copied by *reverse transcriptase*, an enzyme that enters the cell as part of the virus particle. **Reverse transcriptase** is an RNA-dependent DNA polymerase, meaning that the enzyme uses an RNA template to produce a DNA copy. The enzyme then catalyzes the synthesis of a complementary DNA strand, in the end producing a double-stranded DNA copy of the RNA genome. The DNA integrates into the host's chromosome, where it can be transcribed to produce progeny RNA viral genomes and mRNAs for viral proteins. HIV, the virus responsible for AIDS in humans, is a retrovirus. As a result of their similarity to retroviruses, *Ty* elements were hypothesized to transpose not by a DNA-to-DNA mechanism, but by making an RNA copy of the integrated DNA sequence and then creating a new *Ty* element by reverse transcription. The new element would then integrate at a new chromosome location.

Evidence substantiating the hypothesis was obtained through experiments with *Ty* elements modified by DNA manipulation techniques to have special features enabling their transposition to be monitored easily. One compelling piece of evidence came from experiments in which an intron was placed into the *Ty* element (there are no introns in normal *Ty* elements) and the element was monitored from its initial placement through the transposition event. At the new location, the *Ty* element no longer had the intron sequence. This result could only be interpreted to mean that transposition occurred via an RNA intermediate.

Subsequently, it was shown that *Ty* elements encode a reverse transcriptase. Moreover, *Ty* viruslike particles containing *Ty* RNA and reverse transcriptase activity have been identified in yeast cells. Because of their similarity to retroviruses in this regard, *Ty* elements are called **retrotransposons**, and the transposition process is called **retrotransposition**.

Figure 7.26
The *Ty* transposable element of yeast.



***Drosophila* Transposable Elements.** A number of classes of transposable elements have been identified in *Drosophila*. In this organism, it is estimated that about 15% of the genome is mobile—a remarkable percentage.

The *P element* is an example of a family of transposable elements in *Drosophila*. *P* elements vary in length from 500 to 2,900 bp, and each has terminal inverted repeats. The shorter *P* elements are nonautonomous elements, while the longest *P* elements are autonomous elements that encode a transposase needed for transposition of all the *P* elements (Figure 7.27). Insertion of a *P* element into a new site results in a direct repeat of the target site.

P elements are important vectors for transferring genes into the germ line of *Drosophila* embryos, allowing genetic manipulation of the organism. Figure 7.28 illustrates an experiment by Gerald M. Rubin and Allan C. Spradling in which the wild-type *rosy*$^+$ gene was introduced into a strain homozygous for a mutant *rosy* allele (which has a red-brown eye color). The *rosy*$^+$ gene was

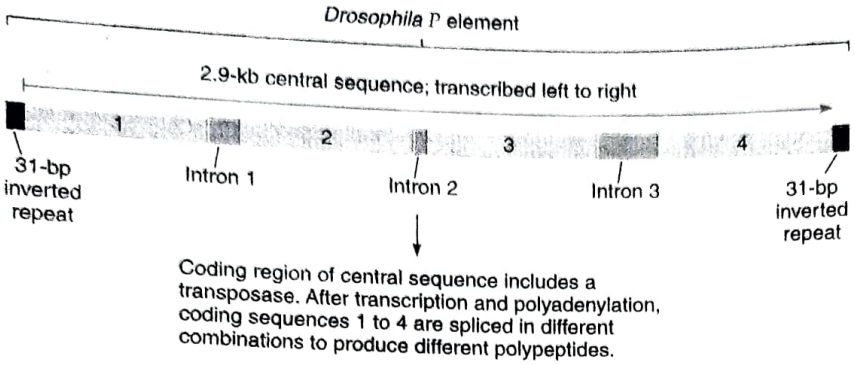
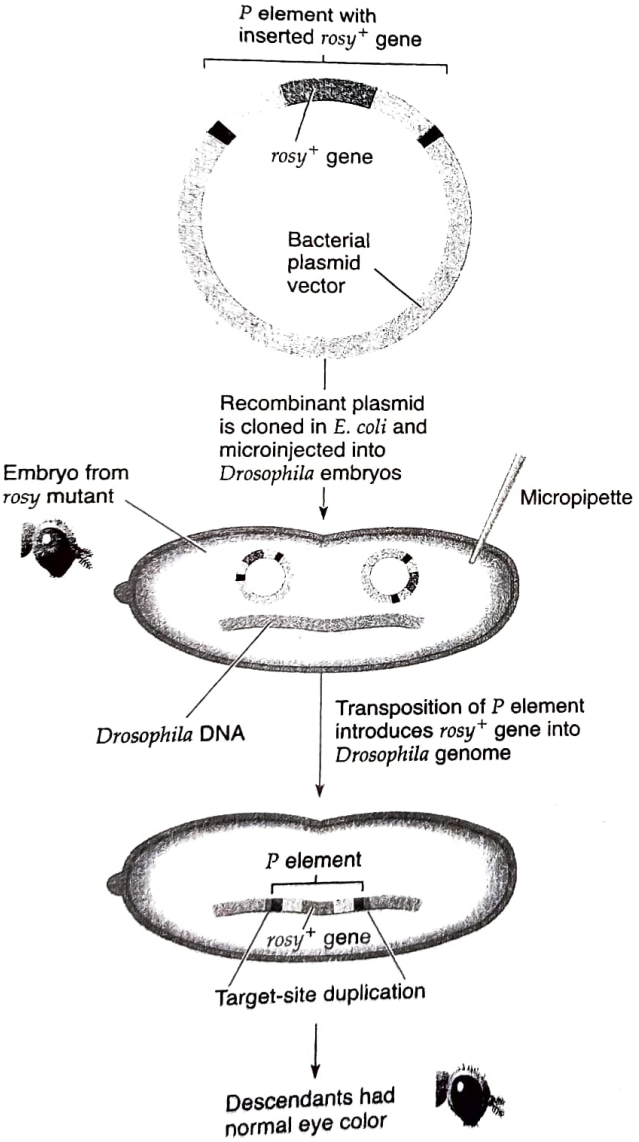


Figure 7.27
Structure of the autonomous *P* transposable element found in *Drosophila melanogaster*.

Figure 7.28
Illustration of the use of *P* elements to introduce genes into the *Drosophila* genome.



introduced into the middle of a *P* element by recombinant DNA techniques and cloned in a plasmid vector (see Chapter 8, pp. 175–176.) The plasmids were then microinjected into *rosy* embryos in the regions that would become the germ-line cells. *P* element-encoded transposase then catalyzed the movement of the *P* element, along with the *rosy*$^+$ gene it contained, to the *Drosophila* genome in some of the germ-line cells. When the flies that developed from these embryos produced gametes, they contained the *rosy*$^+$ gene, so descendants of those flies had normal eye color. In principle, any gene can be transferred into the genome of the fly in this way.

Keynote

Transposable elements in eukaryotes can transpose to new sites while leaving a copy behind in the original site, or they can excise themselves from the chromosome. When the excision is imperfect, deletions can occur; and by various recombination events, other chromosomal rearrangements such as inversions and duplications can occur. Whereas most transposable elements move by using a DNA-to-DNA mechanism, some eukaryotic transposable elements, such as yeast *Ty* elements, transpose via an RNA intermediate (using a transposable elements-encoded reverse transcriptase) and so resemble retroviruses.

Human Retrotransposons. In Chapter 2, pp. 28–30, we discussed the different repetitive classes of DNA sequences found in the genome. Of relevance here are the LINEs (long interspersed sequences) and SINEs (short interspersed sequences) found in the moderately repetitive class of sequences. **LINEs** are repeated sequences 1,000–7,000 bp long, interspersed with unique-sequence DNA. **SINEs** are 100–400-bp repeated sequences interspersed with unique-sequence DNA. Both LINEs and SINEs occur in DNA families whose members are related by sequence.

Like the yeast *Ty* elements, LINEs and SINEs are retrotransposons. Full-length LINEs are autonomous elements that encode the enzymes for their own retrotransposition and for that of LINEs with internal

deletions—nonautonomous derivatives. Those enzymes are also required for the transposition of SINEs, which are nonautonomous elements.

About 20% of the human genome consists of LINEs, with one-quarter of them being L1, the best-studied LINE. The maximum length of L1 elements is 6,500 bp, although only about 3,500 of them in the genome are of that full length, the rest having internal deletions of various length (much as corn Ds elements have). The full-length L1 elements contain a large open reading frame that is homologous to known reverse transcriptases. When the yeast *Ty* element reverse transcriptase gene was replaced with the putative reverse transcriptase gene from L1, the *Ty* element was able to transpose. Point mutations introduced into the sequence abolished the enzyme activity, indicating that the L1 sequence can indeed make a functional reverse transcriptase. Thus, like corn Ac elements, full-length L1 elements (and full-length LINEs of other families) are autonomous elements. L1 and other LINEs do not have LTRs, so they are not closely related to the retrotransposons we have already discussed. Therefore, while transposition is via an RNA intermediate, the mechanism is different. Interestingly, in 1991, two unrelated cases of hemophilia (OMIM 306700) in children were shown to result from insertions of an L1 element into the factor VIII gene, the product of which is required for normal blood clotting. Molecular analysis showed that the insertion was not present in either set of parents, leading to the conclusion that the L1 element had newly transposed. More generally, these results show that L1 elements in humans can transpose and that they can cause disease by insertional mutagenesis (that is, by inserting into genes).

SINEs are also retrotransposons, but none of them encodes enzymes needed for transposition. These nonautonomous elements depend upon the enzymes encoded by LINEs for their transposition. In humans, a very abundant SINE family is the *Alu family*. The repeated sequence in this family is about 300 bp long and is repeated 300,000 to 500,000 times in the genome, amounting to up to 3% of the total genomic DNA. The name for the family refers from the fact that the sequence contains a restriction site for the enzyme *AluI* ("Al-you-one").

Evidence that Alu sequences can transpose has come from the study of a young male patient with neurofibromatosis (OMIM 162200), a genetic disease caused by an autosomal dominant mutation. Individuals with neurofibromatosis develop tumorlike growths (neurofibromas) over the body (see Chapter 13, p. 372). DNA analysis showed that an Alu sequence was present in one of the introns of the neurofibromatosis gene of the patient. RNA transcripts from this gene are longer than those from normal individuals. The presence of the Alu sequence in the intron disrupts the processing of the transcript, causing one exon to be lost completely from the mature mRNA. As a result, the protein encoded is 800 amino acids shorter than normal and is nonfunctional. Neither parent of the patient has neurofibromatosis, and neither has an Alu sequence in the neurofibromatosis gene. Individual members of the Alu family are not identical in sequence, having diverged over evolutionary time. This divergence made it possible to track down the same Alu sequence in the patient's parents. The analysis showed that an Alu sequence probably inserted into the neurofibromatosis gene by retrotransposition in the germ line of the father from a different chromosomal location.

Summary

- Mutations can result in changes in heritable traits.
- Mutation is the process that alters the sequence of base pairs in a DNA molecule. The alteration can be as simple as a single base-pair substitution, insertion, or deletion or as complex as rearrangement, duplication, or deletion of whole sections of a chromosome. Mutations may occur spontaneously, such as through the effects of natural radiation or errors in replication, or they may be induced experimentally by the application of mutagens.
- Mutations at the level of the chromosome are called chromosomal mutations (see Chapter 12). Mutations in the sequences of genes and in other DNA sequences at the level of the base pair are called point mutations.
- The consequences to an organism of a mutation in a gene depend on a number of factors, especially the extent to which the amino acid-coding information for a protein is changed.
- By studying mutants that have defects in certain cellular processes, geneticists have made great progress in understanding how those processes take place. Various screening procedures have been developed to help find mutants of interest after mutagenizing cells or organisms.
- The effects of a gene mutation can be reversed either by reversion of the mutated base-pair sequence or by a mutation at a site distinct from that of the original mutation. The latter is called a suppressor mutation.
- High-energy radiation may damage genetic material by producing chemicals that interact with DNA or by causing unusual bonds between DNA bases. Mutations result if the genetic damage is not repaired. Ionizing radiation may also break chromosomes.

REFERENCE

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