Transposons
Figure 16.1  A major cause of sequence change within a genome is the movement of a transposon to a new site. This may have direct consequences on gene expression. Unequal crossing-over between related sequences causes rearrangements. Copies of transposons can provide targets for such events.
An **insertion sequence** is a small bacterial transposon that carries only the genes needed for its own transposition.

**Inverted terminal repeats** are the short related or identical sequences present in reverse orientation at the ends of some transposons.

**Direct repeats** are identical (or closely related) sequences present in two or more copies in the same orientation in the same molecule of DNA; they are not necessarily adjacent.

**Transposase** is the enzyme activity involved in insertion of transposon at a new site.
Figure 16.2
Transposons have inverted terminal repeats and generate direct repeats of flanking DNA at the target site. In this example, the target is a 5 bp sequence. The ends of the transposon consist of inverted repeats of 9 bp, where the numbers 1 through 9 indicate a sequence of base pairs.
Transposons can carry other genes in addition to those coding for transposition.

**Composite transposons** have a central region flanked by an IS element at each end.

Either one or both of the IS elements of a composite transposon may be able to undertake transposition.

A composite transposon may transpose as a unit, but an active IS element at either end may also transpose independently.

The arms may be in either the same or (more commonly) inverted orientation. So a composite transposon with arms that are direct repeats has the structure

If the arms are inverted repeats, the structure is
**Figure 16.3** A composite transposon has a central region carrying markers (such as drug resistance) flanked by IS modules. The modules have short inverted terminal repeats. If the modules themselves are in inverted orientation, the short inverted terminal repeats at the ends of the transposon are identical.
Figure 16.4  Two IS10 modules create a composite transposon that can mobilize any region of DNA that lies between them. When Tn10 is part of a small circular molecule, the IS10 repeats can transpose either side of the circle.
Figure 16.5  The direct repeats of target DNA flanking a transposon are generated by the introduction of staggered cuts whose protruding ends are linked to the transposon.
Figure 16.6  Replicative transposition creates a copy of the transposon, which inserts at a recipient site. The donor site remains unchanged, so both donor and recipient have a copy of the transposon.
Figure 16.8 Conservative transposition involves direct movement with no loss of nucleotide bonds; compare with lambda integration and excision.
Figure 16.7  Nonreplicative transposition allows a transposon to move as a physical entity from a donor to a recipient site. This leaves a break at the donor site, which is lethal unless it can be repaired.
**Replicative transposition** describes the movement of a transposon by a mechanism in which first it is replicated, and then one copy is transferred to a new site.  
**Transposase** is the enzyme activity involved in insertion of transposon at a new site.  
**Resolvase** is the enzyme activity involved in site-specific recombination between two transposons present as direct repeats in a cointegrate structure.  
**Nonreplicative transposition** describes the movement of a transposon that leaves a donor site (usually generating a double-strand break) and moves to a new site.  
**Conservative transposition** refers to the movement of large elements, originally classified as transposons, but now considered to be episomes. The mechanism of movement resembles that of phage excision and integration.

All transposons use a common mechanism in which staggered nicks are made in target DNA, the transposon is joined to the protruding ends, and the gaps are filled. The order of events and exact nature of the connections between transposon and target DNA determine whether transposition is replicative or nonreplicative.
Transposons cause rearrangement of DNA

**Figure 16.9** Reciprocal recombination between direct repeats excises the material between them; each product of recombination has one copy of the direct repeat.
Figure 16.10  Reciprocal recombination between inverted repeats inverts the region between them.
Deletions are generated by removal of a sequence of DNA, the regions on either side being joined together.

Precise excision describes the removal of a transposon plus one of the duplicated target sequences from the chromosome. Such an event can restore function at the site where the transposon inserted.

Imprecise excision occurs when the transposon removes itself from the original insertion site, but leaves behind some of its sequence.

Homologous recombination between multiple copies of a transposon causes rearrangement of host DNA.

Homologous recombination between the repeats of a transposon may lead to precise or imprecise excision.
Figure 16.11  Transposition is initiated by nicking the transposon ends and target site and joining the nicked ends into a strand transfer complex.
Figure 16.12  Mu transposition passes through three stable stages. MuA transposase forms a tetramer that synapses the ends of phage Mu. Transposase subunits act in *trans* to nick each end of the DNA; then a second *trans* action joins the nicked ends to the target DNA.
Transposition starts by forming a strand transfer complex in which the transposon is connected to the target site through one strand at each end.

The Mu transposase forms the complex by synapsing the ends of Mu DNA, followed by nicking, and then a strand transfer reaction. Replicative transposition follows if the complex is replicated and nonreplicative transposition follows if it is repaired.

Three MuA-binding sites with a 22 bp consensus are located at each end of Mu DNA. L1, L2, and L3 are at the left end; R1, R2, and R3 are at the right end. A monomer of MuA can bind to each site
Figure 16.13  Transposition may fuse a donor and recipient replicon into a cointegrate. Resolution releases two replicons, each containing a copy of the transposon.
Figure 16.14  Mu transposition generates a crossover structure, which is converted by replication into a cointegrate

A **cointegrate structure** is produced by fusion of two replicons, one originally possessing a transposon, the other lacking it; the cointegrate has copies of the transposon present at both junctions of the replicons, oriented as direct repeats.

**Resolution** occurs by a homologous recombination reaction between the two copies of the transposon in a cointegrate. The reaction generates the donor and target replicons, each with a copy of the transposon.

**Resolvase** is the enzyme activity involved in site-specific recombination between two transposons present as direct repeats in a cointegrate structure.
Nonreplicative transposition results when a crossover structure is released by nicking. This inserts the transposon into the target DNA, flanked by the direct repeats of the target, and the donor is left with a double-strand break.
Figure 16.16  Both strands of Tn10 are cleaved sequentially, and then the transposon is joined to the nicked target site.
Figure 16.17  Cleavage of Tn5 from flanking DNA involves nicking, interstrand reaction, and hairpin cleavage.
Figure 16.18 Each subunit of the Tn5 transposase has one end of the transposon located in its active site and also makes contact at a different site with the other end of the transposon.

Nonreplicative transposition results if a crossover structure is nicked on the unbroken pair of donor strands, and the target strands on either side of the transposon are ligated.

Two pathways for nonreplicative transposition differ according to whether the first pair of transposon strands are joined to the target before the second pair are cut (Tn5), or whether all four strands are cut before joining to the target (Tn10).
Figure 16.19 Transposons of the TnA family have inverted terminal repeats, an internal res site, and three known genes.

Replicative transposition of TnA requires a transposase to form the cointegrate structure and a resolvase to release the two replicons.

The action of the resolvase resembles lambda Int protein and belongs to the general family of topoisomerase-like site-specific recombination reactions, which pass through an intermediate in which the protein is covalently bound to the DNA.
Resolution occurs by breaking and rejoining bonds without input of energy. The products identify an intermediate stage in cointegrate resolution; they consist of resolvase covalently attached to both 5’ ends of double-stranded cuts made at the res site. The cleavage occurs symmetrically at a short palindromic region to generate two base extensions. Expanding the view of the crossover region located in site I, we can describe the cutting reaction as:
Tn10 has two IS10 on either ends

module IS10R is active and IS10L is inactive

\( P_{IN} \) generate transposase transcript

\( P_{OUT} \) usually generates a short transcript

\( P_{OUT} \) sometimes activates adjacent host genes

**Figure 16.20** Two promoters in opposite orientation lie near the outside boundary of IS10R. The strong promoter \( P_{OUT} \) sponsors transcription toward the flanking host DNA. The weaker promoter \( P_{IN} \) causes transcription of an RNA that extends the length of IS10R and is translated into the transposase.
Several mechanisms restrain the frequency of Tn10 transposition, by affecting either the synthesis or function of transposase protein. Transposition of an individual transposon is restricted by methylation to occur only after replication. In multicopy situations, cis-preference restricts the choice of target, and OUT/IN RNA pairing inhibits synthesis of transposase.

Multicopy inhibition reduces the rate of transposition of any one copy of a transposon when other copies of the same transposon are introduced into the genome.

Multiple mechanisms affect the rate of transposition.
Transposition Control Mechanism

• Cis preference - Transposase has cis preference
  i.e. increase in copies of Tn10 does not cause increased
  frequency of trasposition that could damage the DNA

• Dam methylation at GATC reduces frequency of
  transposition (frequency of transposition is increased 100X
  in dam- mutants)

• Hemimethylated DNA (GATC) increases PIN transcription
  and enhance binding of transposase to the ends of IS10R

• When is DNA found as hemimethylated?
**Controlling elements** of maize are transposable units originally identified solely by their genetic properties. They may be autonomous (able to transpose independently) or nonautonomous (able to transpose only in the presence of an autonomous element).

A **sector** is a patch of cells made up of a single altered cell and its progeny. **Variegation** of phenotype is produced by a change in genotype during somatic development.

An **acentric fragment** of a chromosome (generated by breakage) lacks a centromere and is lost at cell division.

A **dicentric chromosome** is the product of fusing two chromosome fragments, each of which has a centromere. It is unstable and may be broken when the two centromeres are pulled to opposite poles in mitosis.

The **breakage-fusion-bridge** cycle is a type of chromosomal behavior in which a broken chromatid fuses to its sister, forming a "bridge". When the centromeres separate at mitosis, the chromosome breaks again (not necessarily at the bridge), thereby restarting the cycle.
Figure 16.22  Clonal analysis identifies a group of cells descended from a single ancestor in which a transposition-mediated event altered the phenotype. Timing of the event during development is indicated by the number of cells; tissue specificity of the event may be indicated by the location of the cells.

Transposition in maize can

• be visualized

• cause insertions, excisions or chromosome breaks

• may effect the activity of adjacent genes
Acentric fragment is lost due to lack of a centromer

Figure 16.23  A break at a controlling element causes loss of an acentric fragment; if the fragment carries the dominant markers of a heterozygote, its loss changes the phenotype. The effects of the dominant markers, Cl, Bz, Wx, can be visualized by the color of the cells or by appropriate staining.
Figure 16.24  *Ds* provides a site to initiate the chromatid fusion-bridge-breakage cycle. The products can be followed by clonal analysis.

**DS activity leads to two unusual chromosomes**

1. U shape acentric fragment
2. U shape dicentric chromosome

**Result:** duplications and deletions
An autonomous controlling element in maize is an active transposon with the ability to transpose.

A nonautonomous controlling element is a transposon in maize that encodes a non-functional transposase; it can transpose only in the presence of a trans-acting autonomous member of the same family.
**Figure 16.26** The Ac element has two open reading frames; Ds elements have internal deletions.

- **Nonautonomous elements are stable** – do not transpose

- Ac has 11 bp inverted repeats
- Ds9 has 104 bp deletion
- Ds retains only 2 kb
**Figure 16.27**  *Spm/En* has two genes. *tnpA* consists of 11 exons that are transcribed into a spliced 2500 base mRNA. *tnpB* may consist of a 6000 base mRNA containing ORF1 + ORF2.

Spm and En are identical autonomous element and differ only at < 10 positions.

Spm elements affect gene expression at their sites of insertion, when the TnpA protein binds to its target sites at the ends of the transposon.

TnpB may bind to the 13 bp terminal inverted repeats to cleave the termini for transposition.

Spm elements are inactivated by methylation.
Figure 16.28  Hybrid dysgenesis is asymmetrical; it is induced by P male x M female crosses, but not by M male x P female crosses.

P elements are transposons that are carried in P strains of *D. melanogaster* but not in M strains.

When a P male is crossed with an M female, transposition is activated.

The insertion of P elements at new sites in these crosses inactivates many genes and makes the cross infertile.
P elements are activated in the germline of a P male when crossed with a M female because a tissue-specific splicing event removes one intron, generating the coding sequence for the transposase.

The P element also produces a repressor of transposition, which is inherited maternally in the cytoplasm. The presence of the repressor explains why M male × P female crosses remain fertile.

The primary transcript extends for 2.5 kb or 3.0 kb, the difference probably reflecting merely the leakiness of the termination site.
Figure 16.30  Hybrid dysgenesis is determined by the interactions between P elements in the genome and 66 kD repressor in the cytotype of female.