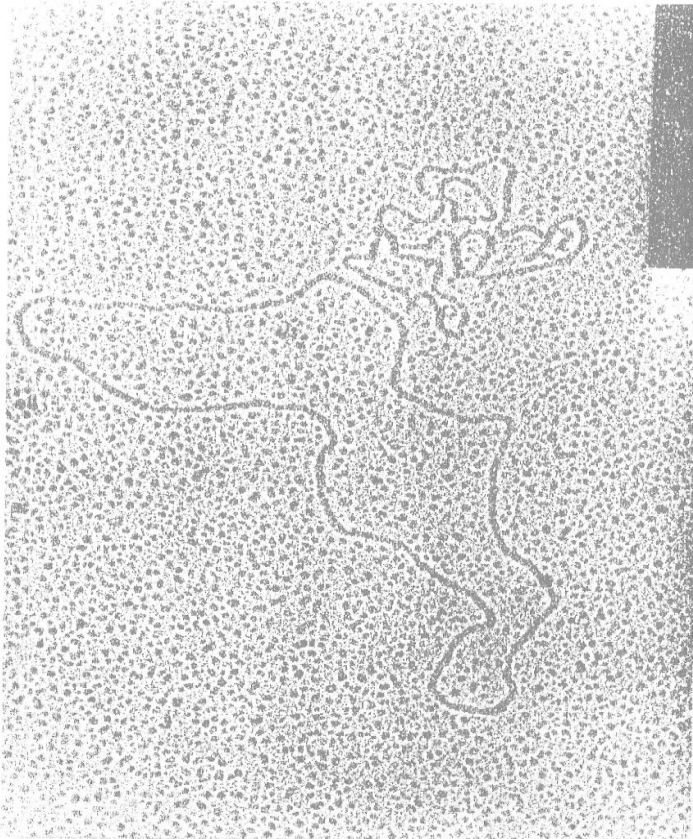
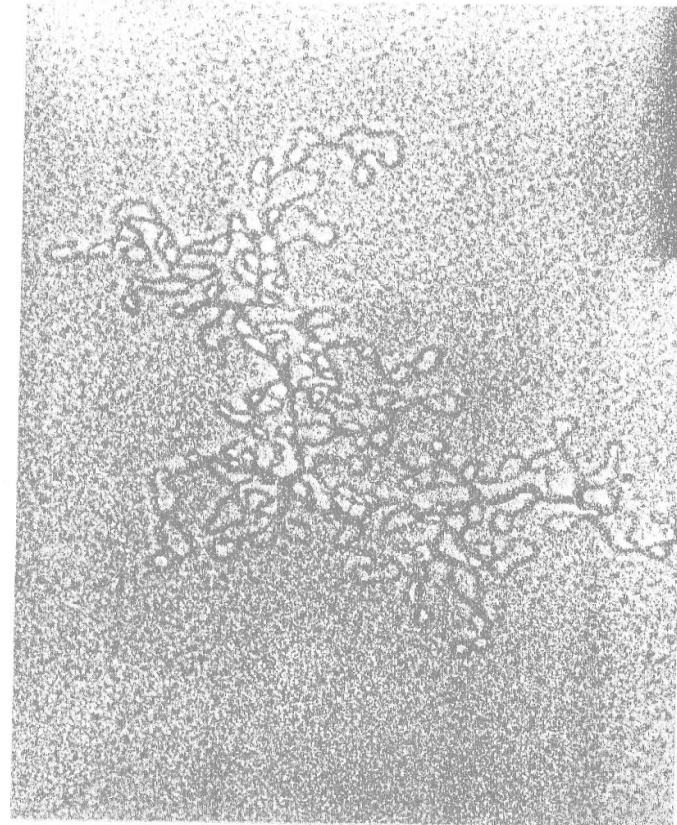


## Bacterial Plasmids

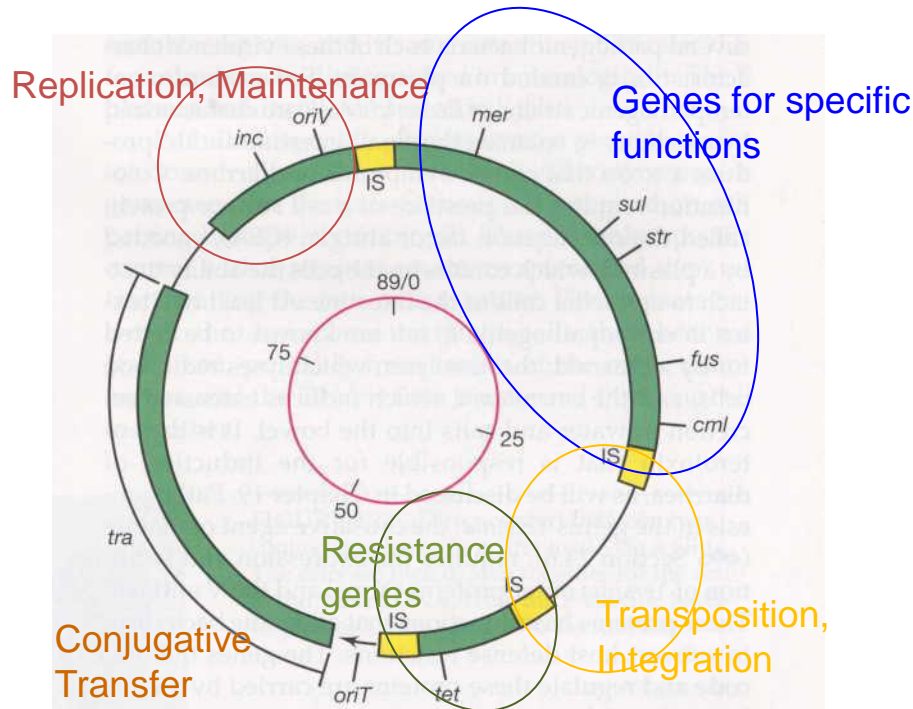
Open circular form



Covalently closed circles (ccc-form)



# Bacterial plasmids



**FIGURE 9.21** Genetic map of the resistance plasmid R100. The inner circle shows the size of the plasmid in kilobase pairs. The outer circle shows the location of major antibiotic resistance genes and other key functions: *inc*, incompatibility genes; *oriV*, origin of replication site; *oriT*, origin of conjugative transfer; *mer*, mercuric ion resistance; *sul*, sulfonamide resistance; *tet*, tetracycline resistance; *tra*, transfer functions. The locations of insertion sequences (IS) are also shown.

## Replication

- Origin of replication (*oriV*)
- Regulatory functions for replication (*rep*, *trf*)
- copy number
- Host range
- incompatibility

## Maintenance

- Partitioning systems (*par*)
- Multimer resolution systems (*mrs*)
- Addiction systems (e.g. *hok-sok*)
- Stable maintenance of plasmids upon cell division

## Conjugative transfer

- Complete Transfer regions (*tra*)
- Mobilization regions (*mob*, *oriT*, *nic*, *bom*)
- Autonomous In vivo transfer of plasmids
- In vivo transfer mediated by helper functions

## Bacterial Plasmids

### Copy Number

Replication & its control

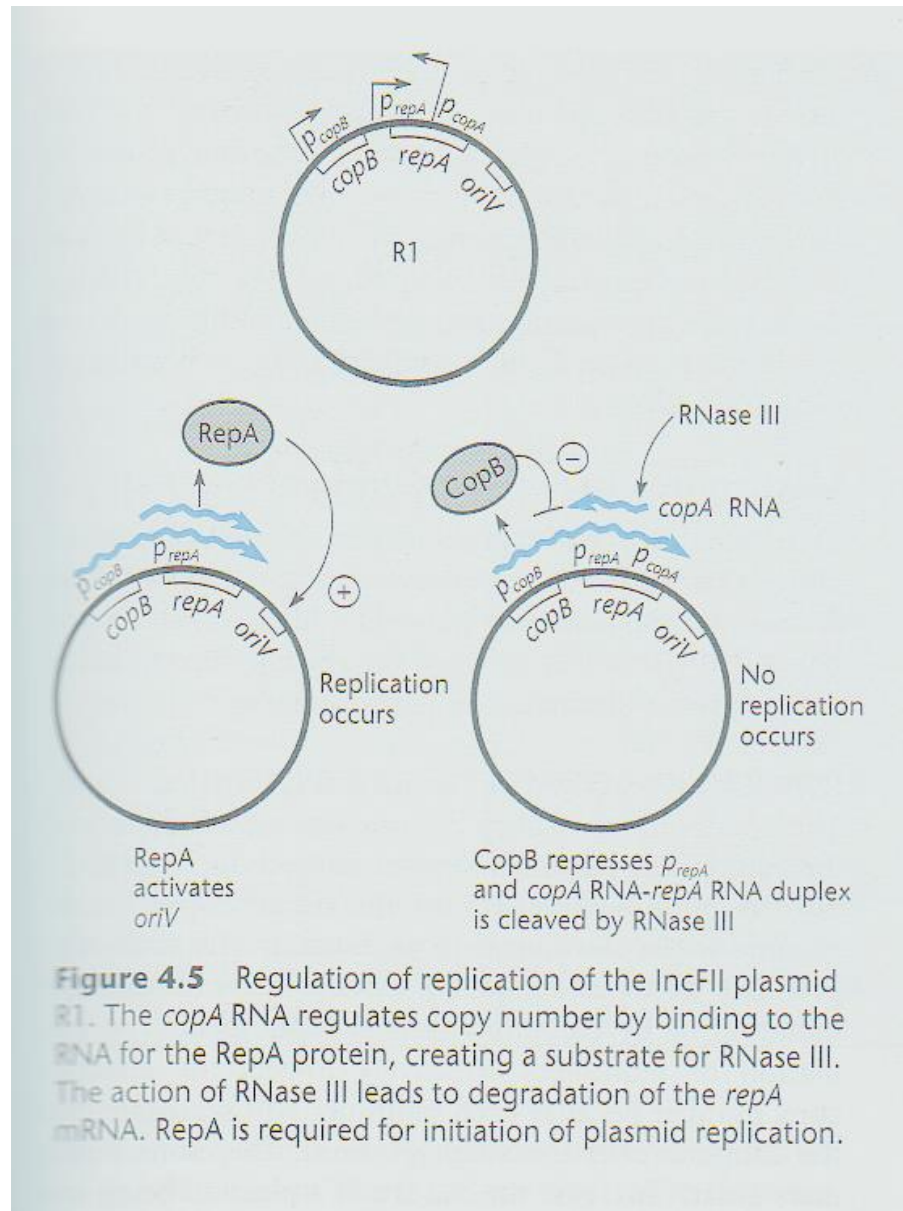
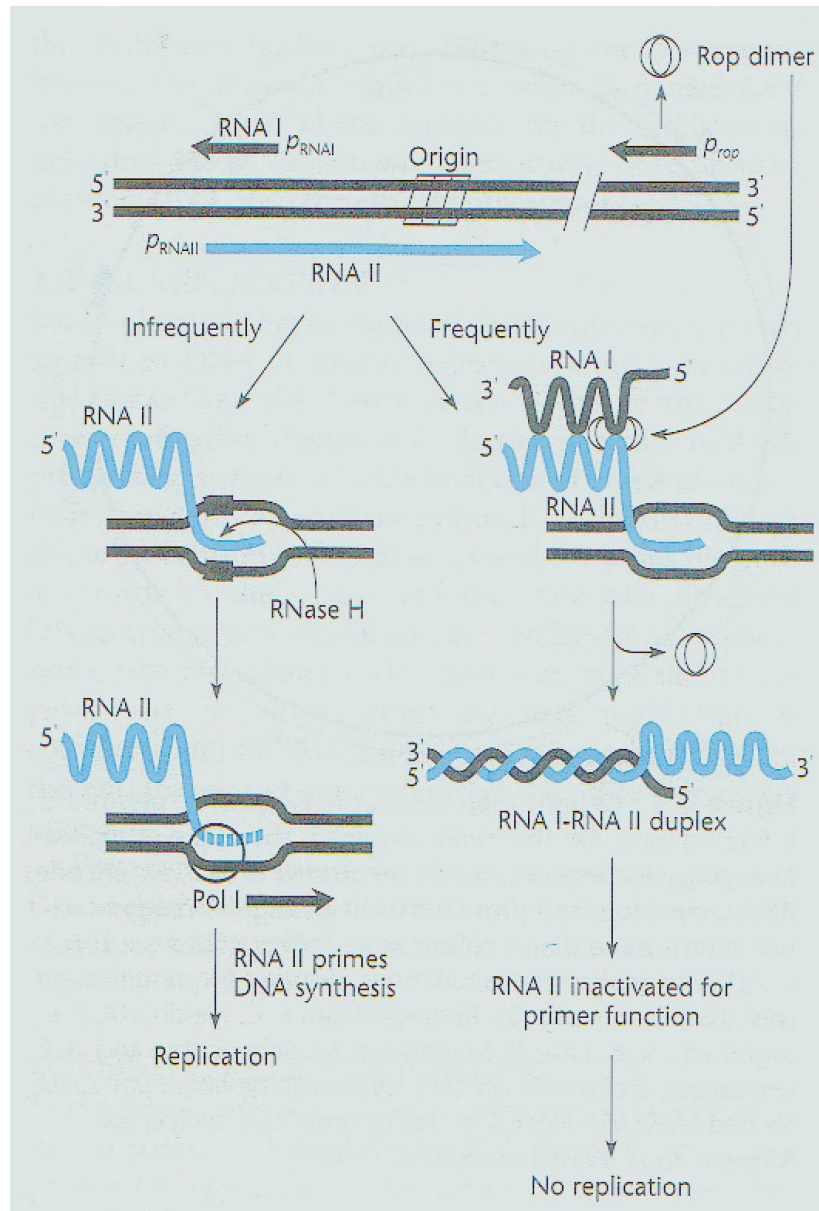
Stringent control: low copy plasmids  
F, R1, RP4/RK2 (1-6)

Relaxed Control: high copy number  
ColE1, pBR322, pUC18

### Incompatibility:

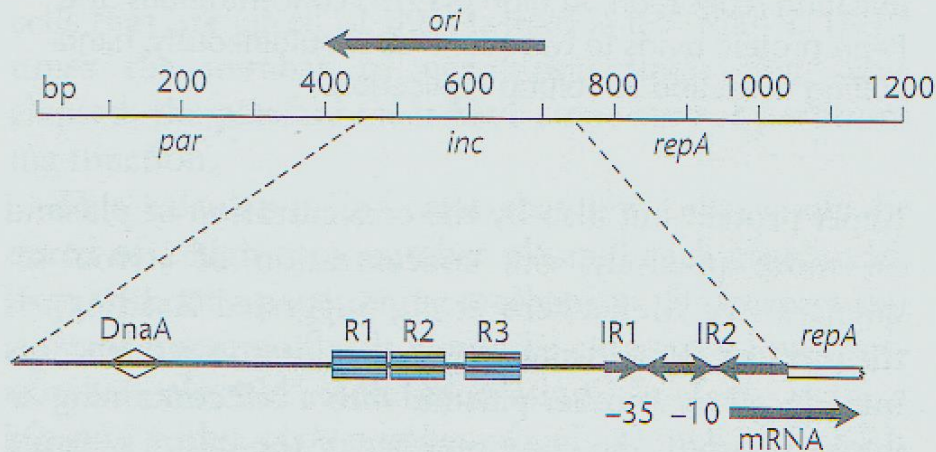
Replication / Control  
Partitioning



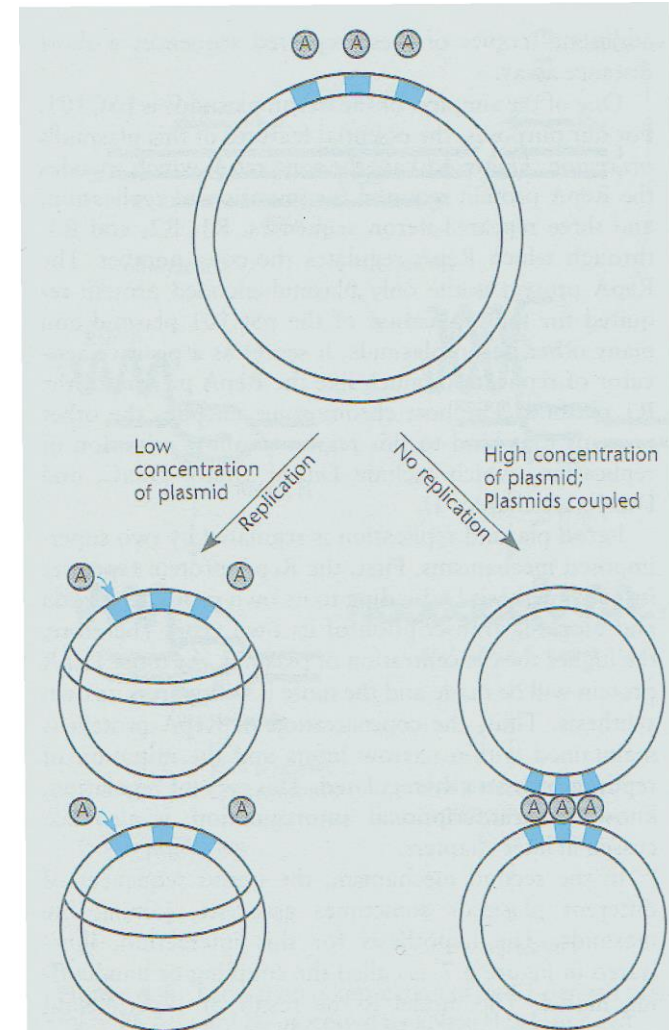


**Figure 4.5** Regulation of replication of the IncFII plasmid R1. The *copA* RNA regulates copy number by binding to the RNA for the RepA protein, creating a substrate for RNase III. The action of RNase III leads to degradation of the *repA* mRNA. RepA is required for initiation of plasmid replication.

## Iteron Model



**Figure 4.6** The *ori* region of pSC101. R1, R2, and R3 are the three iteron sequences (CAAAGGTCTAGCAGCAGAATT-TACAGA for R3) to which RepA binds to handcuff two plasmids. RepA autoregulates its own synthesis by binding to the inverted repeats IR1 and IR2. The location of the partitioning site *par* (see the section on Partitioning) and the binding sites for the host protein DnaA are also shown.



**Figure 4.7** The "handcuffing" or "coupling" model for regulation of iteron plasmids. At low concentrations of plasmids, the RepA protein only binds to one plasmid at a time, initiating replication. At high plasmid concentrations, the RepA protein binds to two plasmids simultaneously, handcuffing them and inhibiting replication.

## Plasmids

### Conjugative Transfer:

Gram-negative:	F, RP4/RK2,
Gram-positive:	pAM $\beta$ 1, SCP2*
Plants	Ti

### Bacteriocin-/ Microcin-Production

### Antibiotic Resistance:

$\beta$ -Lactam Antibiotics:	$\beta$ -Lactamases
Chloramphenicol:	Acetyltransferases
Aminoglycoside-Ab.:	Phosphotransferases
Tetracyclins:	Membrane transfer
Sulfonamides:	Bypass
Trimethoprim	

### Heavy Metal Resistance:

Mercury, Hg-organic compounds,  
Tellurium  
Arsenic, Antimony, Cadmium,  
Copper, Silver

## Plasmids

### Degradative Plasmids:

Aromatic, heterocyclic compounds  
Carbohydrates (sucrose)  
specific metabolites (Nopalin, Octopin)

### Specific metabolic pathways

Nitrogen fixation  
Hydrogen oxidation

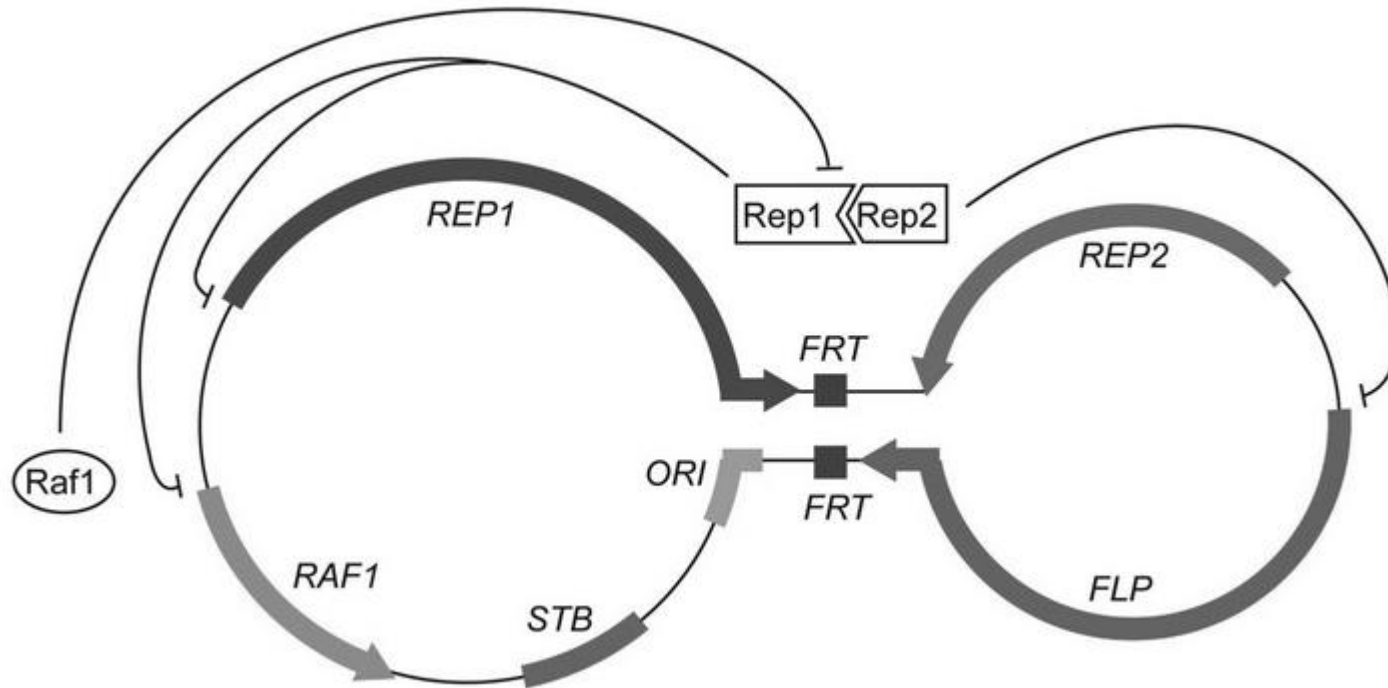
### Symbiosis factors

Rhizobia

### Medically relevant features

Colonizing factors  
Invasins  
Toxins  
Siderophores

A



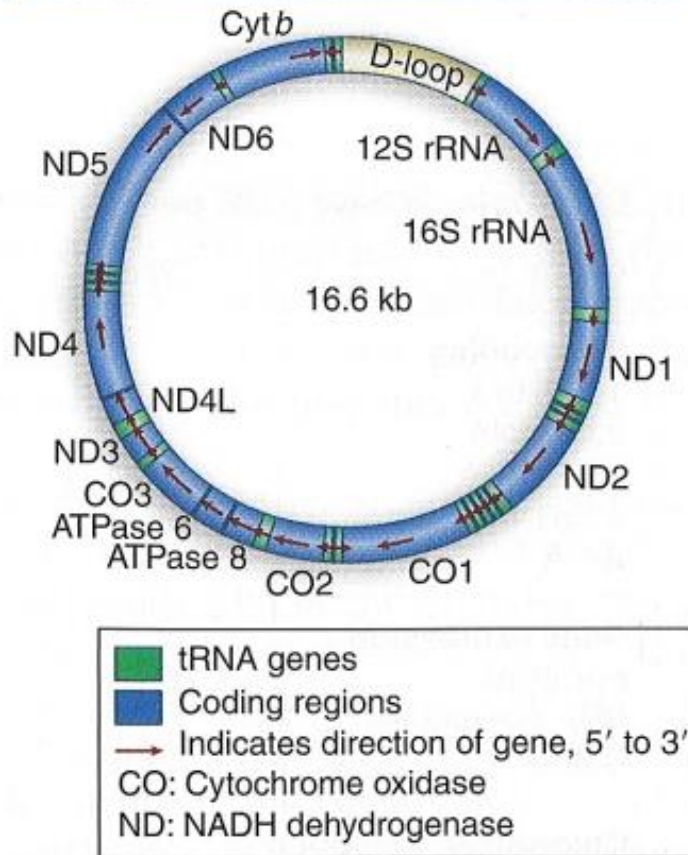
The genetic organization of the 2µm plasmid of *S. cerevisiae*: regional and point centromeres (A) The double-stranded circular plasmid is shaped as a dumb-bell to denote a long inverted repeat sequence that divides the genome into two unique regions. The *Rep1* and *Rep2* proteins, together with the *STB* locus, constitute the plasmid partitioning system. The *Flp* recombinase, along with the *FRT* sites, is responsible for plasmid copy number maintenance. The *Raf1* protein is a positive regulator of amplification. *ORI* denotes the plasmid replication origin. The regulatory network, comprising the *Rep1*, *Rep2* and *Raf1* proteins, that controls plasmid gene expression is indicated.





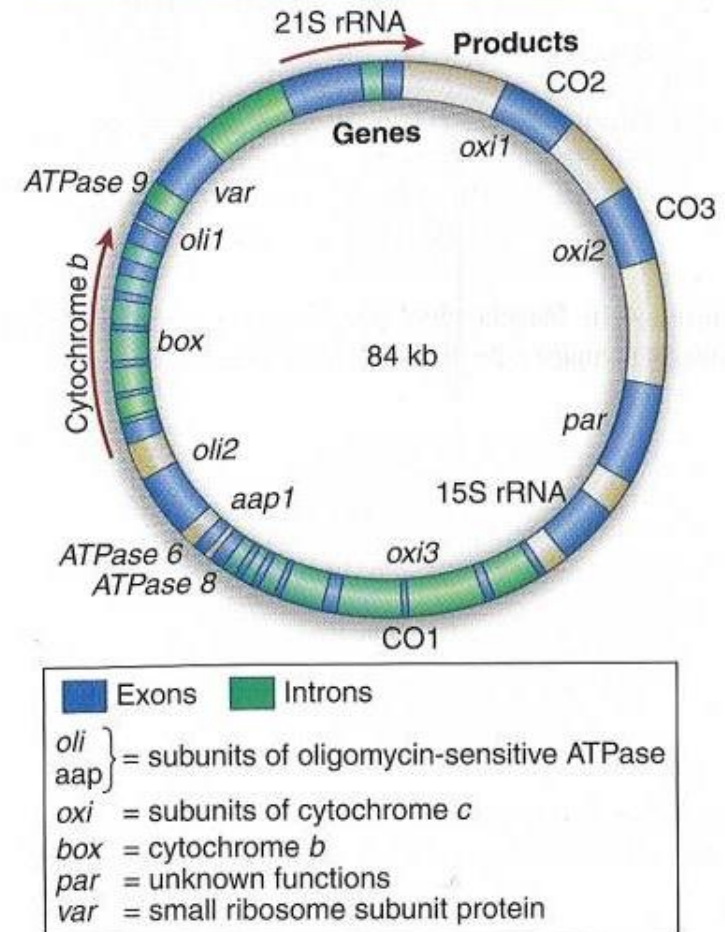
Fig. 2a,b. *Saccharomyces cerevisiae*: structure of the 2 μm plasmid: (a) double-stranded plasmid; (b) homoduplex of the 2 μm plasmid. The self-annealing of the inverted repeats of the plasmid yields typical "dumb-bell" structures (from C.P. Hollenberg)

### All human mtDNA is expressed



**Figure 4.17** Human mitochondrial DNA has 22 tRNA genes, 2 rRNA genes, and 13 protein-coding regions. 14 of the 15 protein-coding or rRNA-coding regions are transcribed in the same direction. 14 of the tRNA genes are expressed in the clockwise direction and 8 are read counterclockwise.

### Yeast mtDNA has the same genes as human



**Figure 4.18** The mitochondrial genome of *S. cerevisiae* contains both interrupted and uninterrupted protein-coding genes, rRNA genes, and tRNA genes (positions not indicated). Arrows indicate direction of transcription.

20.10.15

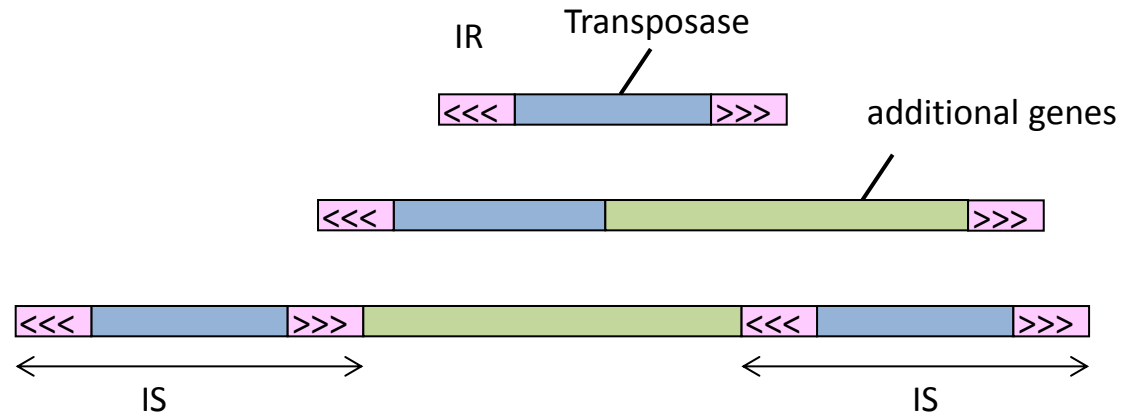
# Transposable Elements - Insertion Sequences and Transposons

## DNA – DNA Transposition

IS Elements

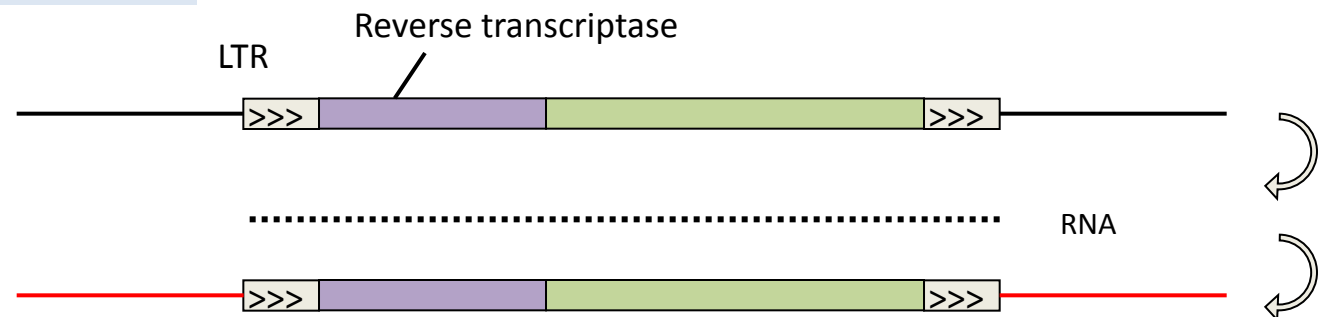
Simple Transposons

Composite Transposons



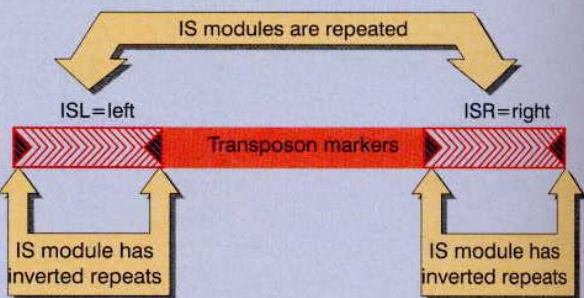
## DNA – RNA – DNA Transposition

Retrotransposons

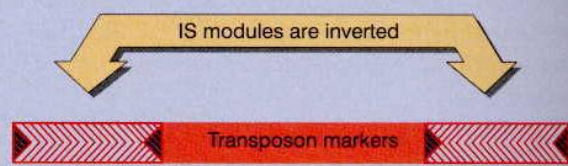




**Figure 15.2** A composite transposon has a central region carrying markers (such as drug resistance) flanked by IS modules. The modules have short inverted terminal repeats. If the modules themselves are in inverted orientation (as drawn), the short inverted terminal repeats at the ends of the transposon are identical.

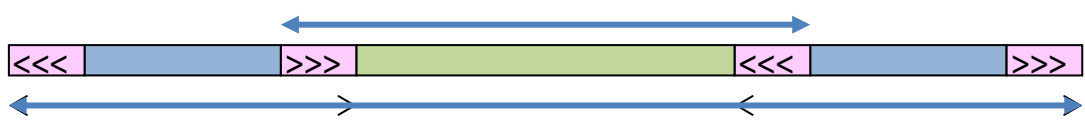


**Example**  
 Tn9 IS1 *cam*<sup>R</sup> IS modules identical both functional

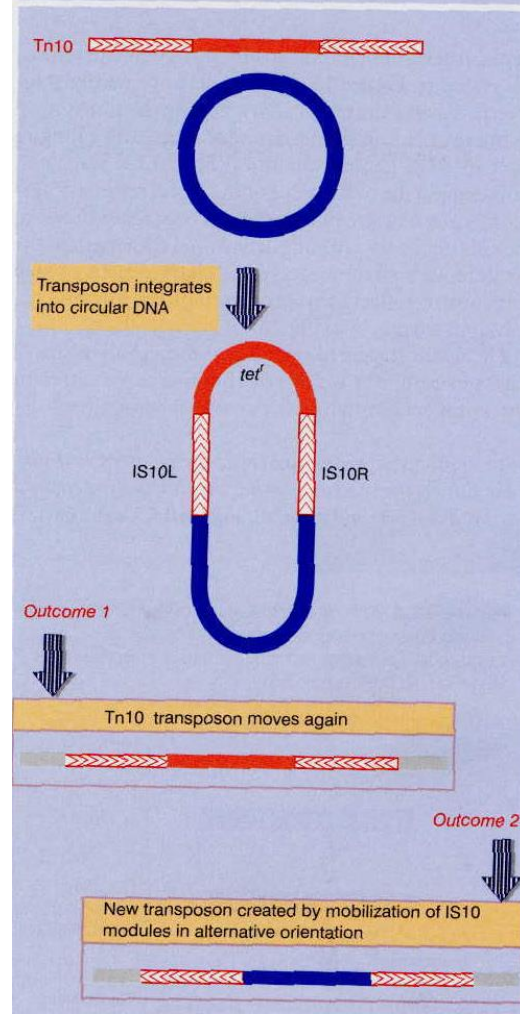


Transposon	Left end	Markers	Right end
Tn903	IS903	<i>kan</i> <sup>R</sup>	both IS ends functional
Tn10	IS10L nonfunctional	<i>tet</i> <sup>R</sup>	IS10R functional
Tn5	IS50L nonfunctional	<i>kan</i> <sup>R</sup>	IS50R functional

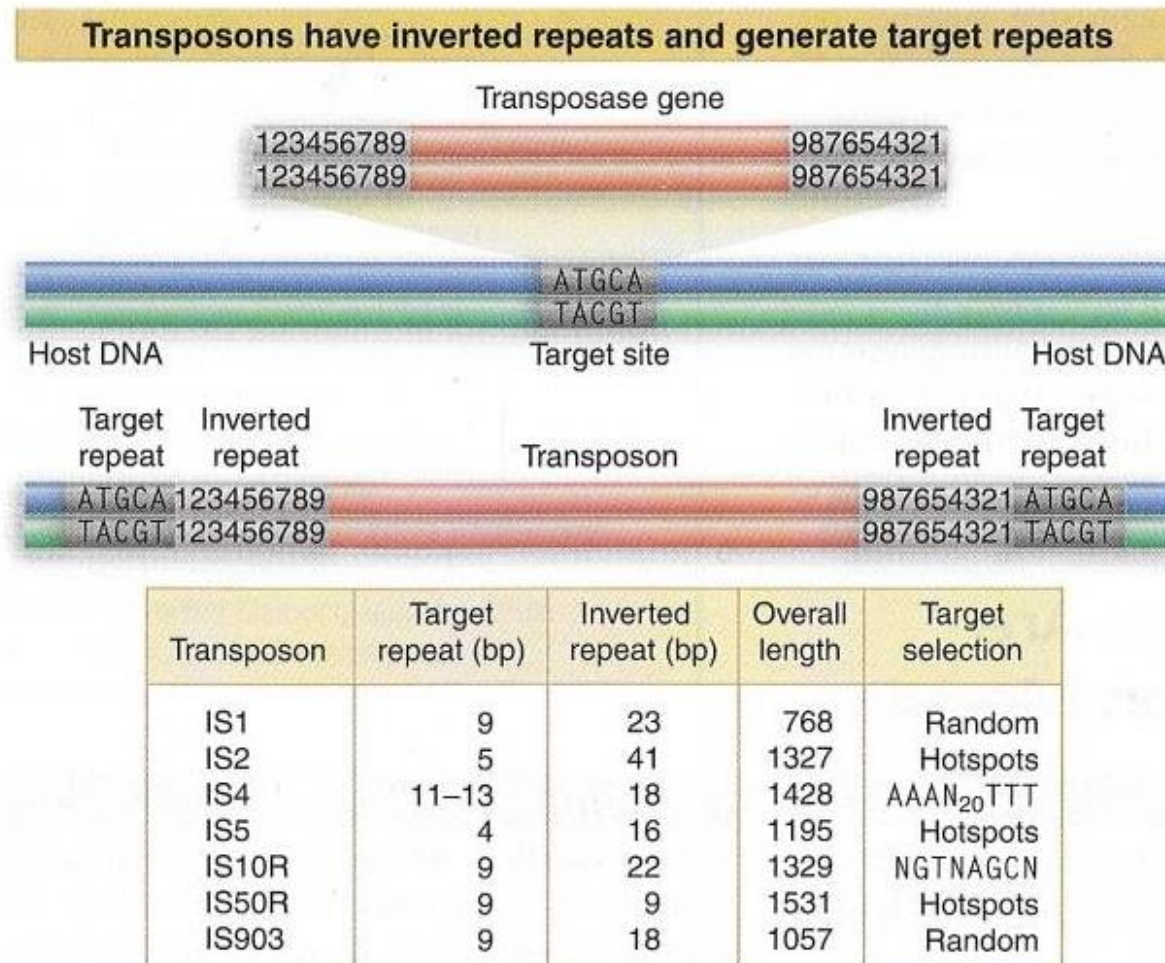
IS elements and transposons can generate new transposable elements by integration at adjacent positions into a genome



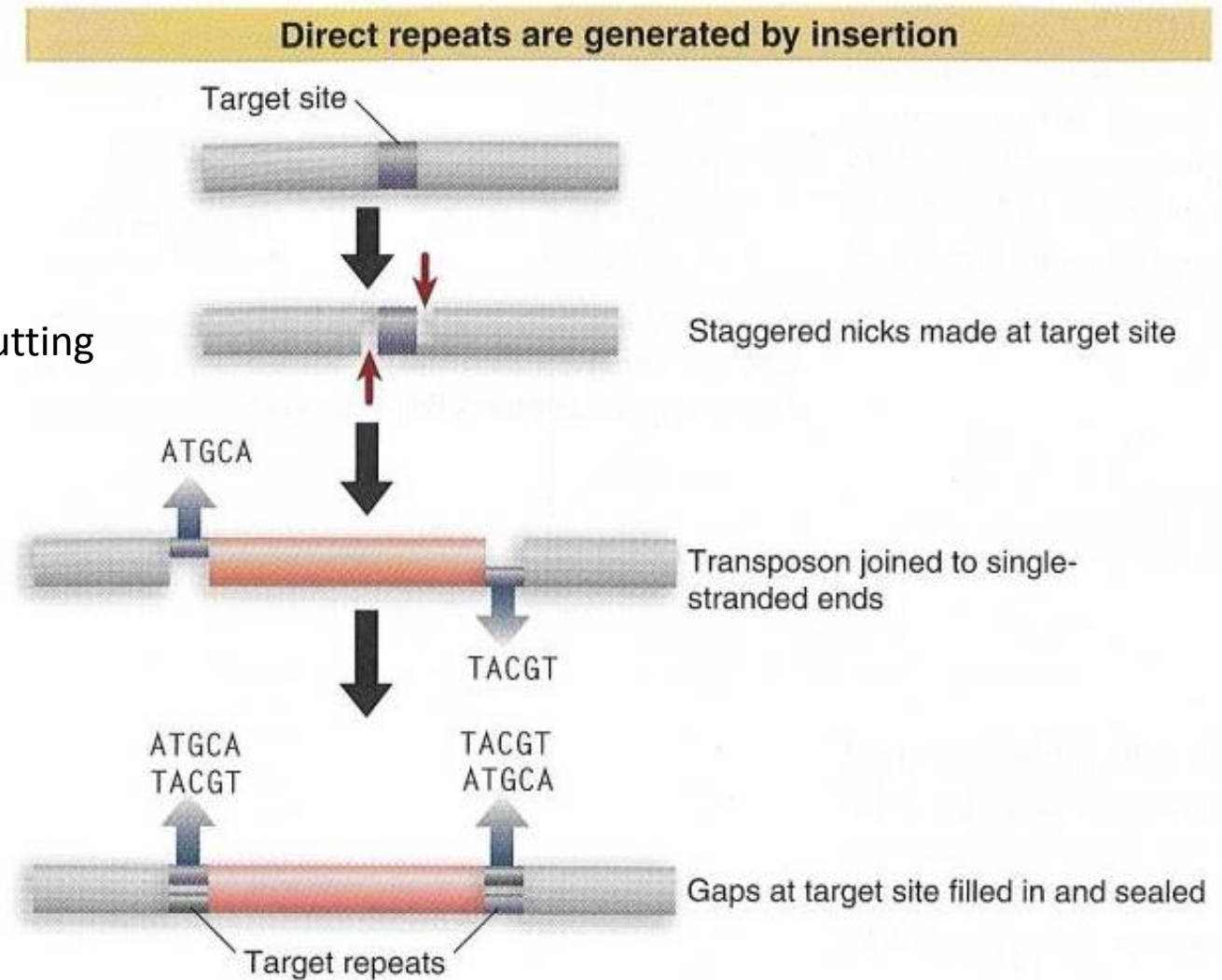
**Figure 15.3** Two IS10 modules create a composite transposon that can mobilize any region of DNA that lies between them. When Tn10 is part of a small circular molecule, the IS10 repeats can transpose either side of the circle.



## Target site duplication



**Figure 21.1** 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.



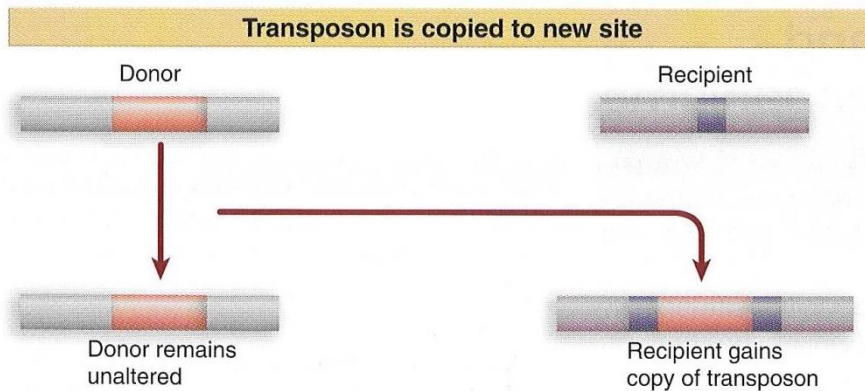
**Figure 21.4** The direct repeats of target DNA flanking a transposon are generated by the introduction of staggered cuts whose protruding ends are linked to the transposon.

Target site duplication  
caused by staggered cutting



## Replicative Transposition

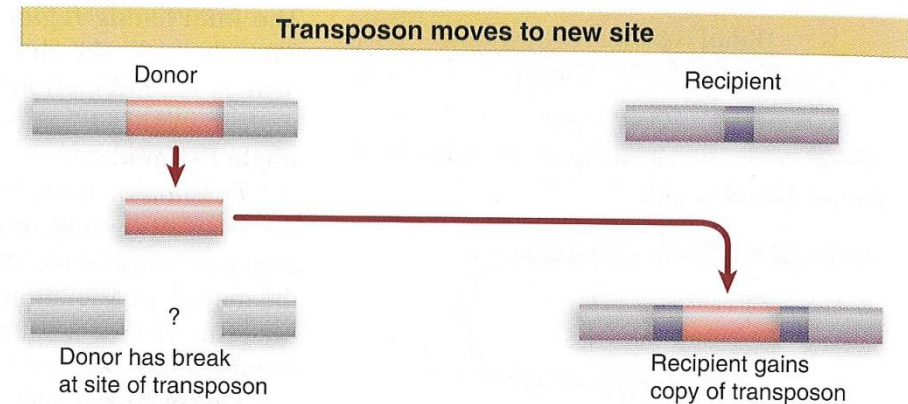
Recipient and donor contain Tn



**Figure 21.5** 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.

## Non-replicative Transposition

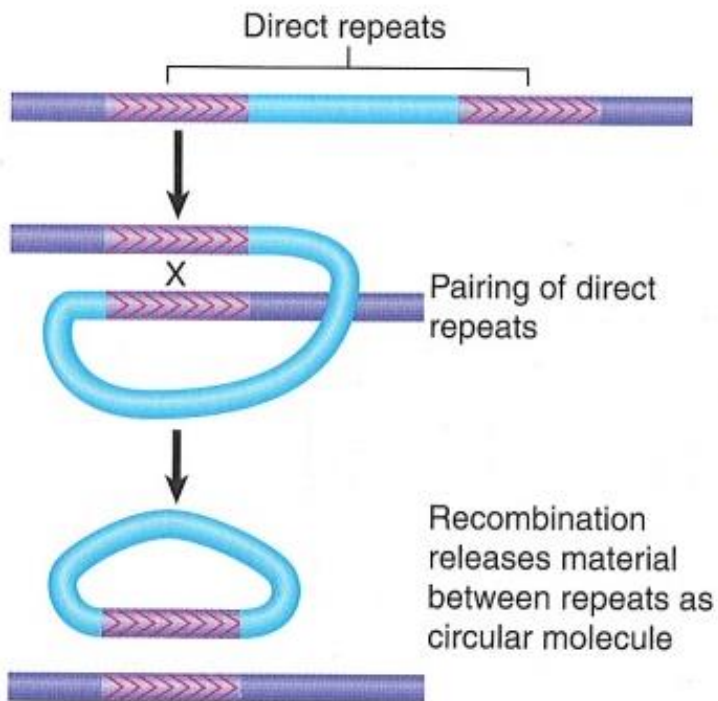
Only recipient contains Tn,  
Donor loses Tn



**Figure 21.6** 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 repaired.

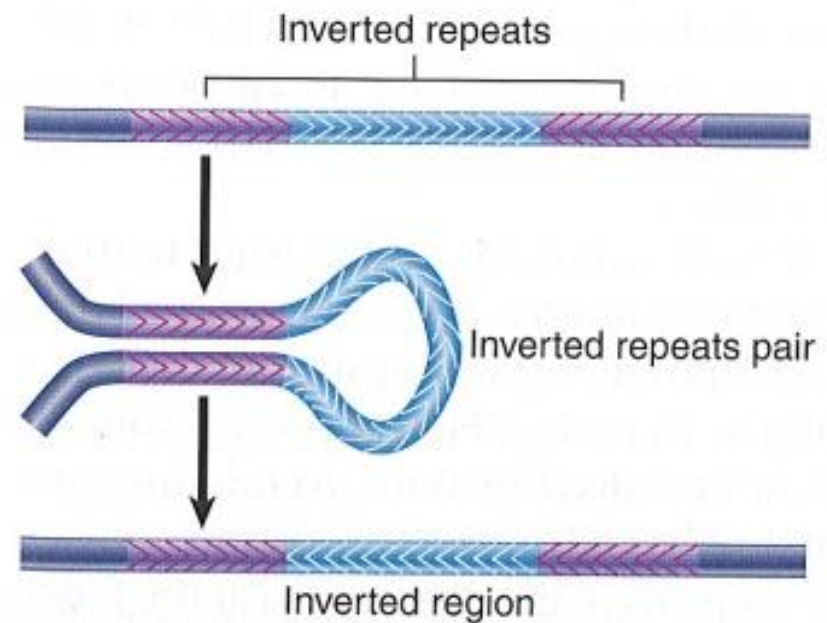


## Deletion

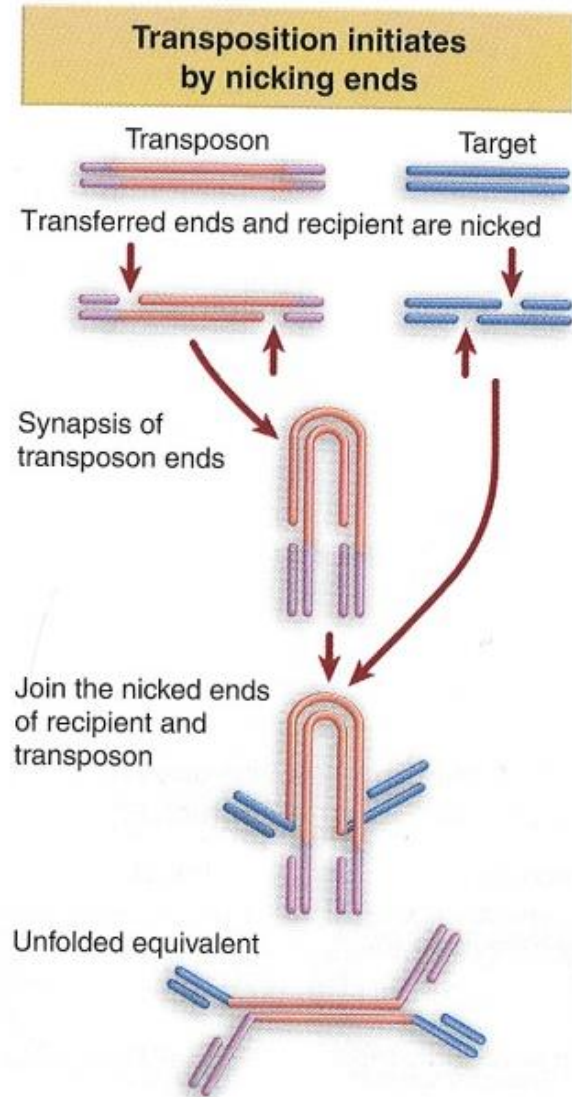


**FIGURE 17.7** Reciprocal recombination between direct repeats excises the material between them; each product of recombination has one copy of the direct repeat.

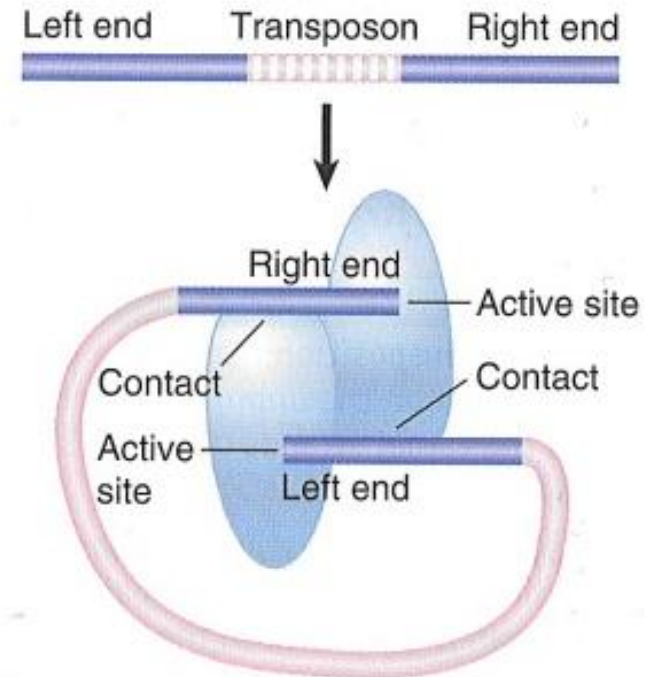
## Inversion



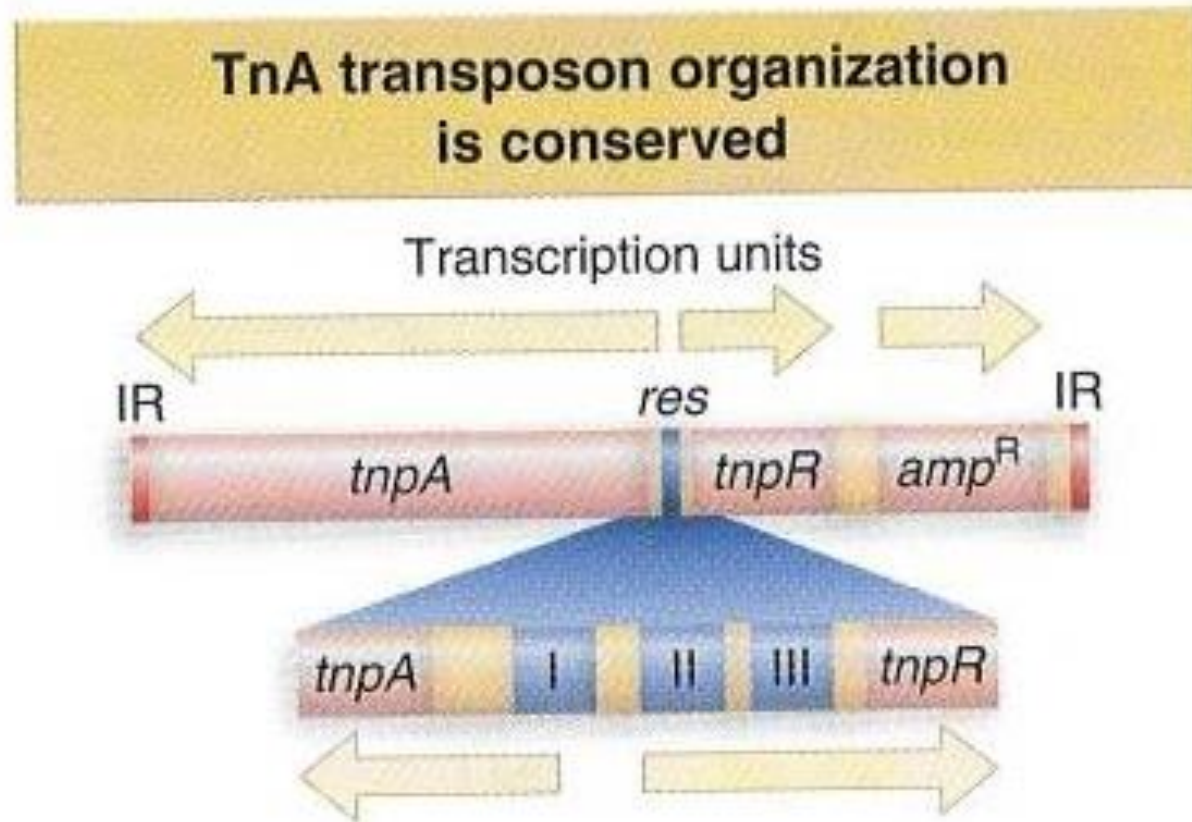
**FIGURE 17.8** Reciprocal recombination between inverted repeats inverts the region between them.



**Figure 21.9** Transposition is initiated by nicking the transposon ends and target site and joining the nicked ends into a strand transfer complex.

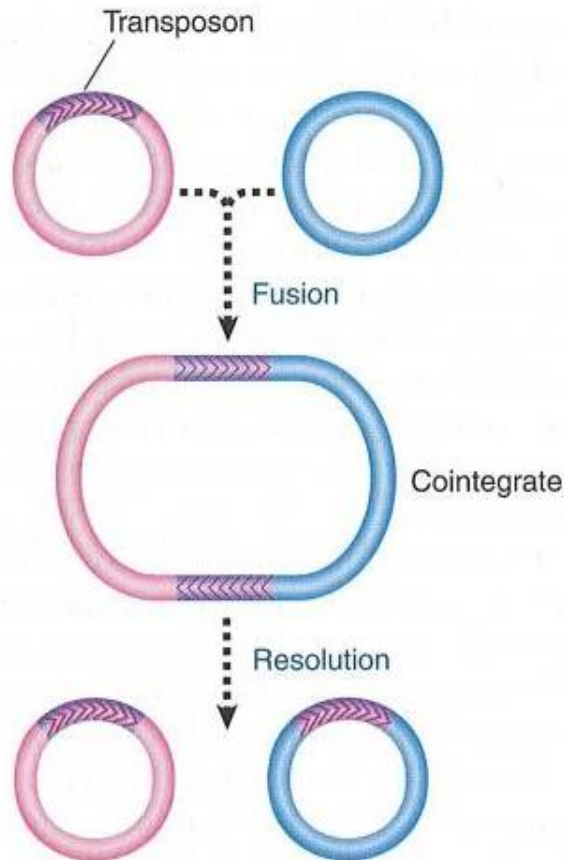


**FIGURE 17.14** Each subunit of the Tn5 transposase has one end of the transposon located in its active site and also makes contact at a different site with the other end of the transposon.

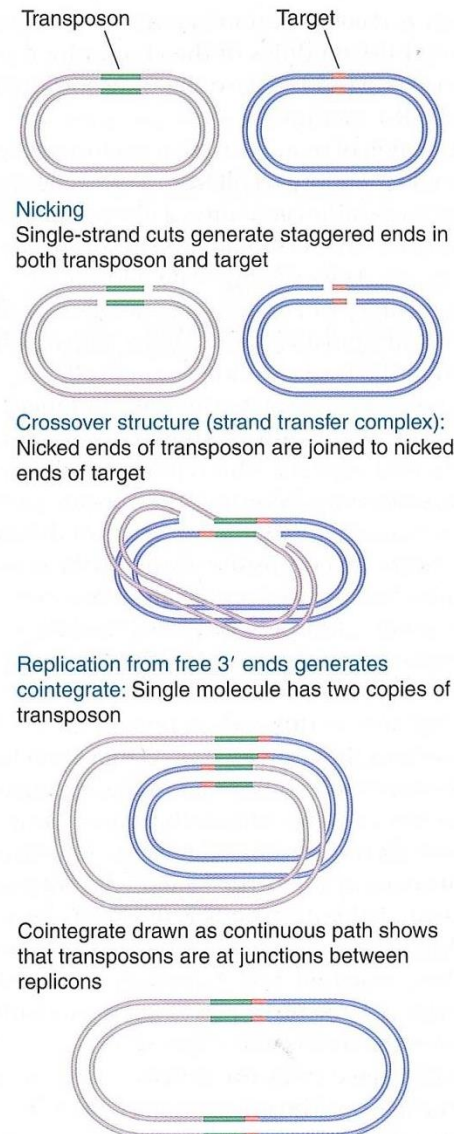


**Figure 21.17** Transposons of the TnA family have inverted terminal repeats, an internal *res* site, and three known genes.

## Cointegrate formation and resolution



**FIGURE 17.9** Transposition may fuse a donor and recipient replicon into a cointegrate. Resolution releases two replicons, each containing a copy of the transposon.

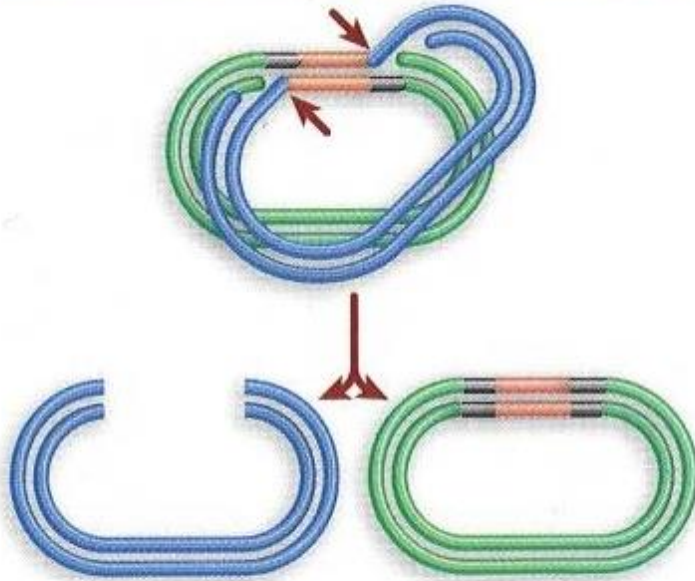


**FIGURE 17.10** Mu transposition generates a crossover structure, which is converted by replication into a cointegrate.

## Replicative Transposition



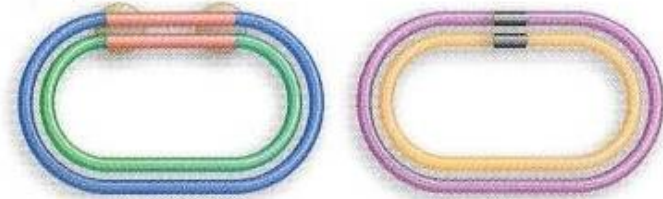
### A crossover can be released by nicking



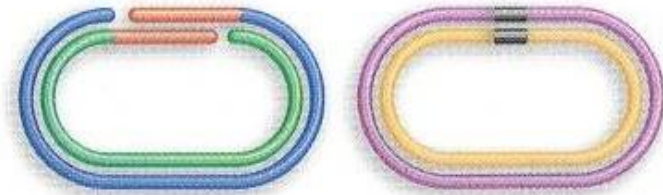
**Figure 21.13** Nonreplicative transposition results when a crossover structure is released by nicking. This inserts the transposon into the target DNA, flanked by the direct repeats of the target, and the donor is left with a double-strand break.

### Transposition can use cleavage and ligation

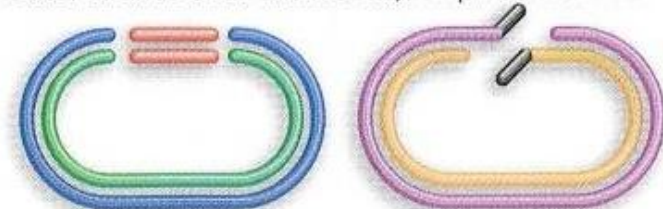
Transposase binds to both ends of Tn



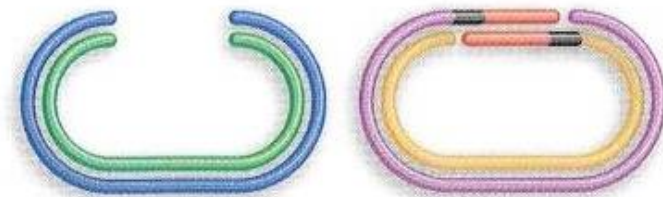
Transferred ends are nicked



Other strands ends are nicked, recipient is nicked



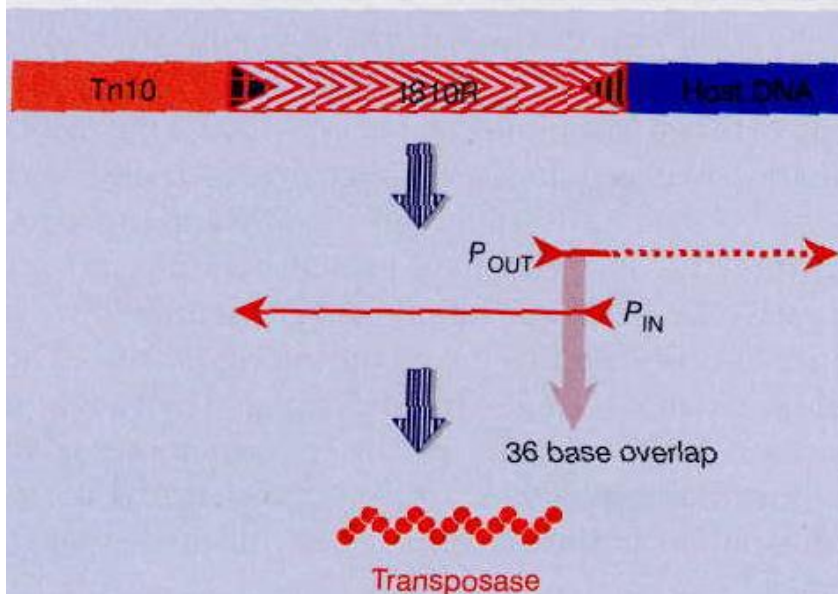
Donor is released, Tn joined to target



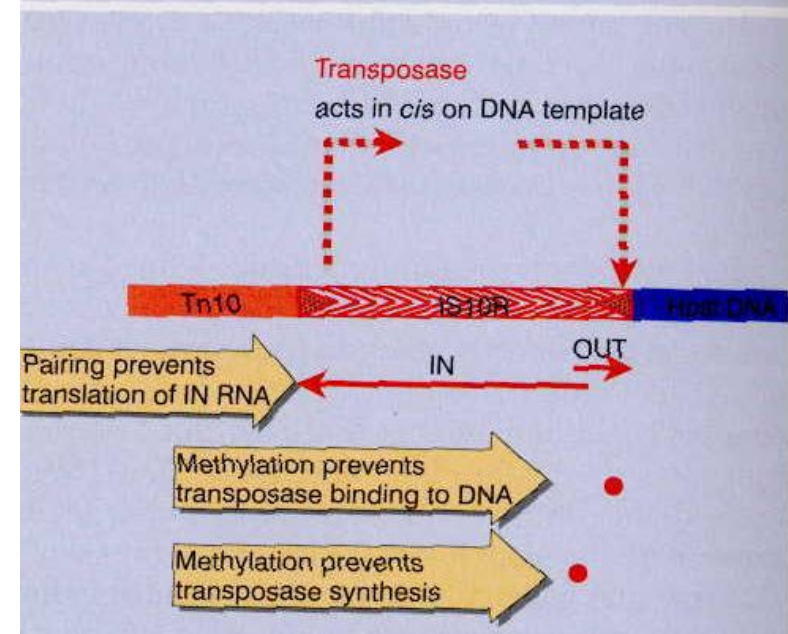
**Figure 21.14** Both strands of Tn10 are cleaved sequentially, and then the transposon is joined to the nicked target site.

## Transposons can influence expression of genes flanking integration site

**Figure 15.17** 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.

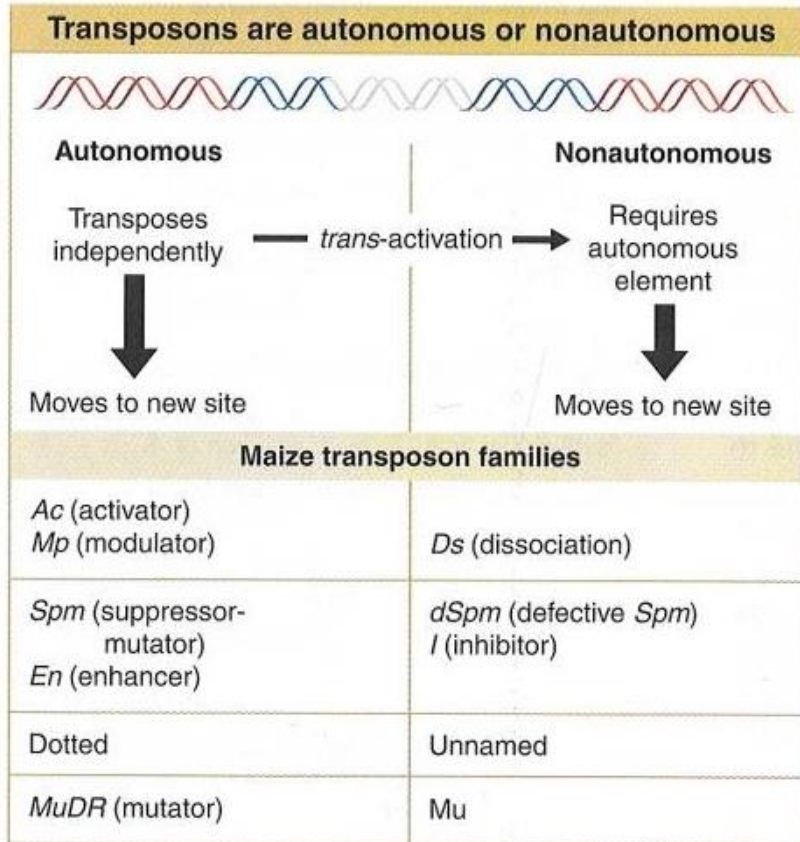


**Figure 15.18** Several mechanisms restrain the frequency of Tn10 transposition, by affecting either the synthesis or function of transposase protein. Transposition of an individual transposon is restricted by methylation to occur only after replication. In multicopy situations, *cis*-preference restricts the choice of target, and OUT/IN RNA pairing inhibits synthesis of transposase.

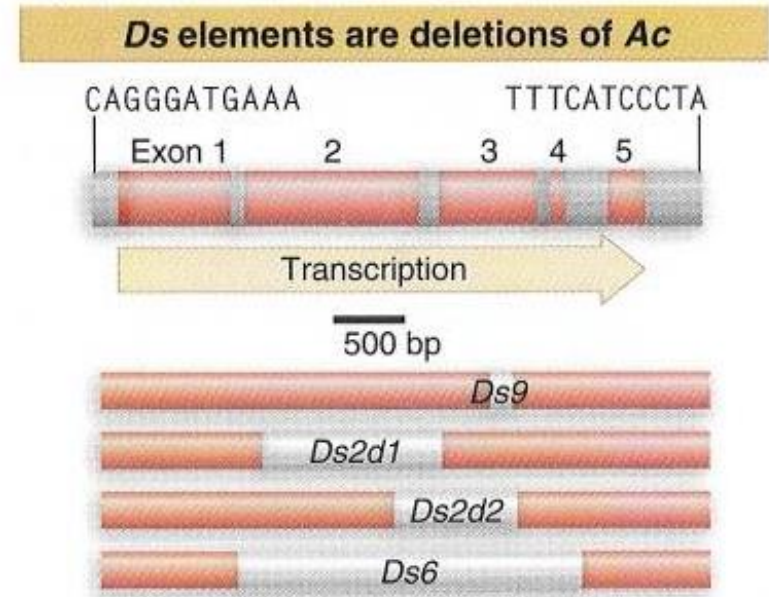




## Transposons of Eukaryotes

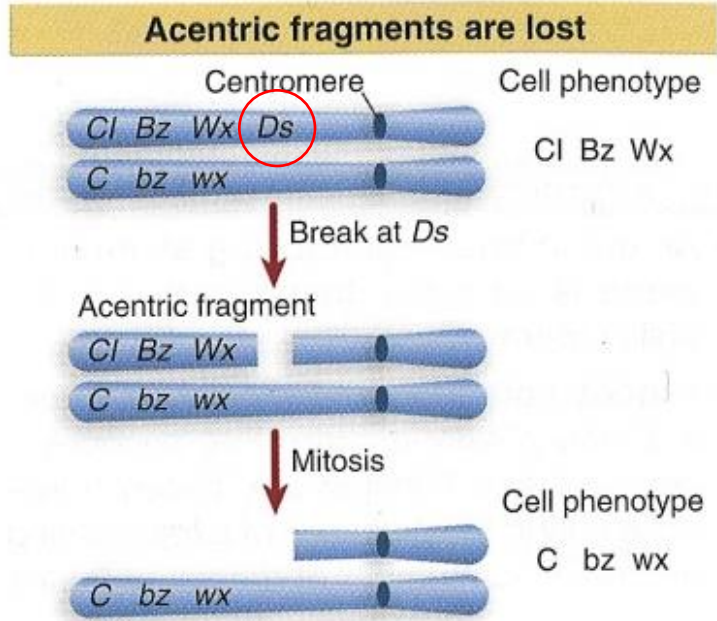


**Figure 21.21** Each controlling element family has both autonomous and nonautonomous members. Autonomous elements are capable of transposition. Nonautonomous elements are deficient in transposition. Pairs of autonomous and nonautonomous elements can be classified in >4 families.



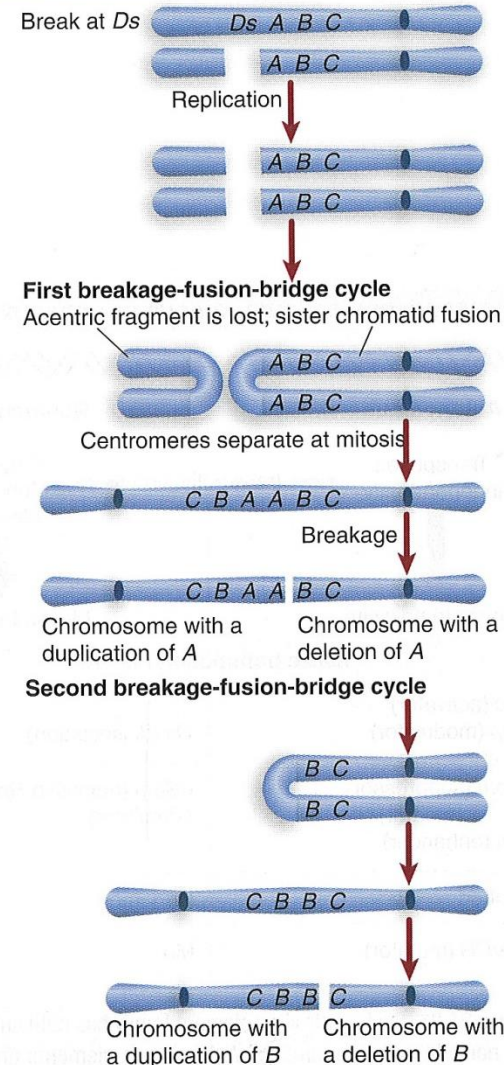
**Figure 21.22** The *Ac* element has five exons that code for a transposase; *Ds* elements have internal deletions.

## Transposons of Eukaryotes



**Figure 21.19** 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.

### *Ds* causes a breakage-fusion-bridge cycle

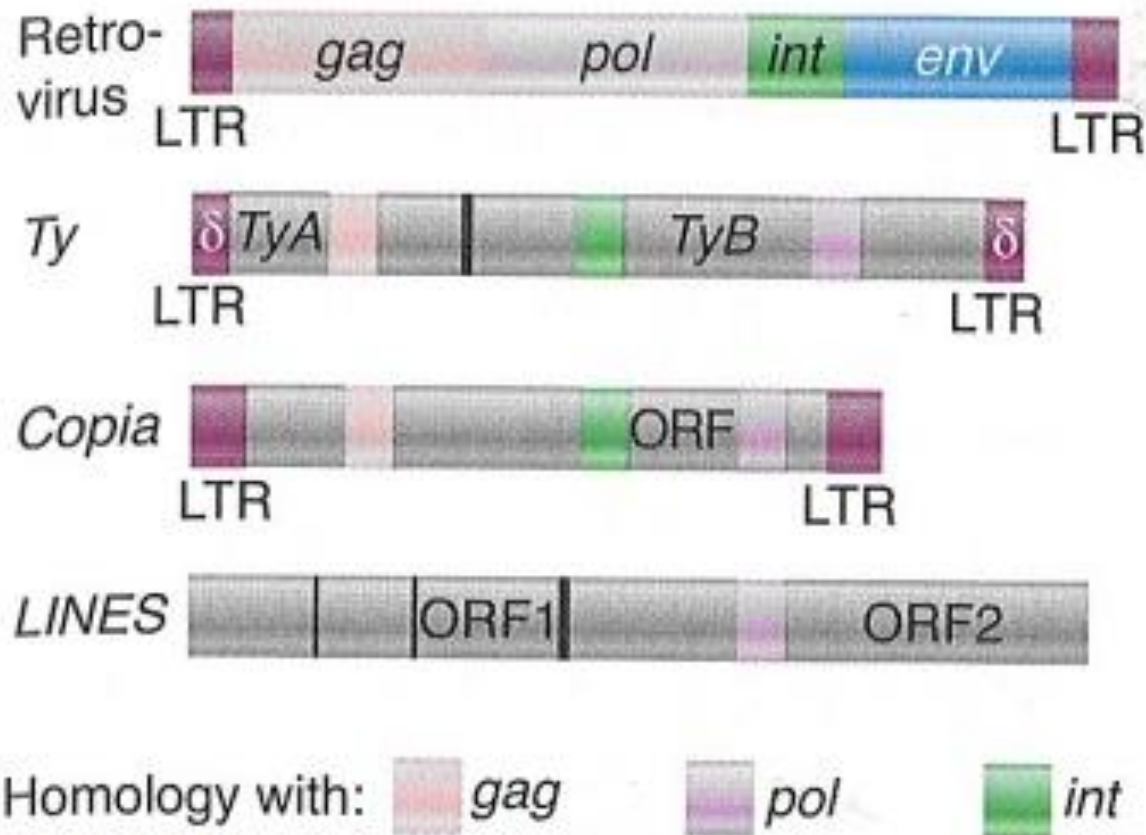


Controlling elements in maize

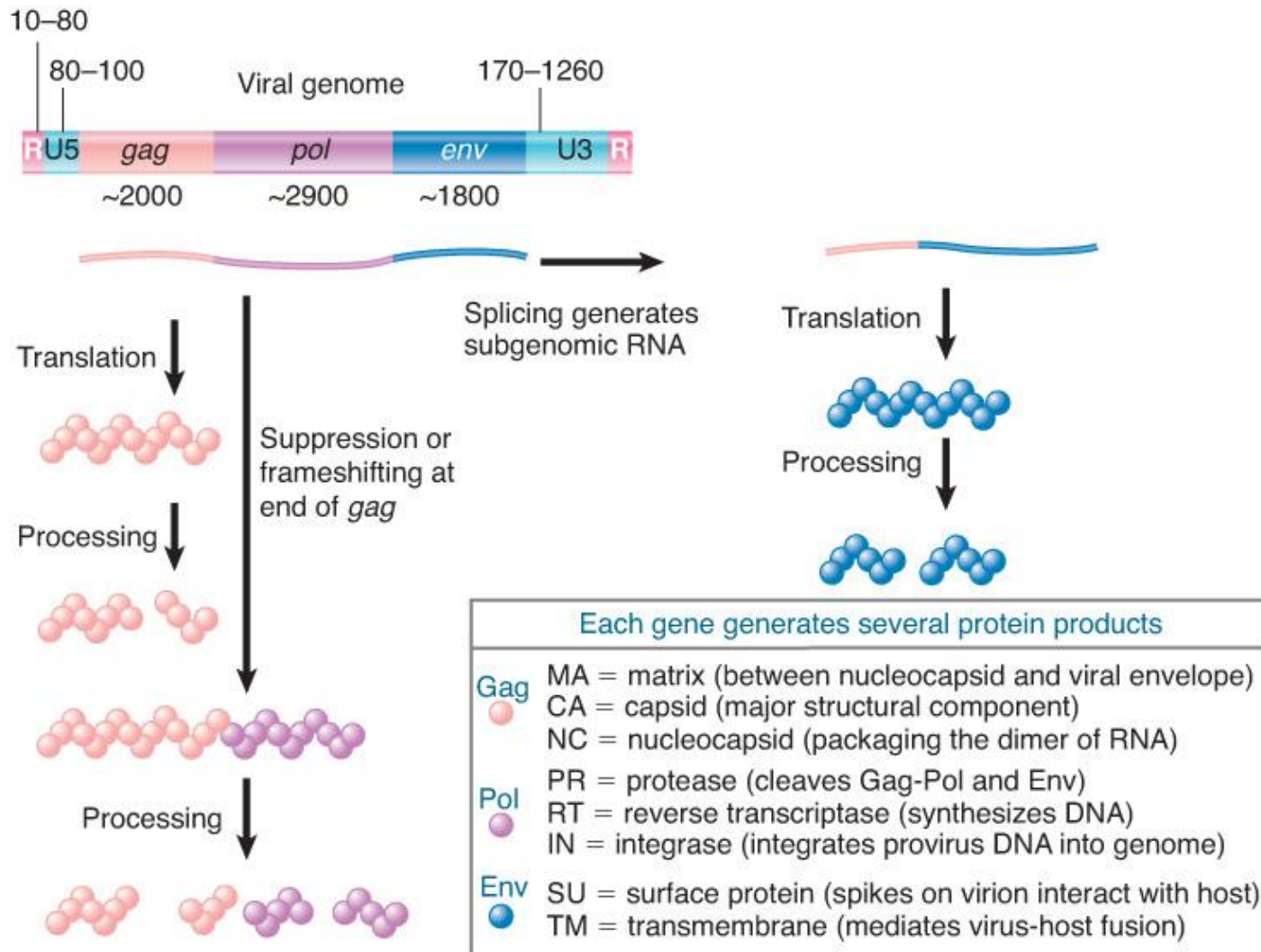
**Figure 21.20** *Ds* provides a site to initiate the chromatid breakage-fusion-bridge cycle. The products can be followed by clonal analysis.



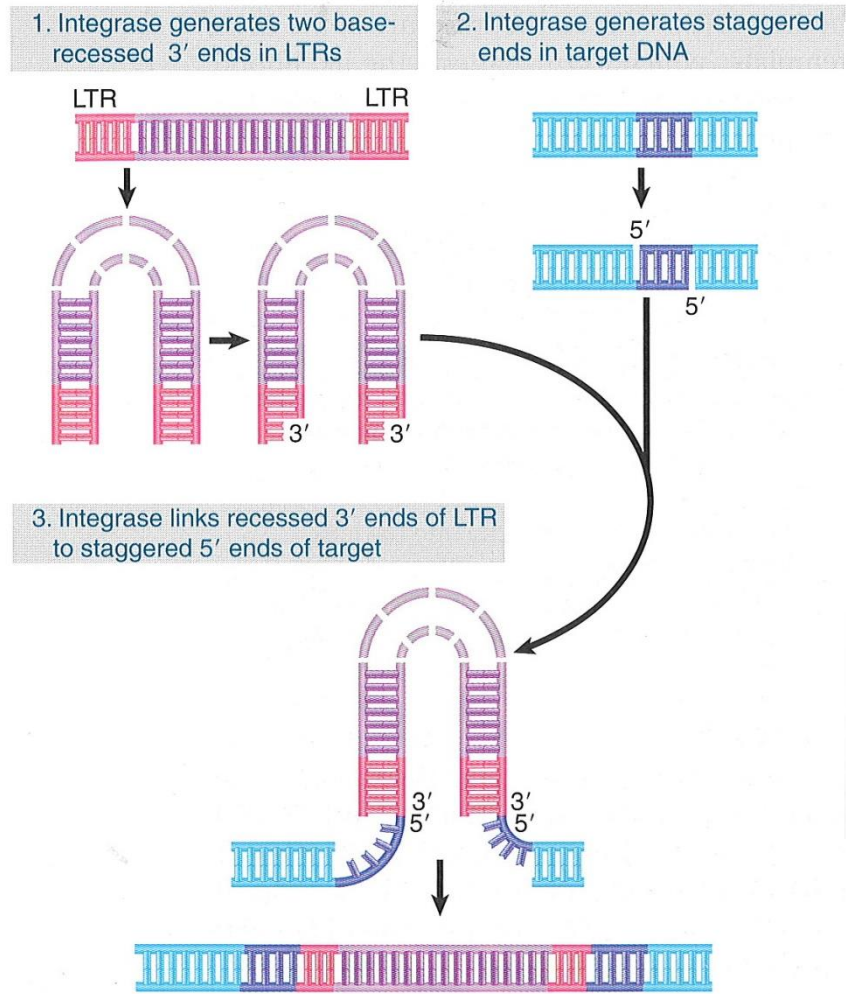
## Retrotransposons



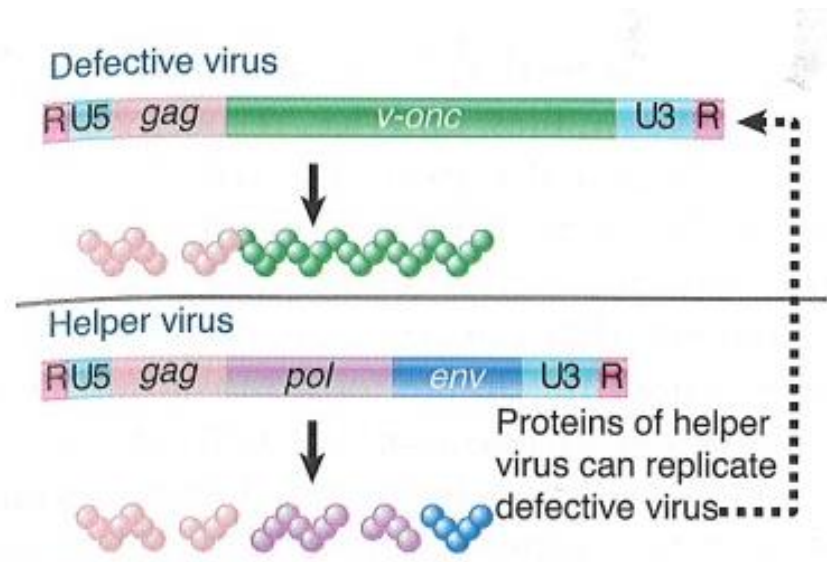
**FIGURE 17.31** Retrotransposons that are closely related to retroviruses have a similar organization, but non-LTR retrotransposons such as LINEs share only the reverse transcriptase activity and lack LTRs.



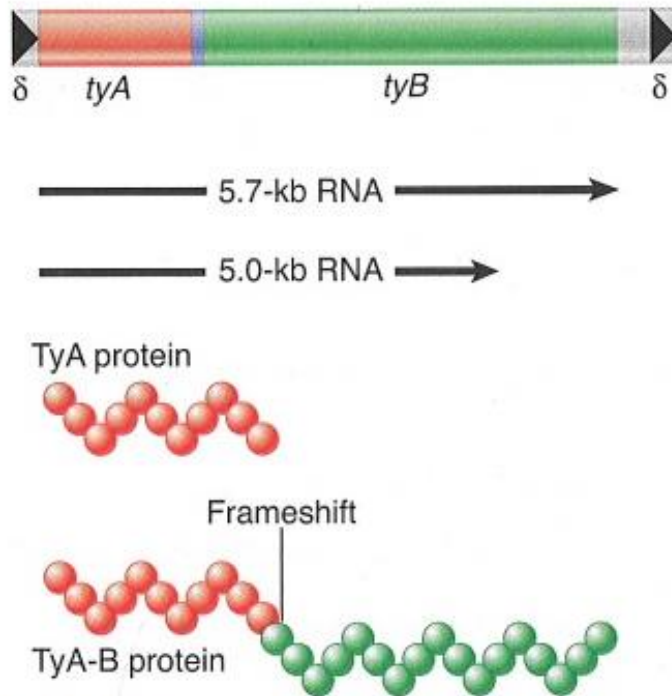
**FIGURE 17.21** The genes of the retrovirus are expressed as polyproteins that are processed into individual products.



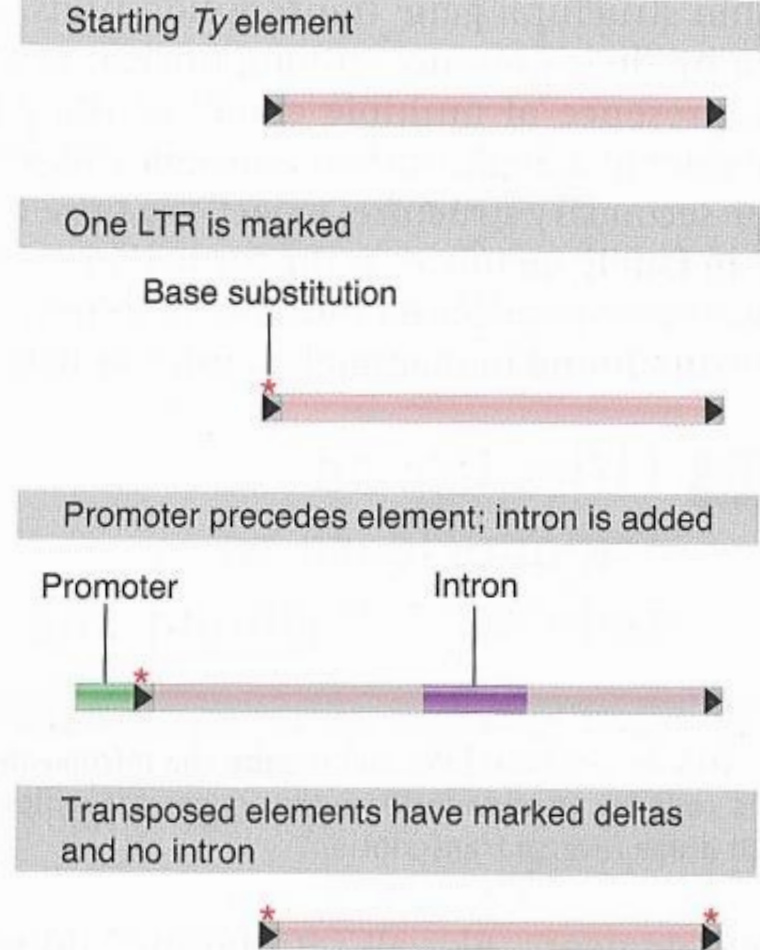
**FIGURE 17.27** Integrase is the only viral protein required for the integration reaction, in which each LTR loses 2 bp and is inserted between 4-bp repeats of target DNA.



**FIGURE 17.28** Replication-defective transforming viruses have a cellular sequence substituted for part of the viral sequence. The defective virus may replicate with the assistance of a helper virus that carries the wild-type functions.



**FIGURE 17.33** *Ty* elements terminate in short direct repeats and are transcribed into two overlapping RNAs. They have two reading frames, with sequences related to the retroviral *gag* and *pol* genes.



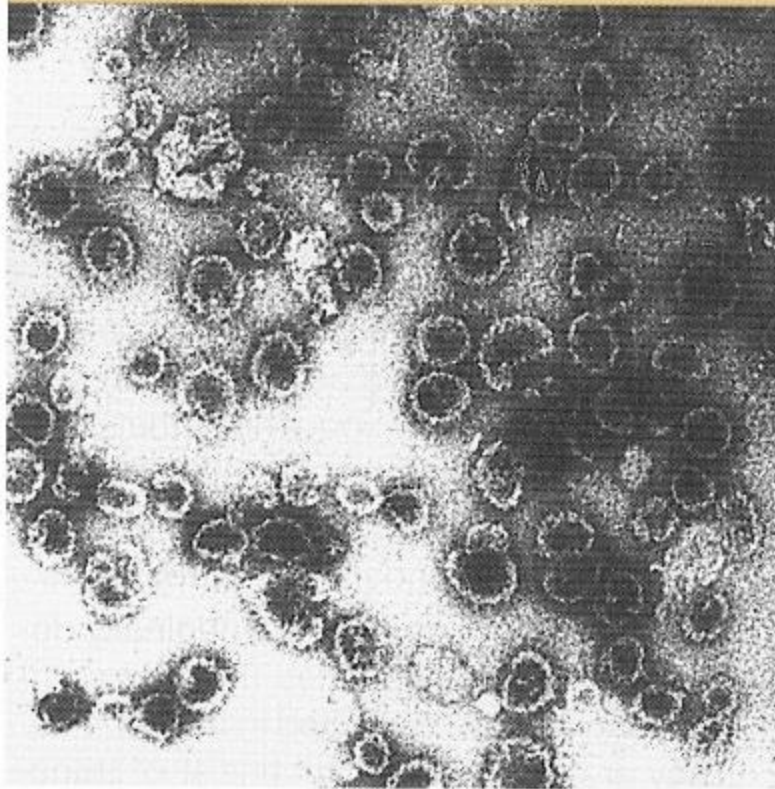
**FIGURE 17.34** A unique *Ty* element, engineered to contain an intron, transposes to give copies that lack the intron. The copies possess identical terminal repeats, which are generated from one of the termini of the original *Ty* element.



<b>Eukaryotic genomes have three types of retroposons</b>			
	Viral superfamily	LINES	Nonviral superfamily
<b>Common types</b>	<i>Ty</i> ( <i>S. cerevisiae</i> ) <i>copia</i> ( <i>D. melanogaster</i> )	L1 (human) B1, B2 ID, B4 (mouse)	SINES (mammals) pseudogenes of pol III transcripts
<b>Termini</b>	Long terminal repeats	No repeats	No repeats
<b>Target repeats</b>	4–6 bp	7–21 bp	7–21 bp
<b>Enzyme activities</b>	Reverse transcriptase and/or integrase	Reverse transcriptase/ endonuclease	None (or none coding for transposon products)
<b>Organization</b>	May contain introns (removed in subgenomic mRNA)	1 or 2 uninterrupted ORFs	No introns

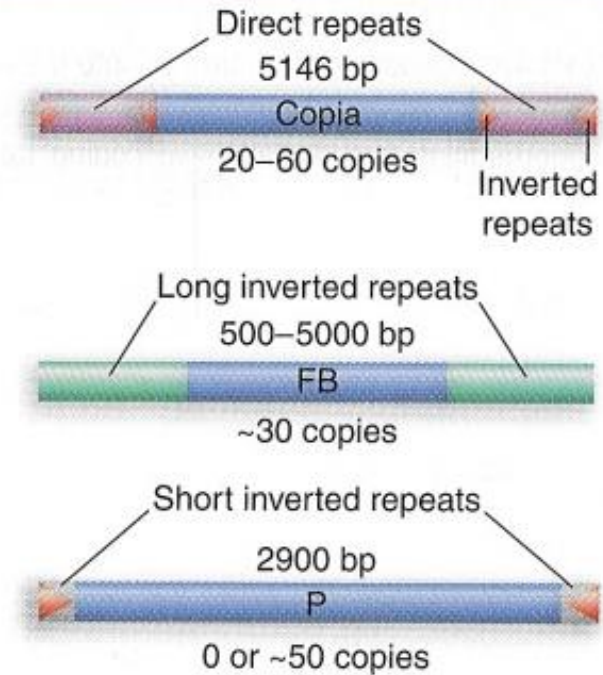
**Figure 22.15** Retroposons can be divided into the viral superfamilies that are either retroviruslike or LINES and the nonviral superfamilies that do not have coding functions.

### Ty elements resemble viruses



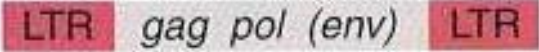
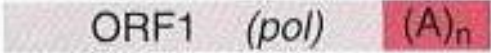

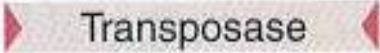
**Figure 22.13** Ty elements generate viruslike particles. Photograph kindly provided by Alan Kingsman, Oxford Bio-Medica plc.

### *D. melanogaster* has several transposons



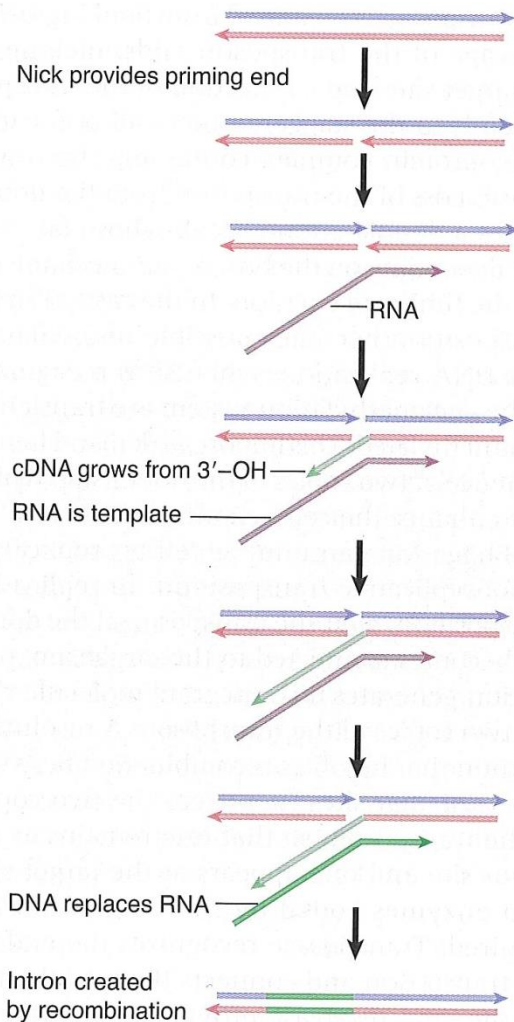
**Figure 22.14** Three types of transposable element in *D. melanogaster* have different structures.

### Retroviruses and transposons constitute half the human genome

Element	Organization	Length (kb)	Human genome Number	Fraction
Retrovirus/ retroposon		1–11	450,000	8%
LINES (autonomous) e.g., L1		6–8	850,000	17%
SINES (nonautonomous) e.g., Alu		<0.3	1,500,000	15%
DNA transposon		2–3	300,000	3%

**Figure 22.17** Four types of transposable elements constitute almost half of the human genome.





**FIGURE 17.36** Retrotransposition of non-LTR retroposons occurs by nicking the target to provide a primer for cDNA synthesis on an RNA template. The arrowheads indicate 3' ends.

## Reverse Transcription → Tool for Genetic Variation

**Figure 16.18**

Pseudogenes could arise by reverse transcription of RNA to give duplex DNAs that become integrated into the genome.

