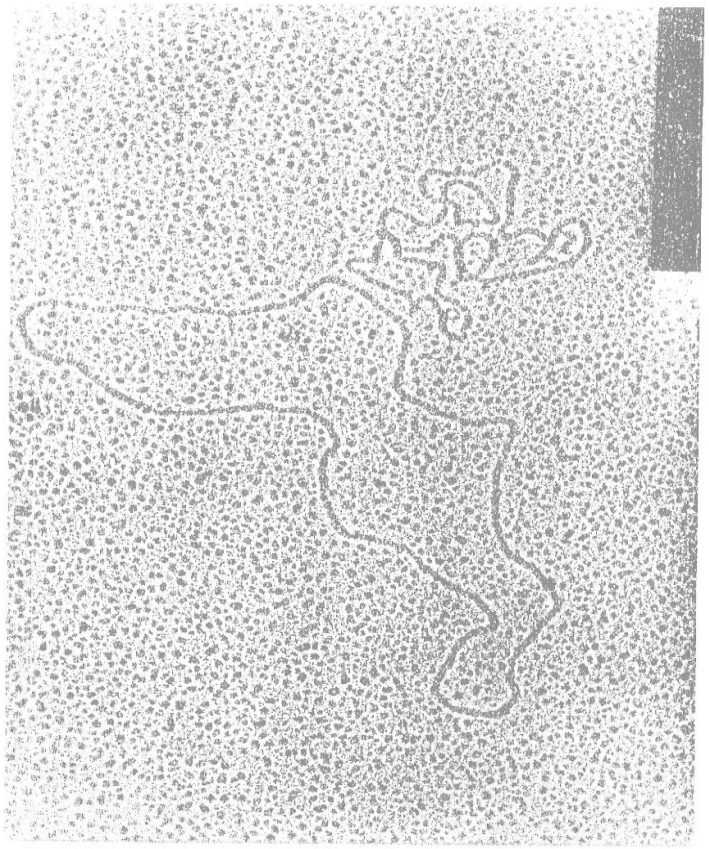
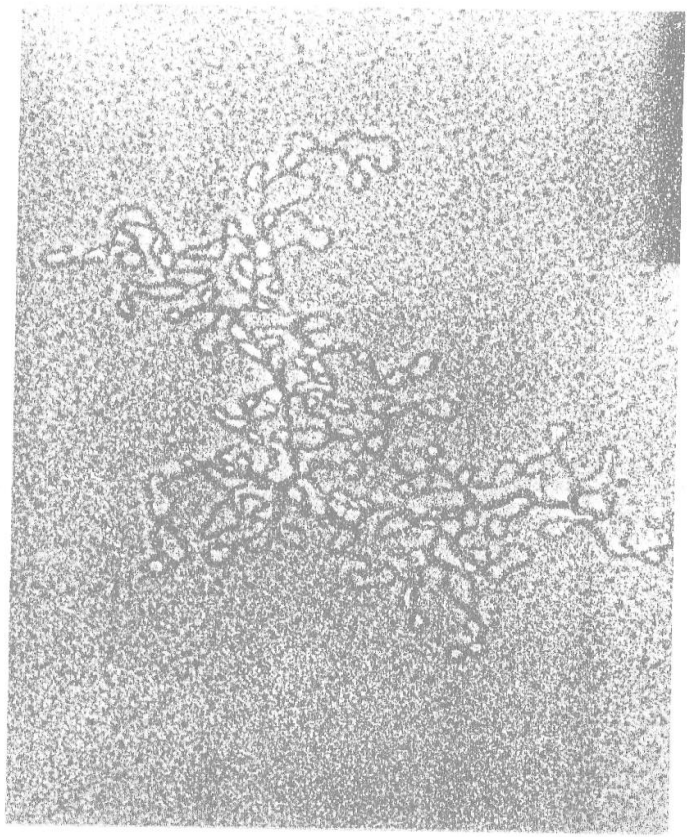
Bacterial Plasmids

Open circular form



Covalently closed circles (ccc-form)



Bacterial plasmids

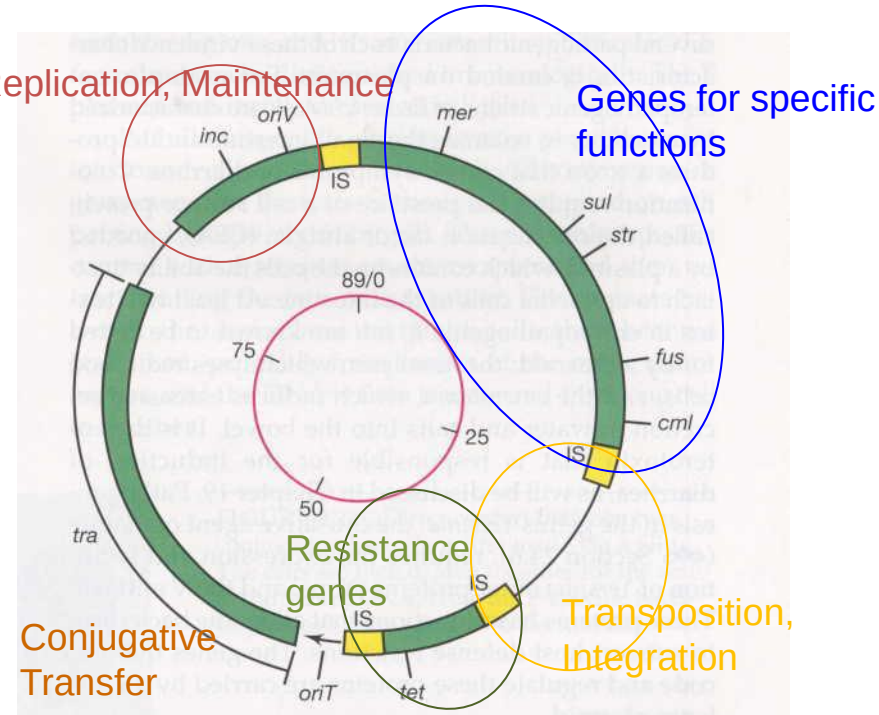


FIGURE 9.21 Genetic map of the resistance plasmid R100. The inner circle shows the size of the plasmid in kilobase pairs. The outer circle shows the location of major antibiotic resistance genes and other key functions: *inc*, incompatibility genes; *oriV*, origin of replication site; *oriT*, origin 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 regons (*mob, oriT, nic, bom*)

→ Autonomous In vivo transfer of plasmids
→ In vivo transfer mediated by helper functions

Bacterial Plasmids

Copy Number

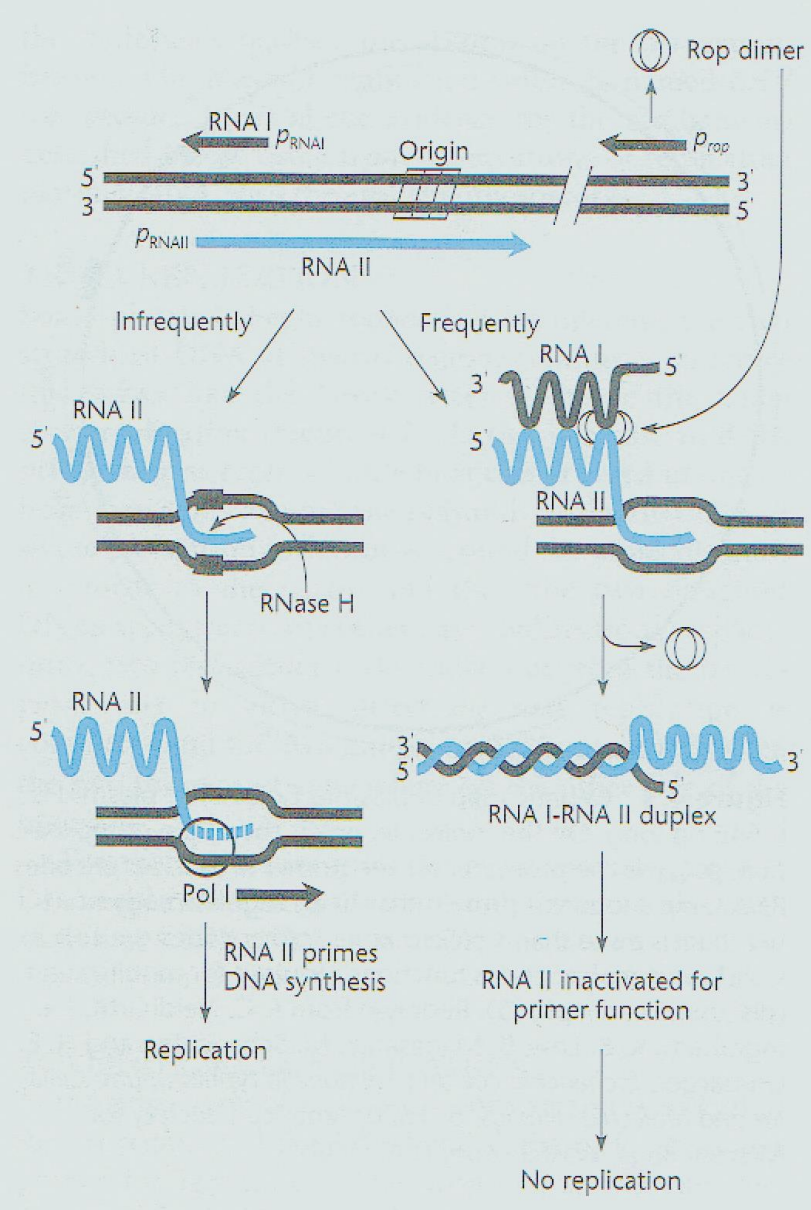
Replication & its control

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

Relaxed Control: high copy number
ColE1, pBR322, pUC18

Incompatibility:

Replication / Control
Partitioning



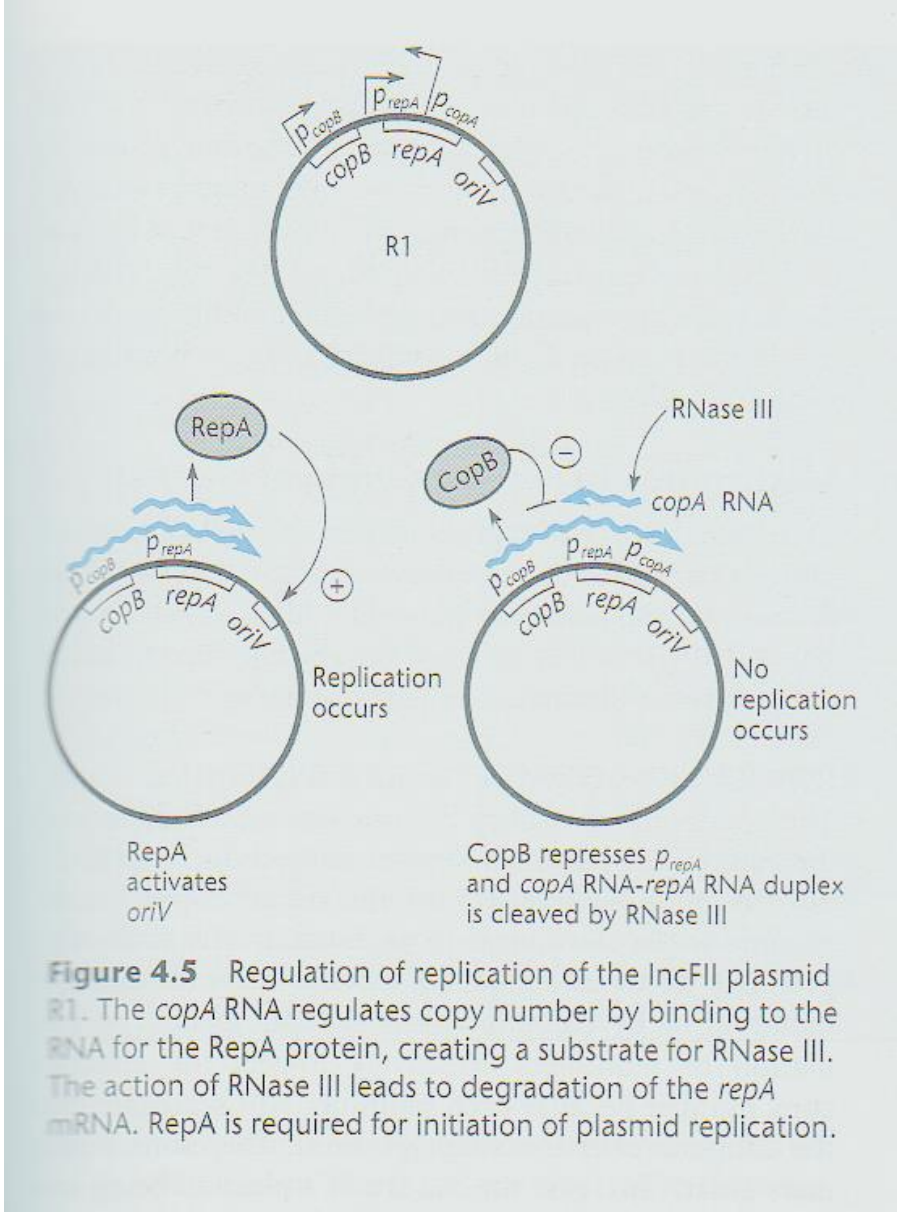


Figure 4.5 Regulation of replication of the IncFII plasmid R1. The *copA* RNA regulates copy number by binding to the RNA for the RepA protein, creating a substrate for RNase III. The action of RNase III leads to degradation of the *repA* mRNA. RepA is required for initiation of plasmid replication.

Iteron Model

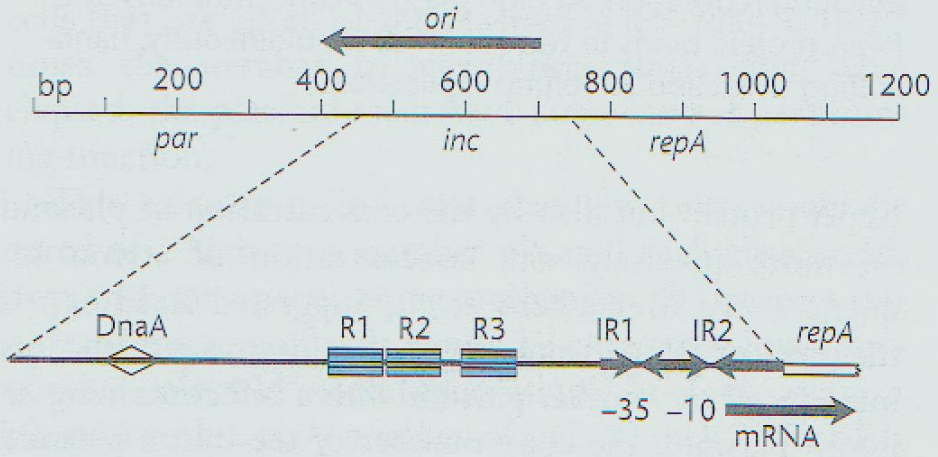


Figure 4.6 The *ori* region of pSC101. R1, R2, and R3 are the three iteron sequences (CAAAGGTCTAGCAGCAGAATTTACAGA for R3) to which RepA binds to handcuff two plasmids. RepA autoregulates its own synthesis by binding to the inverted repeats IR1 and IR2. The location of the partitioning site *par* (see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

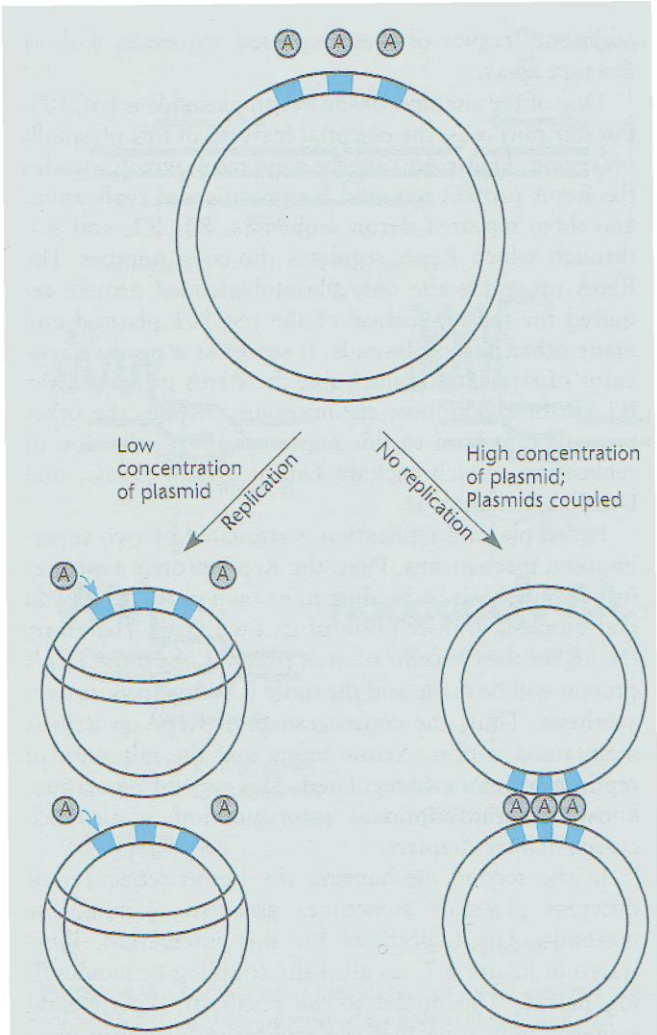


Figure 4.7 The "handcuffing" or "coupling" model for regulation of iteron plasmids. At low concentrations of plasmids, the RepA protein only binds to one plasmid at a time, initiating replication. At high plasmid concentrations, the RepA protein binds to two plasmids simultaneously, handcuffing them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

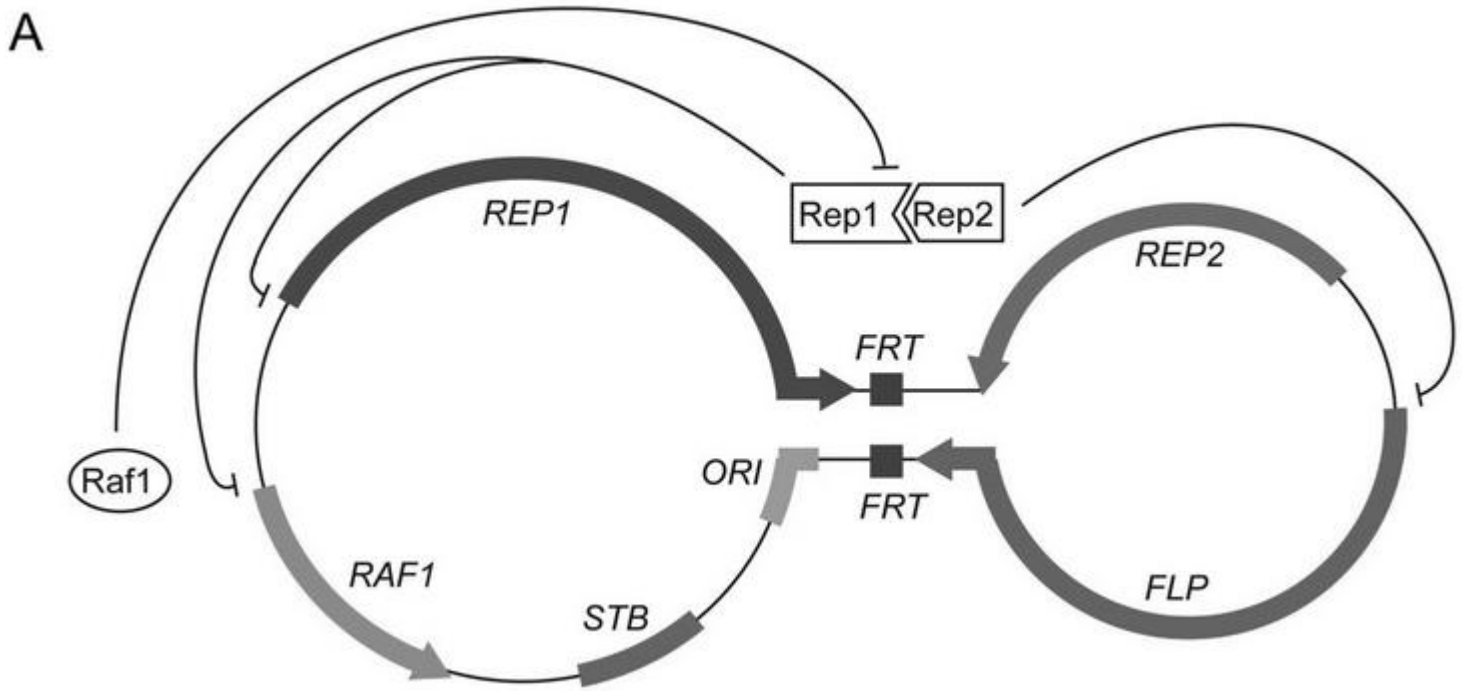
Plasmids

Conjugative Transfer:	Gram-negative:	F, RP4/RK2,
	Gram-positive:	pAMß1, SCP2*
	Plants	Ti
Bacteriocin-/ Microcin-Production		
Antibiotic Resistance:	ß-Lactam Antibiotics:	ß-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

Plasmids in Eukaryotes - Yeast



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.

Biochemical Society Transactions Apr 01, 2013, 41 (2) 501-507; DOI: 10.1042/BST20120224

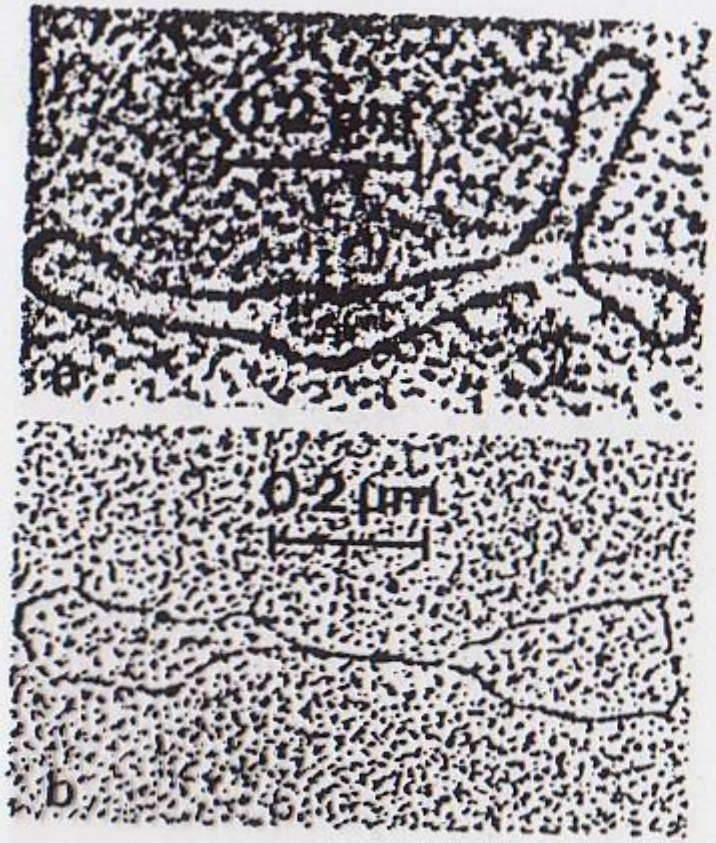


Fig. 2a,b. *Saccharomyces cerevisiae*: structure of the 2 µm plasmid: (a) double-stranded plasmid; (b) homoduplex of the 2 µm plasmid. The self-annealing of the inverted repeats of the plasmid yields typical "dumb-bell" structures (from C.P. Hollenberg)

All human mtDNA is expressed

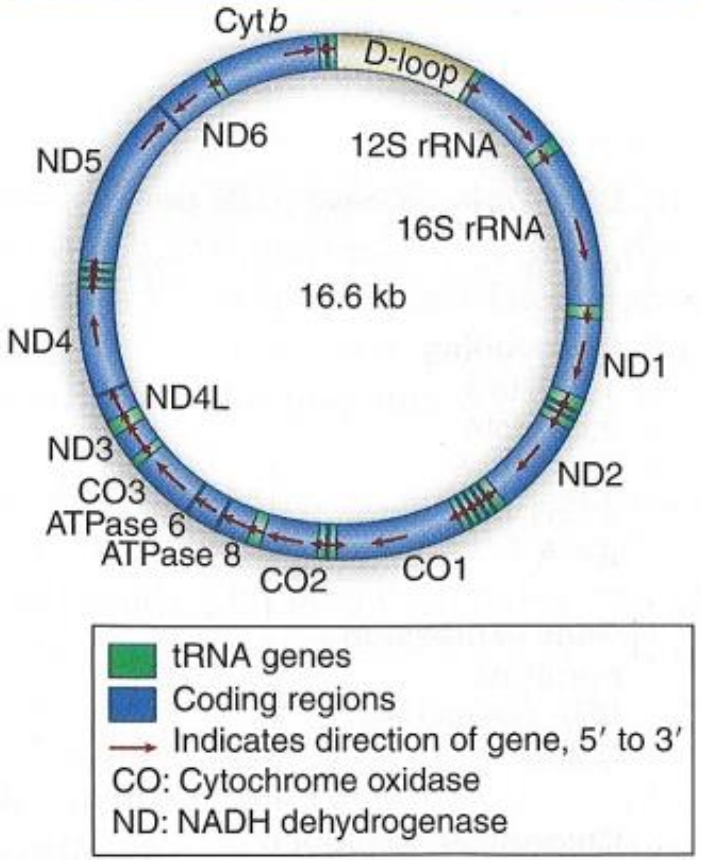


Figure 4.17 Human mitochondrial DNA has 22 tRNA genes, 2 rRNA genes, and 13 protein-coding regions. 14 of the 15 protein-coding or rRNA-coding regions are transcribed in the same direction. 14 of the tRNA genes are expressed in the clockwise direction and 8 are read counterclockwise.

Yeast mtDNA has the same genes as human

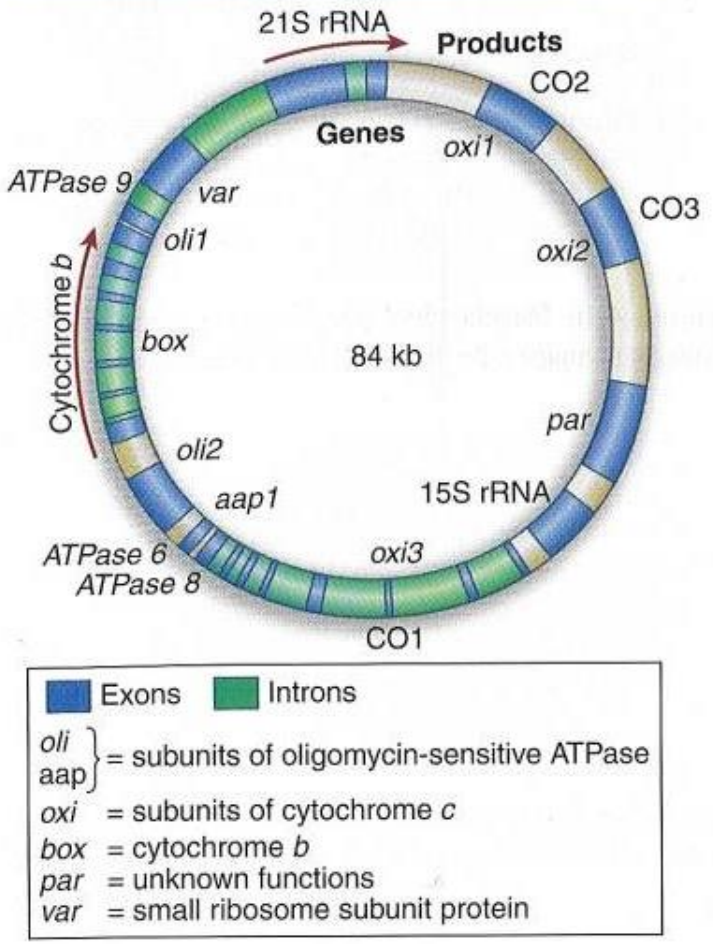


Figure 4.18 The mitochondrial genome of *S. cerevisiae* contains both interrupted and uninterrupted protein-coding genes, rRNA genes, and tRNA genes (positions not indicated). Arrows indicate direction of transcription.

Taken from: B.Lewin, „Essential Genes“, Pearson Education International

20.10.15

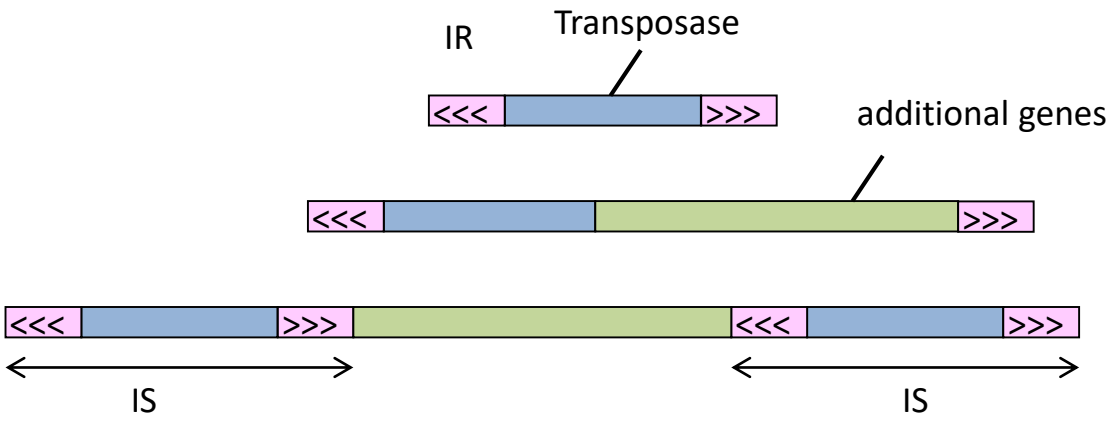
Transposable Elements - Insertion Sequences and Transposons

DNA – DNA Transposition

IS Elements

Simple Transposons

Composite Transposons



DNA – RNA – DNA Transposition

Retrotransposons

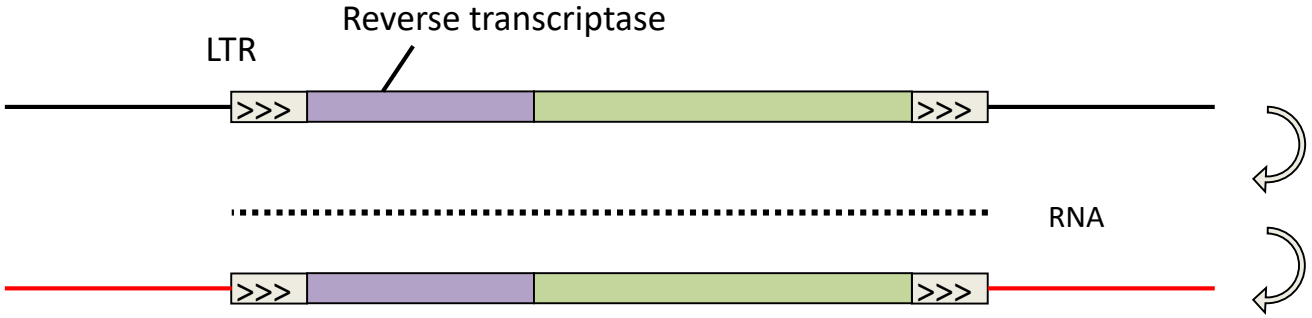
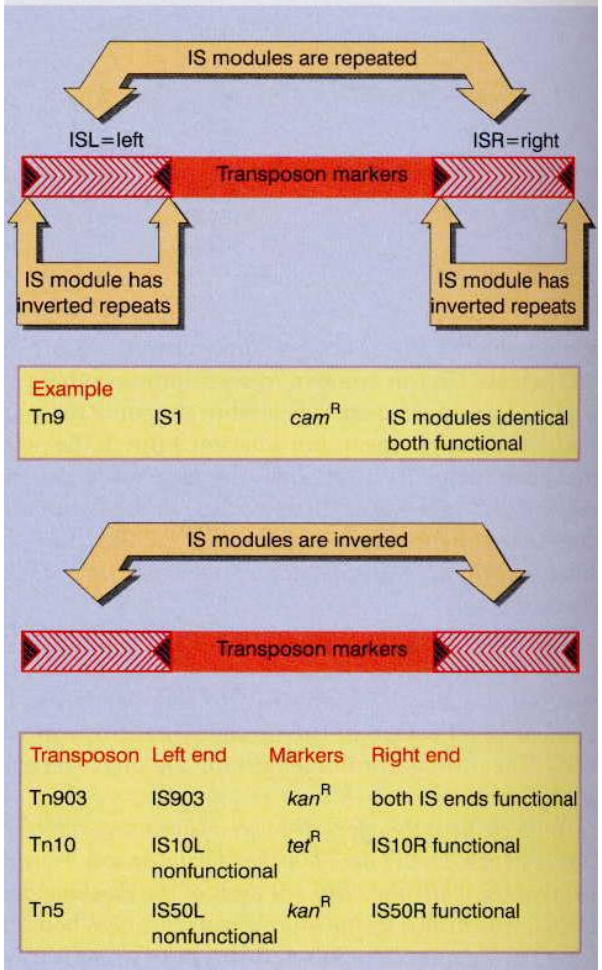
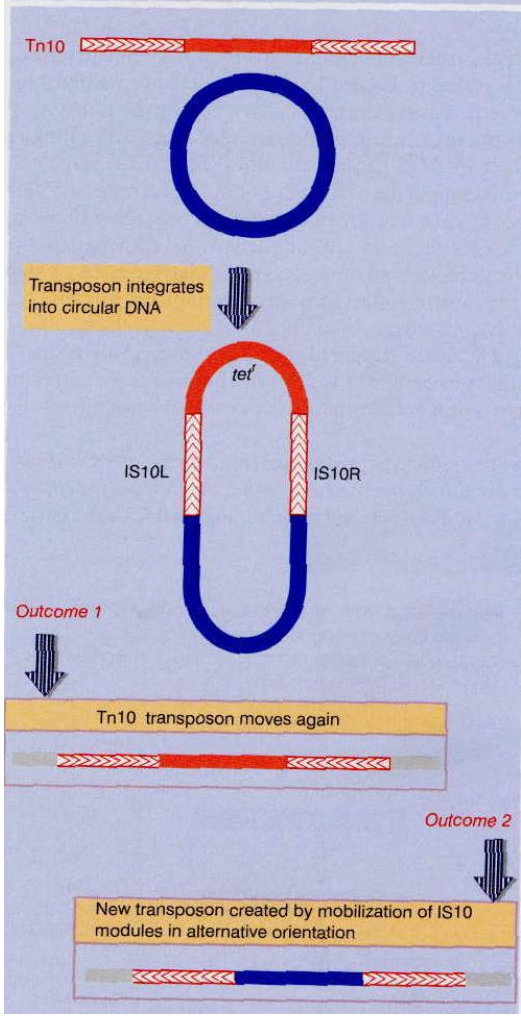


Figure 15.2 A composite transposon has a central region carrying markers (such as drug resistance) flanked by IS modules. The modules have short inverted terminal repeats. If the modules themselves are in inverted orientation (as drawn), the short inverted terminal repeats at the ends of the transposon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

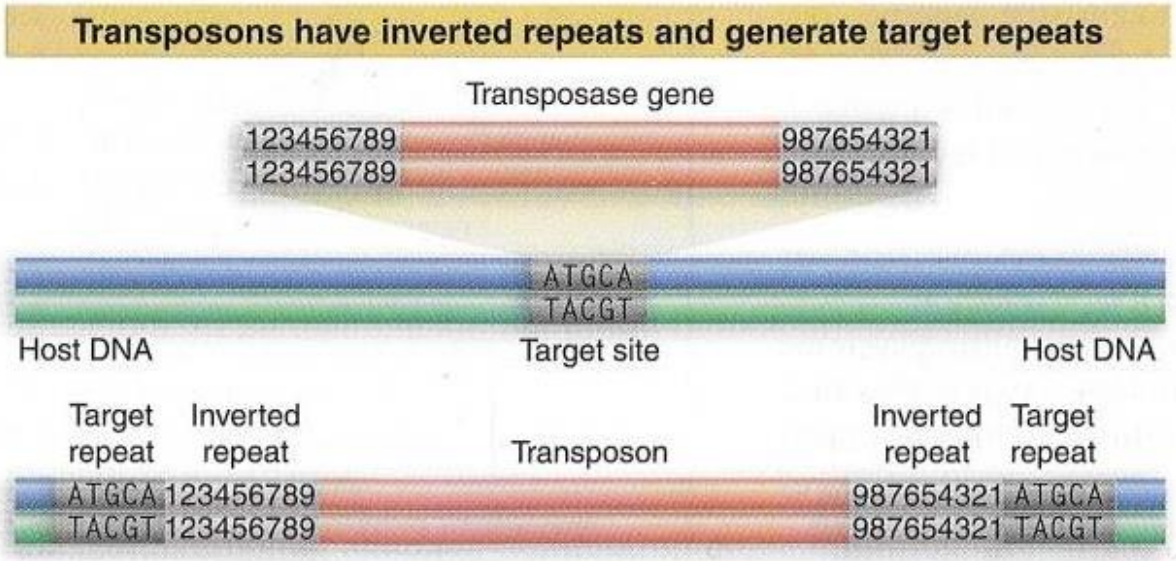


IS elements and transposons can generate new transposable elements by integration at adjacent positions into a genome

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



Transposon	Target repeat (bp)	Inverted repeat (bp)	Overall length	Target selection
IS1	9	23	768	Random
IS2	5	41	1327	Hotspots
IS4	11–13	18	1428	$AAAN_{20}TTT$
IS5	4	16	1195	Hotspots
IS10R	9	22	1329	NGTNAGCN
IS50R	9	9	1531	Hotspots
IS903	9	18	1057	Random

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.

Taken from: B.Lewin, „Essential Genes“, Pearson Education International

Target site duplication
caused by staggered cutting

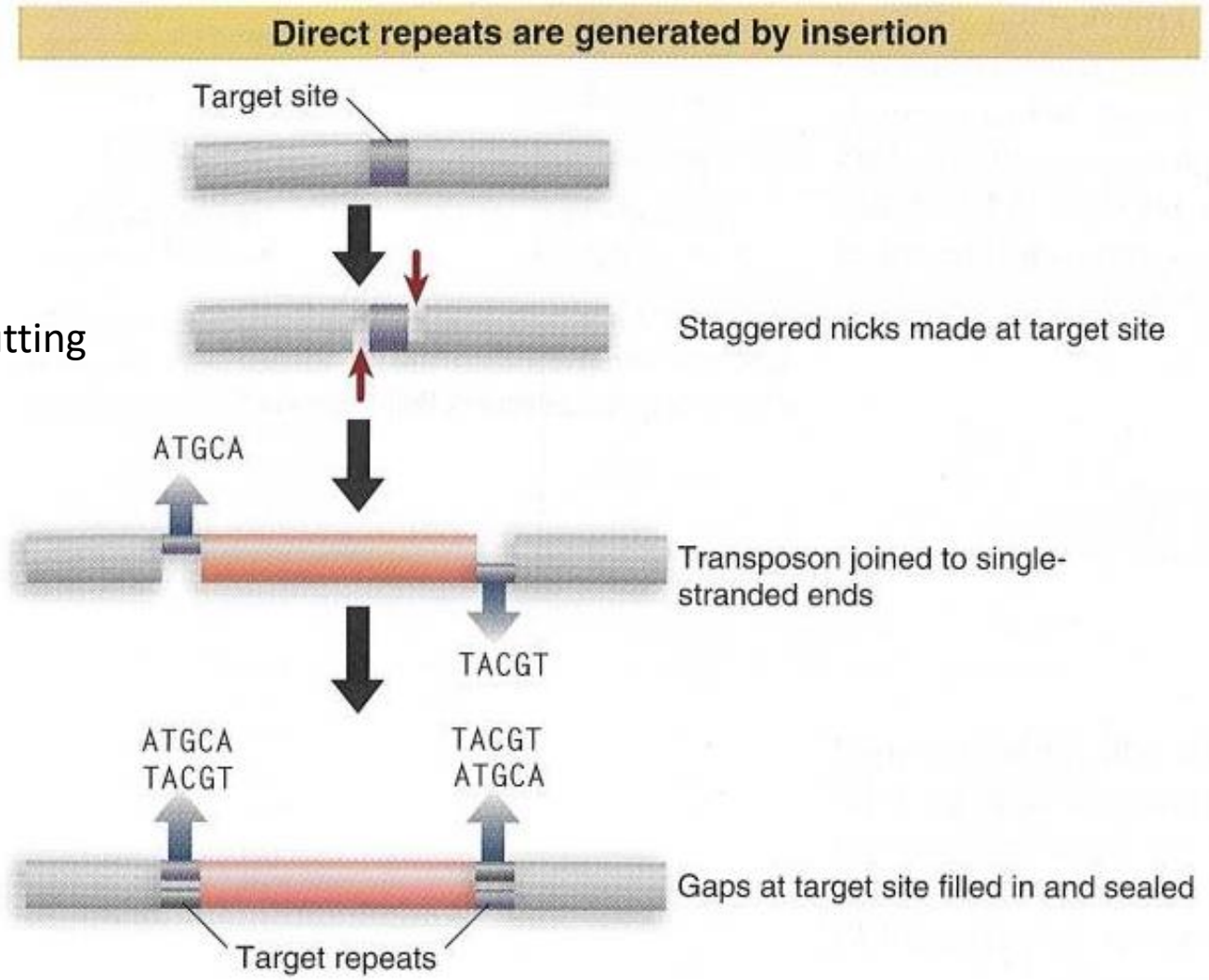


Figure 21.4 The direct repeats of target DNA flanking a transposon are generated by the introduction of staggered cuts whose protruding ends are linked to the transposon.

Taken from: B.Lewin, „Essential Genes“, Pearson Education International

Replicative Transposition

Recipient and donor contain Tn

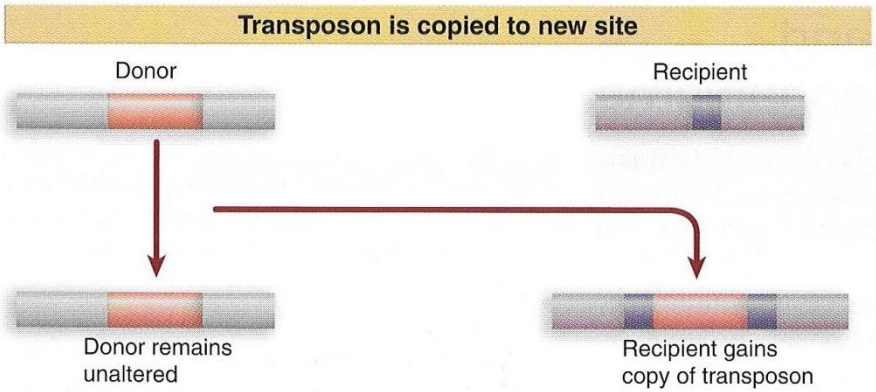


Figure 21.5 Replicative transposition creates a copy of the transposon, which inserts at a recipient site. The donor site remains unchanged, so both donor and recipient have a copy of the transposon.

Non-replicative Transposition

Only recipient contains Tn,
Donor looses Tn

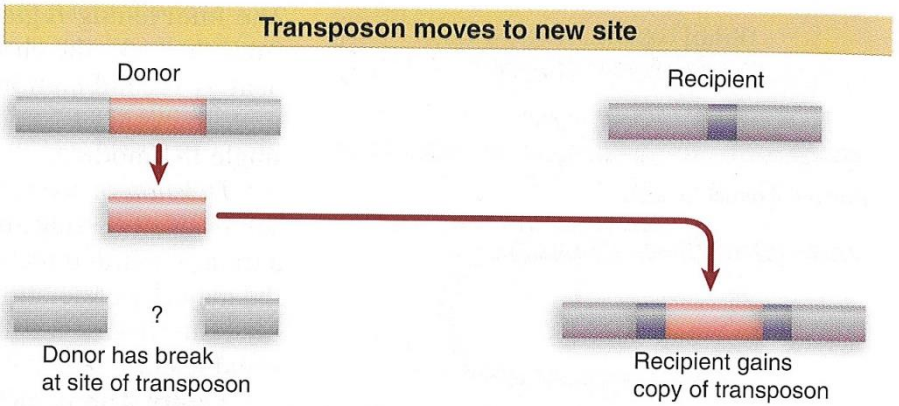


Figure 21.6 Nonreplicative transposition allows a transposon to move as a physical entity from a donor to a recipient site. This leaves a break at the donor site, which is lethal unless repaired.

Taken from: B.Lewin, „Essential Genes“, Pearson Education International

Deletion

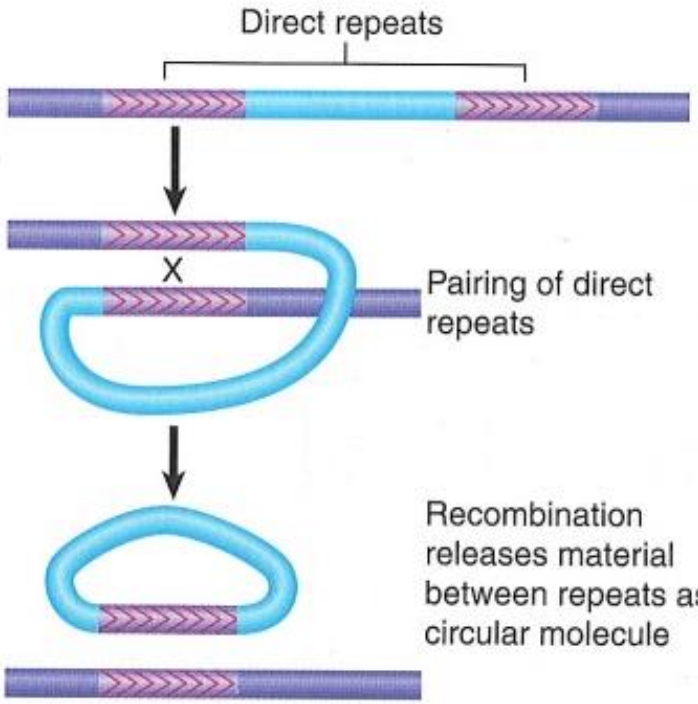


FIGURE 17.7 Reciprocal recombination between direct repeats excises the material between them; each product of recombination has one copy of the direct repeat.

Inversion

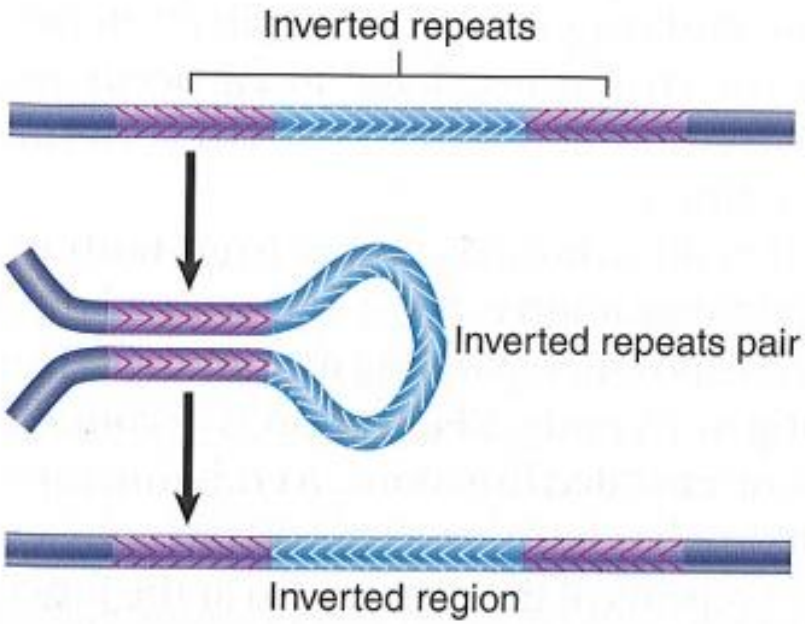


FIGURE 17.8 Reciprocal recombination between inverted repeats inverts the region between them.

Taken from: J.E. Krebs, E.S. Goldstein, S.T. Kilpatrick; „Lewin´s Genes XI"; Jones&Bsrtlett Learning

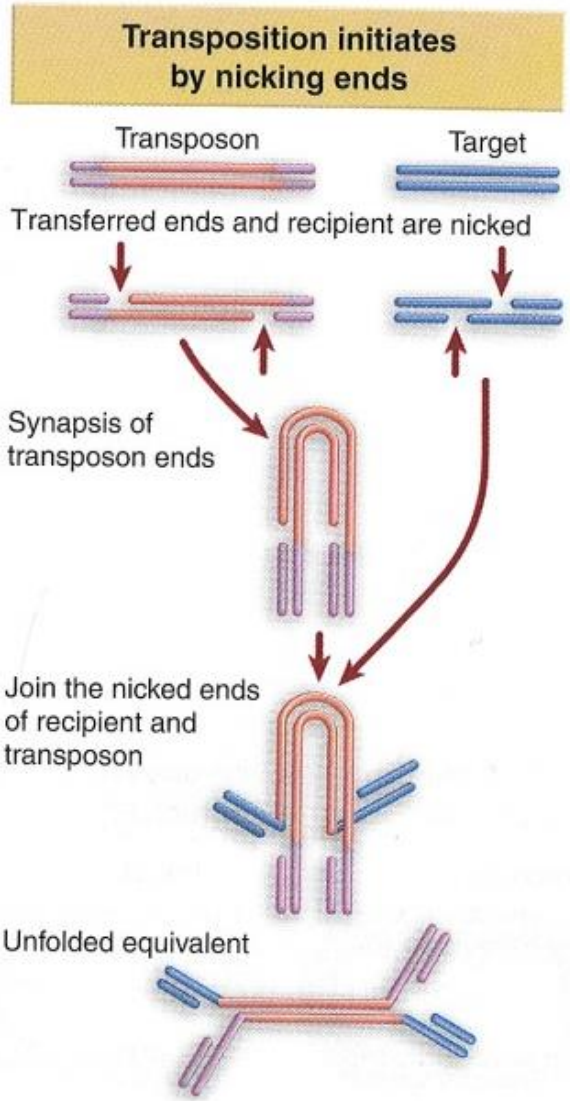


Figure 21.9 Transposition is initiated by nicking the transposon ends and target site and joining the nicked ends into a strand transfer complex.

Taken from: B.Lewin, „Essential Genes“, Pearson Education International

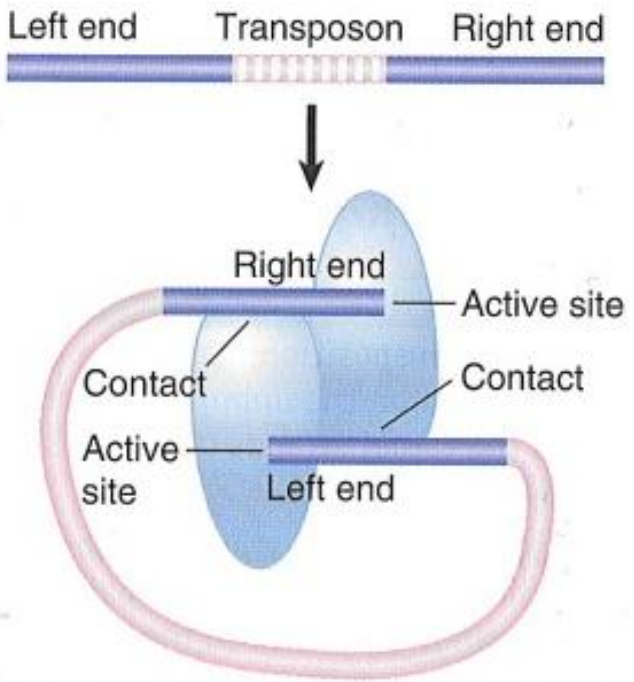


FIGURE 17.14 Each subunit of the Tn5 transposase has one end of the transposon located in its active site and also makes contact at a different site with the other end of the transposon.

Taken from: J.E. Krebs, E.S. Goldstein, S.T. Kilpatrick; „Lewin´s Genes XI“; Jones&Bsrtlett Learning

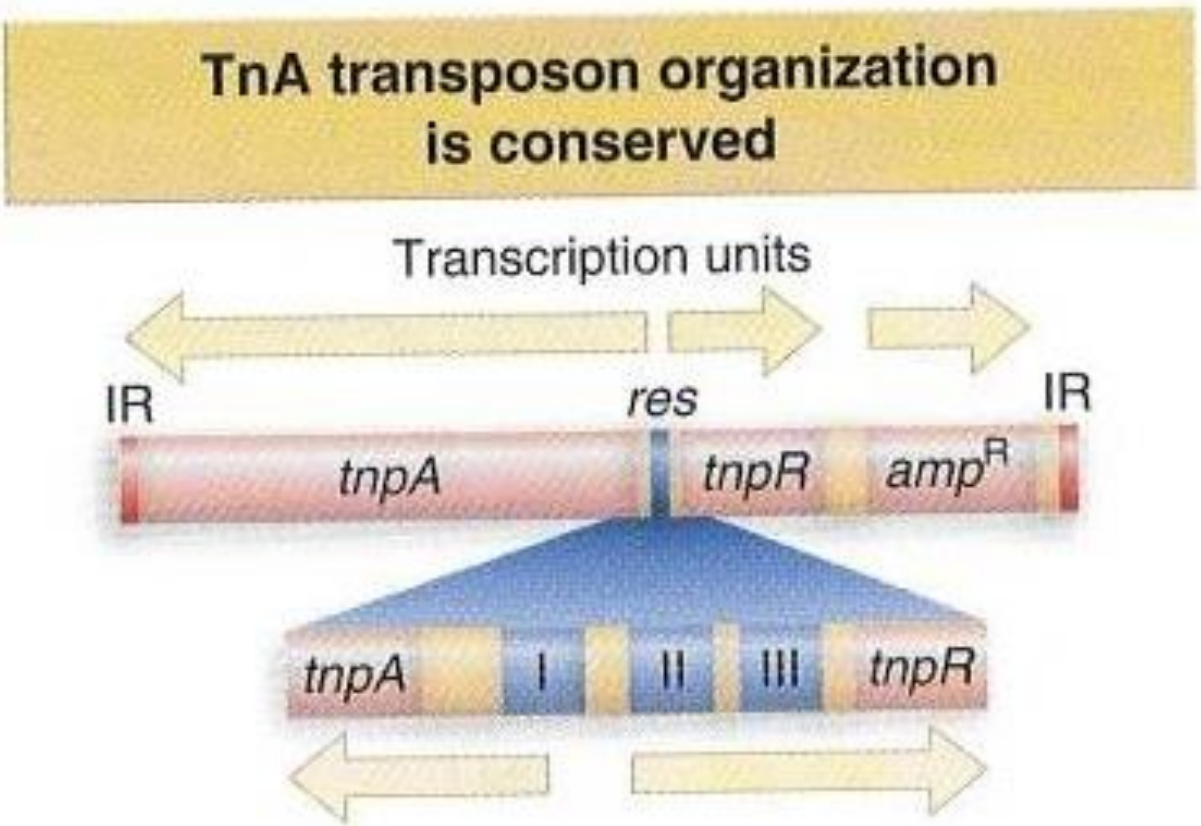


Figure 21.17 Transposons of the TnA family have inverted terminal repeats, an internal *res* site, and three known genes.

Taken from: B.Lewin, „Essential Genes“, Pearson Education International

Cointegrate formation and resolution

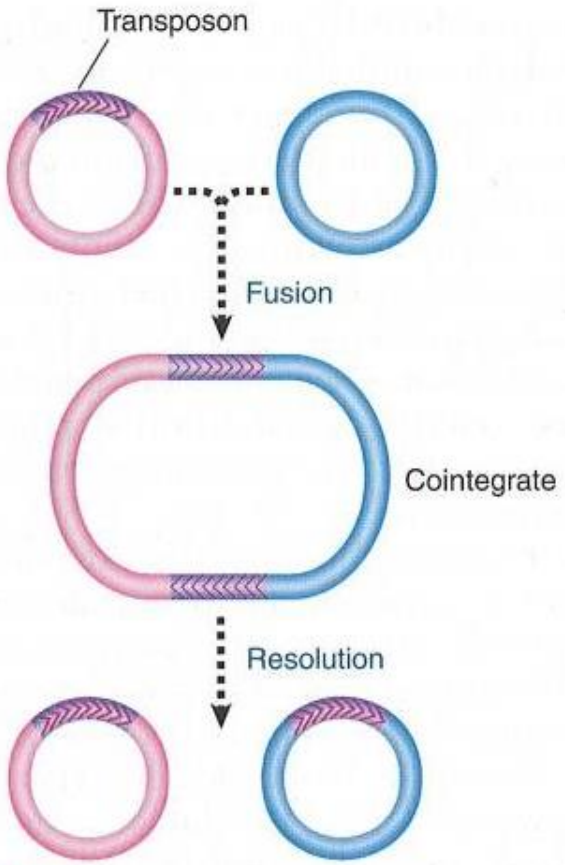


FIGURE 17.9 Transposition may fuse a donor and recipient replicon into a cointegrate. Resolution releases two replicons, each containing a copy of the transposon.

Replicative Transposition

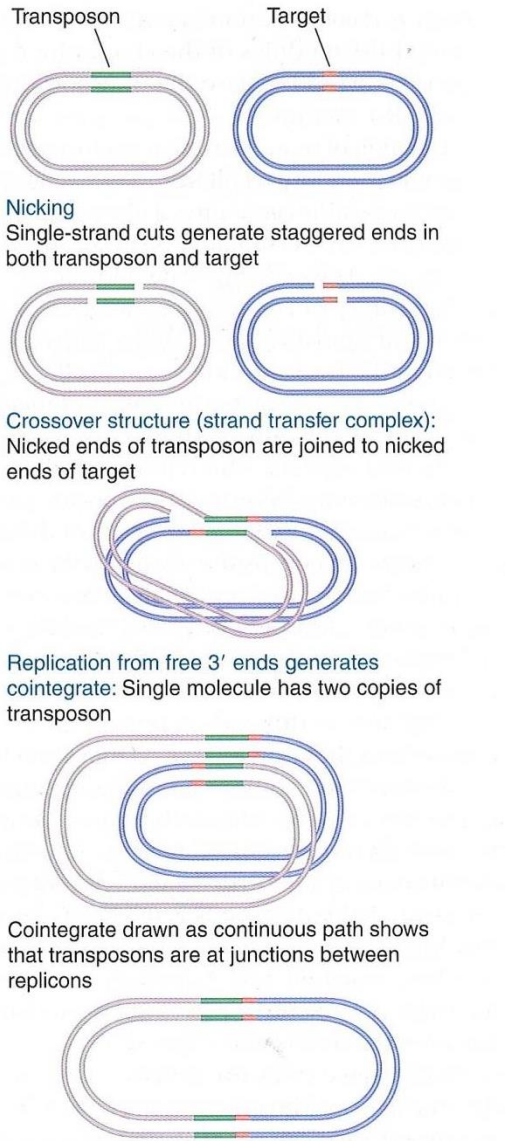


FIGURE 17.10 Mu transposition generates a crossover structure, which is converted by replication into a cointegrate.

Taken from: J.E. Krebs, E.S. Goldstein, S.T. Kilpatrick; „Lewin´s Genes XI"; Jones&Bsrtlett Learning

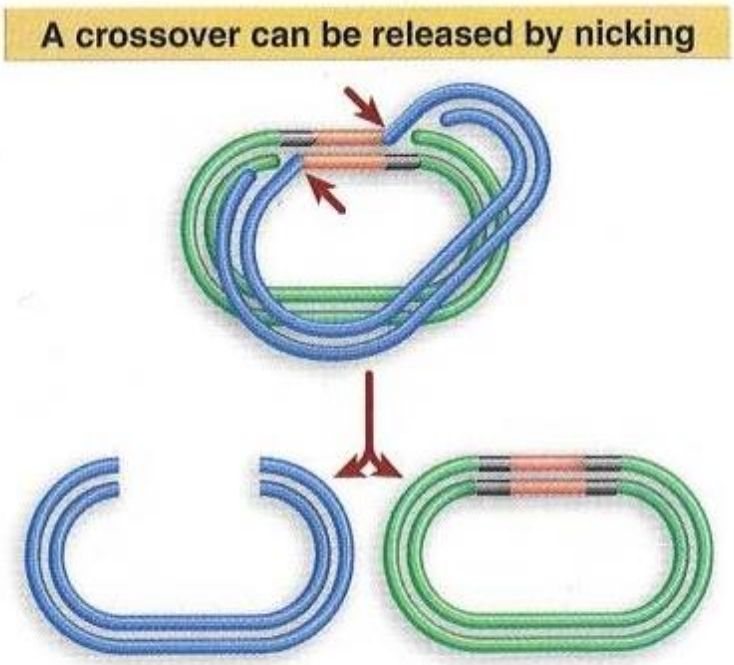


Figure 21.13 Nonreplicative transposition results when a crossover structure is released by nicking. This inserts the transposon into the target DNA, flanked by the direct repeats of the target, and the donor is left with a double-strand break.

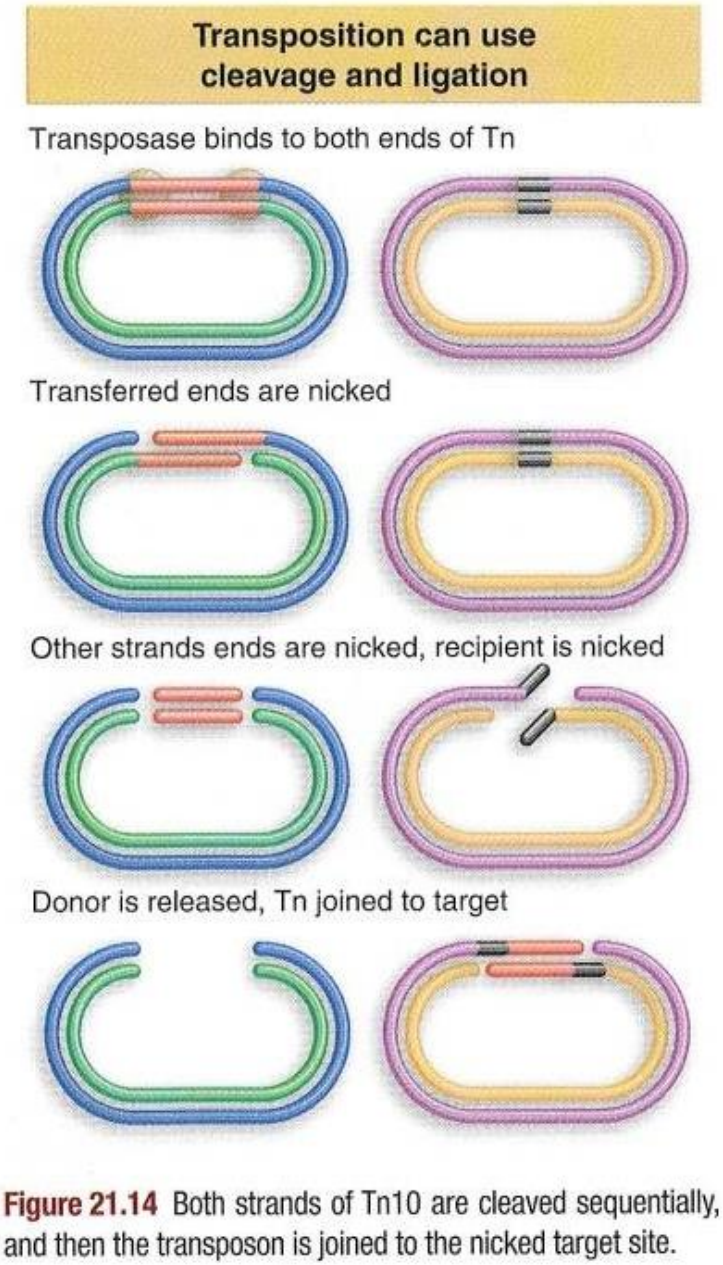


Figure 21.14 Both strands of Tn10 are cleaved sequentially, and then the transposon is joined to the nicked target site.

Taken from: B.Lewin, „Essential Genes“, Pearson Education International

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.

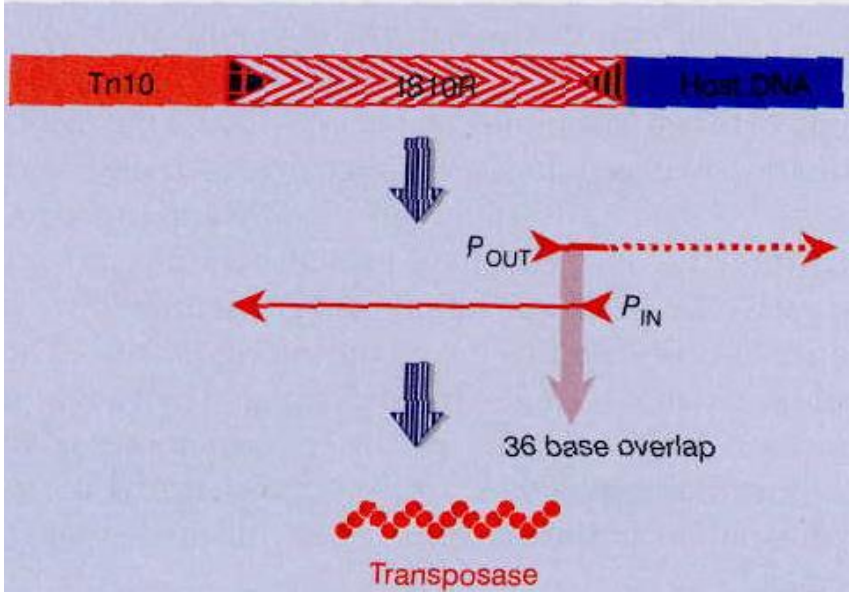
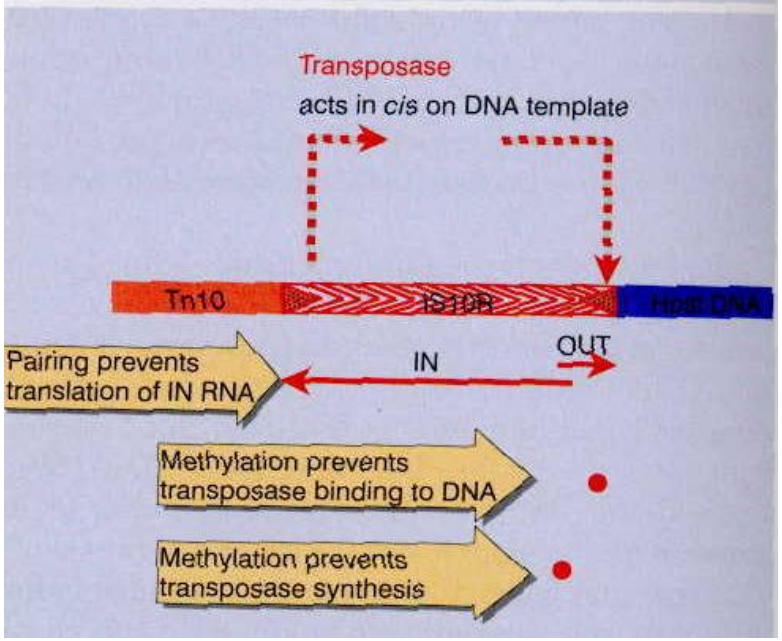


Figure 15.18 Several mechanisms restrain the frequency of Tn10 transposition, by affecting either the synthesis or function of transposase protein. Transposition of an individual transposon is restricted by methylation to occur only after replication. In multicopy situations, *cis*-preference restricts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



Transposons of Eukaryotes

Transposons are autonomous or nonautonomous

Autonomous	Nonautonomous
Transposes independently	Requires autonomous element
trans-activation	
Moves to new site	Moves to new site

Maize transposon families	
Ac (activator) *Mp* (modulator)	*Ds* (dissociation)
Spm (suppressor-mutator) *En* (enhancer)	*dSpm* (defective *Spm*) *I* (inhibitor)
Dotted	Unnamed
MuDR (mutator)	Mu

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.

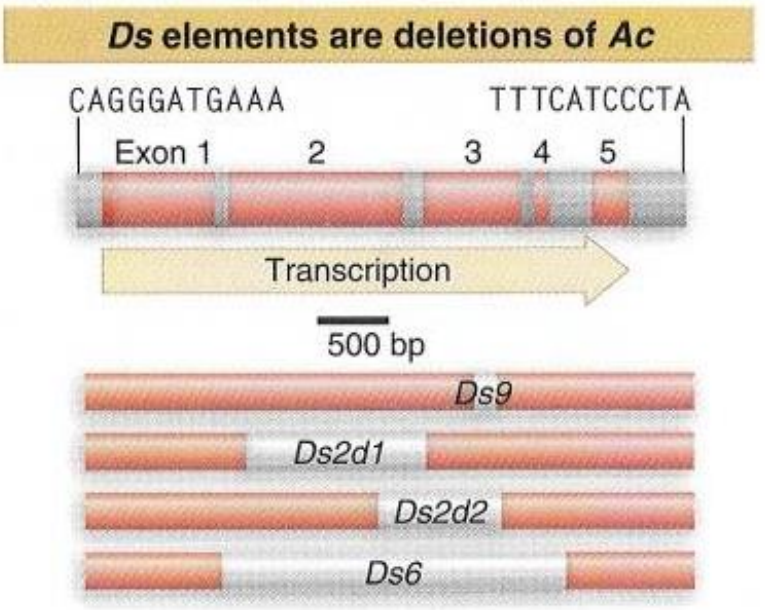


Figure 21.22 The *Ac* element has five exons that code for a transposase; *Ds* elements have internal deletions.

Taken from: B.Lewin, „Essential Genes“, Pearson Education International

Transposons of Eukaryotes

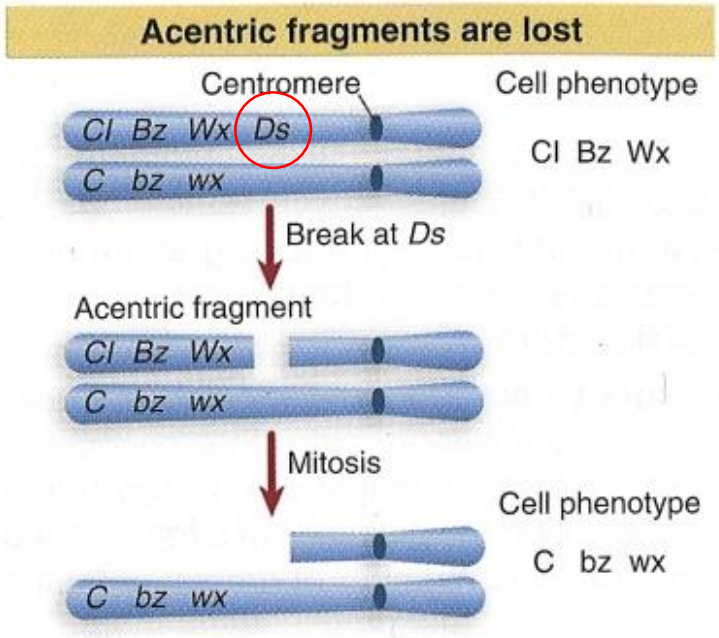


Figure 21.19 A break at a controlling element causes loss of an acentric fragment; if the fragment carries the dominant markers of a heterozygote, its loss changes the phenotype. The effects of the dominant markers, *Cl*, *Bz*, *Wx*, can be visualized by the color of the cells or by appropriate staining.

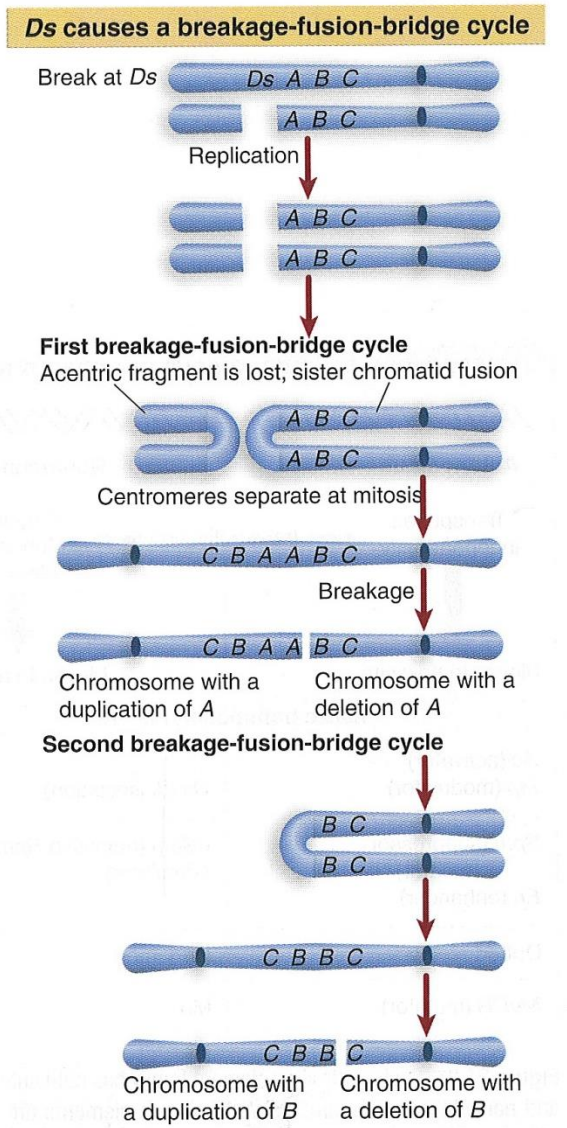


Figure 21.20 *Ds* provides a site to initiate the chromatid breakage-fusion-bridge cycle. The products can be followed by clonal analysis.

Controlling elements in maize

Taken from: B.Lewin, „Essential Genes“, Pearson Education International

Retrotransposons

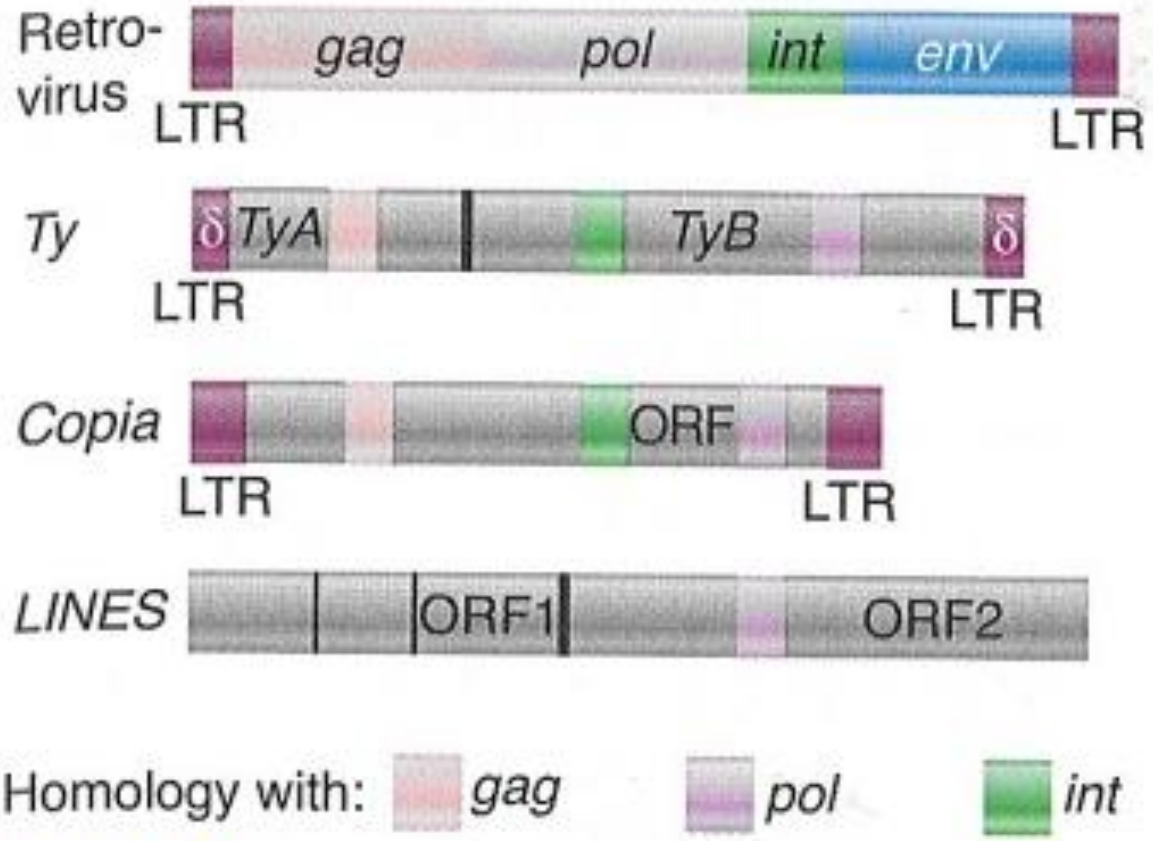


FIGURE 17.31 Retrotransposons that are closely related to retroviruses have a similar organization, but non-LTR retroposons such as LINEs share only the reverse transcriptase activity and lack LTRs.

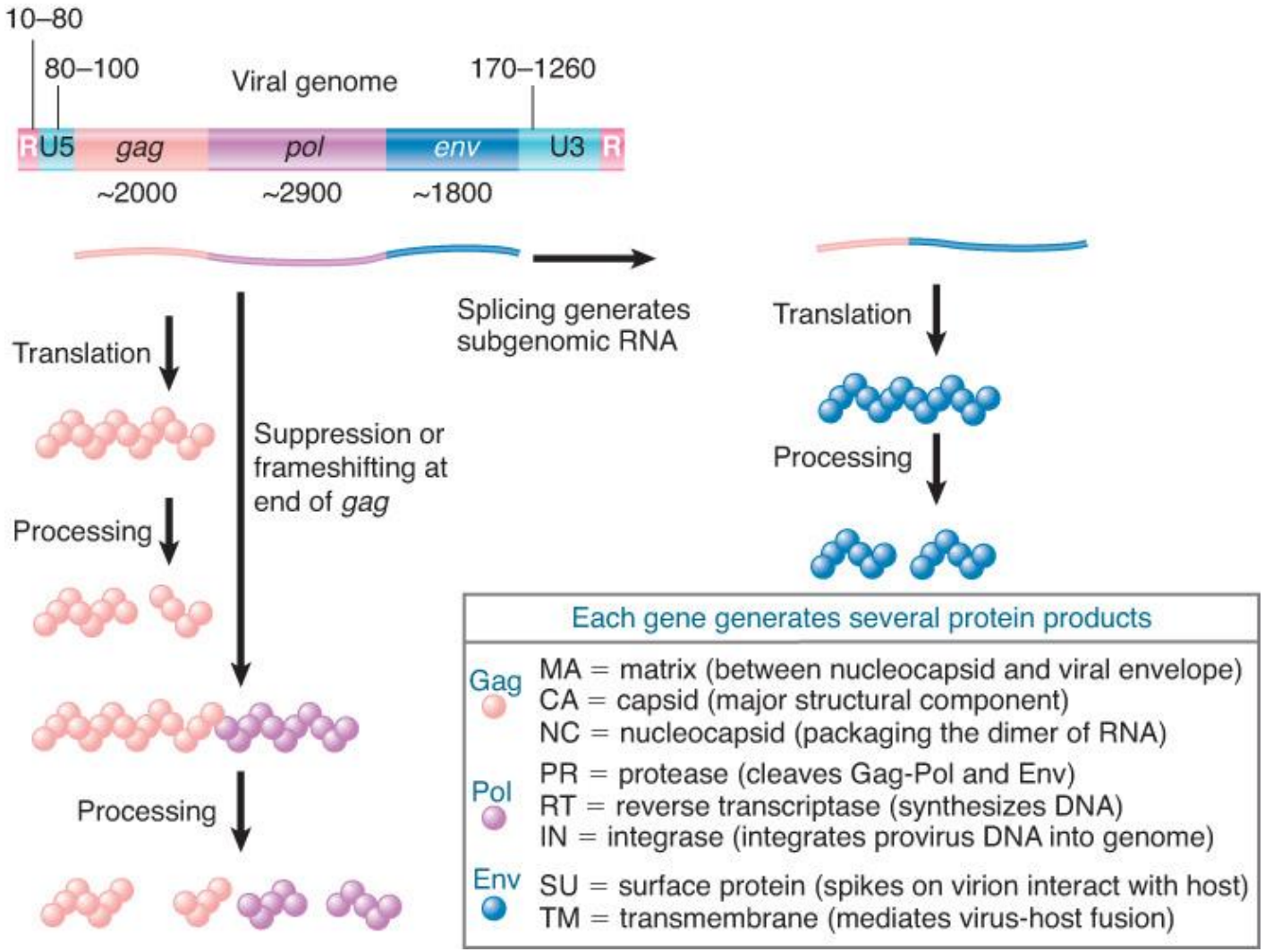


FIGURE 17.21 The genes of the retrovirus are expressed as polyproteins that are processed into individual products.

Taken from: J.E. Krebs, E.S. Goldstein, S.T. Kilpatrick; „Lewin´s Genes XI"; Jones&Bsrtlett Learning

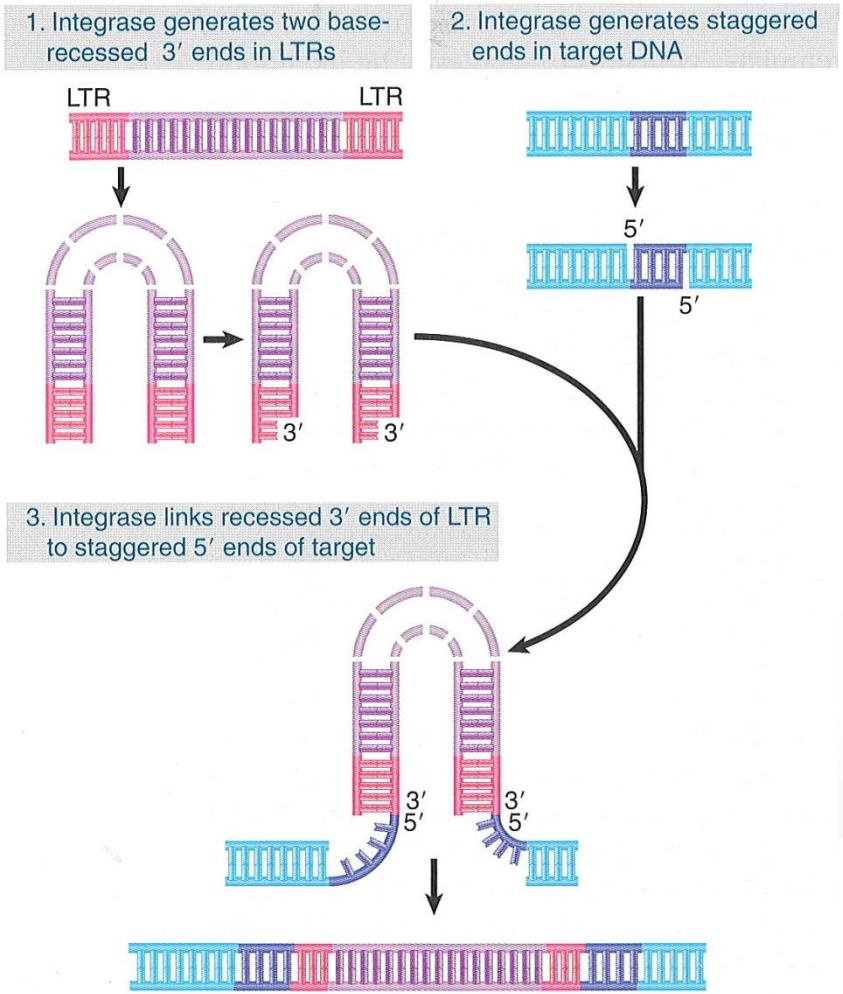


FIGURE 17.27 Integrase is the only viral protein required for the integration reaction, in which each LTR loses 2 bp and is inserted between 4-bp repeats of target DNA.

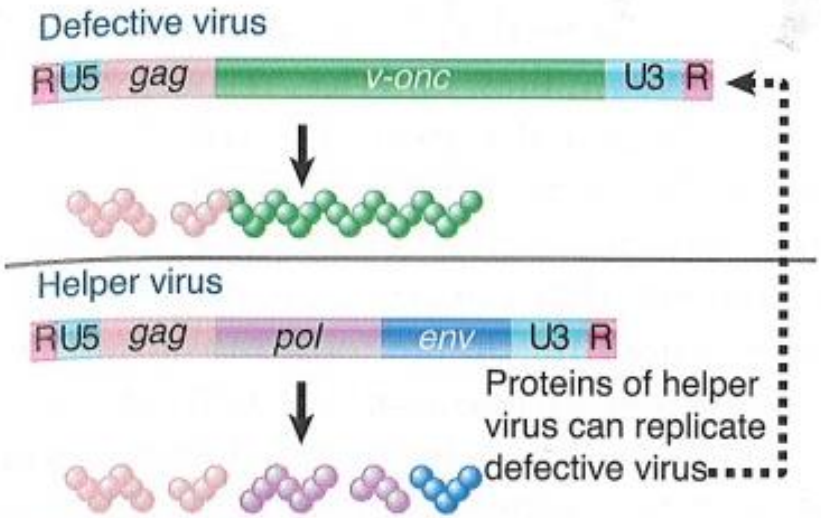


FIGURE 17.28 Replication-defective transforming viruses have a cellular sequence substituted for part of the viral sequence. The defective virus may replicate with the assistance of a helper virus that carries the wild-type functions.

Taken from: J.E. Krebs, E.S. Goldstein, S.T. Kilpatrick; „Lewin´s Genes XI"; Jones&Bsrtlett Learning

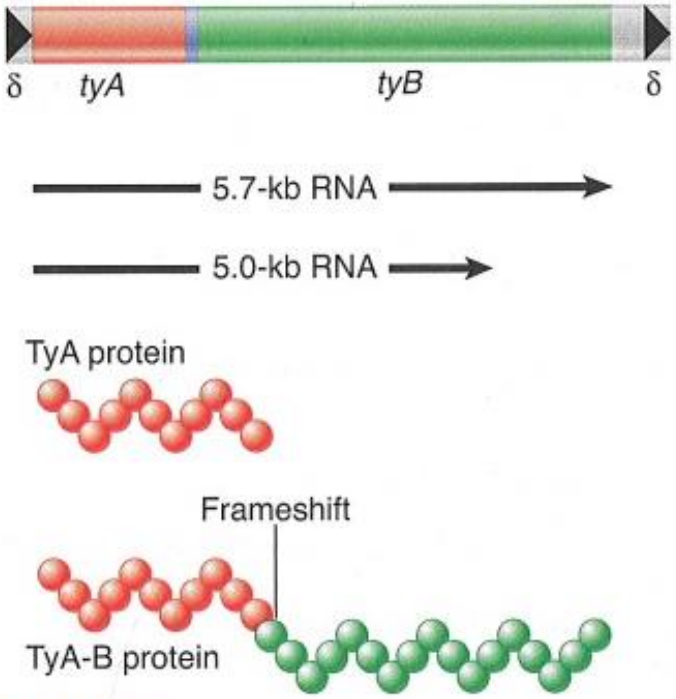


FIGURE 17.33 *Ty* elements terminate in short direct repeats and are transcribed into two overlapping RNAs. They have two reading frames, with sequences related to the retroviral *gag* and *pol* genes.

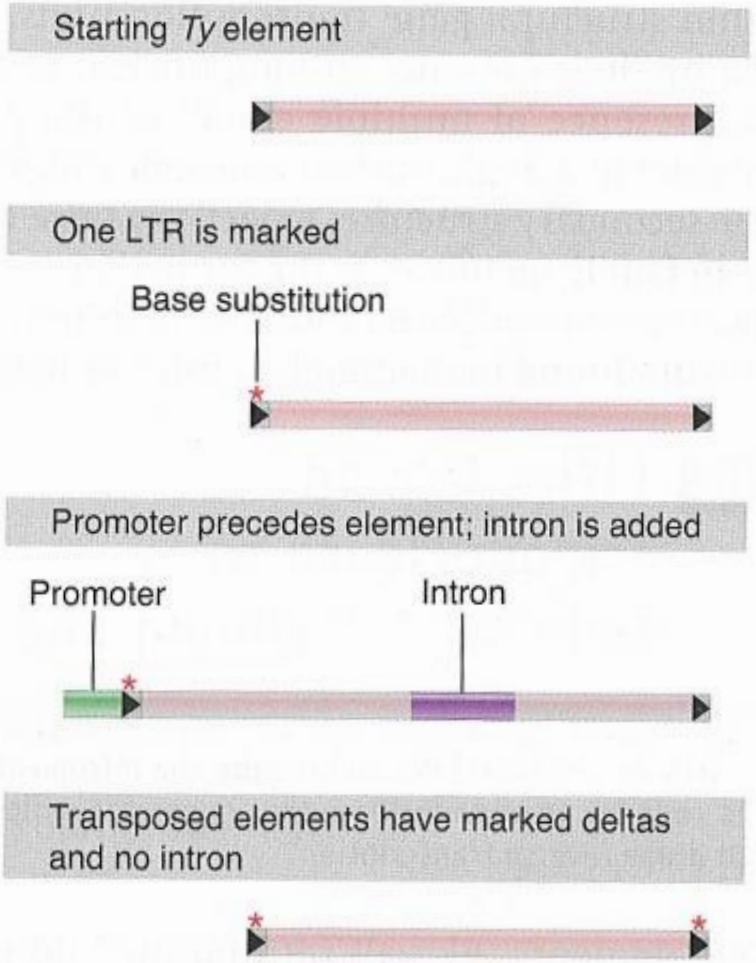


FIGURE 17.34 A unique *Ty* element, engineered to contain an intron, transposes to give copies that lack the intron. The copies possess identical terminal repeats, which are generated from one of the termini of the original *Ty* element.

Taken from: J.E. Krebs, E.S. Goldstein, S.T. Kilpatrick; „Lewin´s Genes XI"; Jones&Bsrtlett Learning

Eukaryotic genomes have three types of retroposons

	Viral superfamily	LINES	Nonviral superfamily
Common types	*Ty (S. cerevisiae)* *copia (D. melanogaster)*	L1 (human) B1, B2 ID, B4 (mouse)	SINES (mammals) pseudogenes of pol III transcripts
Termini	Long terminal repeats	No repeats	No repeats
Target repeats	4–6 bp	7–21 bp	7–21 bp
Enzyme activities	Reverse transcriptase and/or integrase	Reverse transcriptase/ endonuclease	None (or none coding for transposon products)
Organization	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.

Taken from: B.Lewin, „Essential Genes“, Pearson Education International

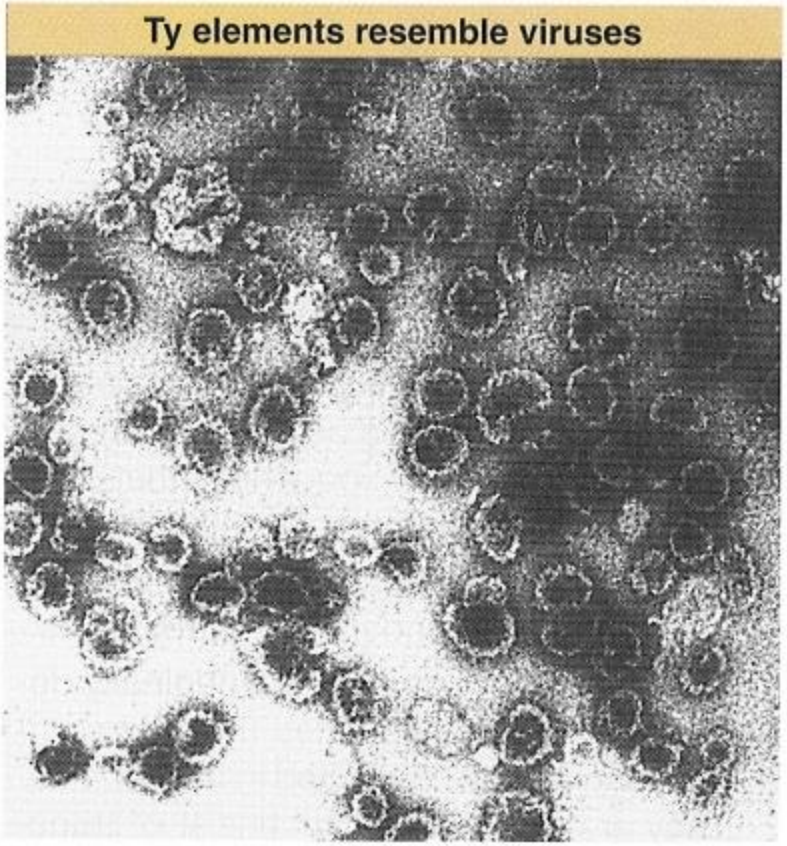


Figure 22.13 *Ty* elements generate viruslike particles. Photograph kindly provided by Alan Kingsman, Oxford Bio-Medica plc.

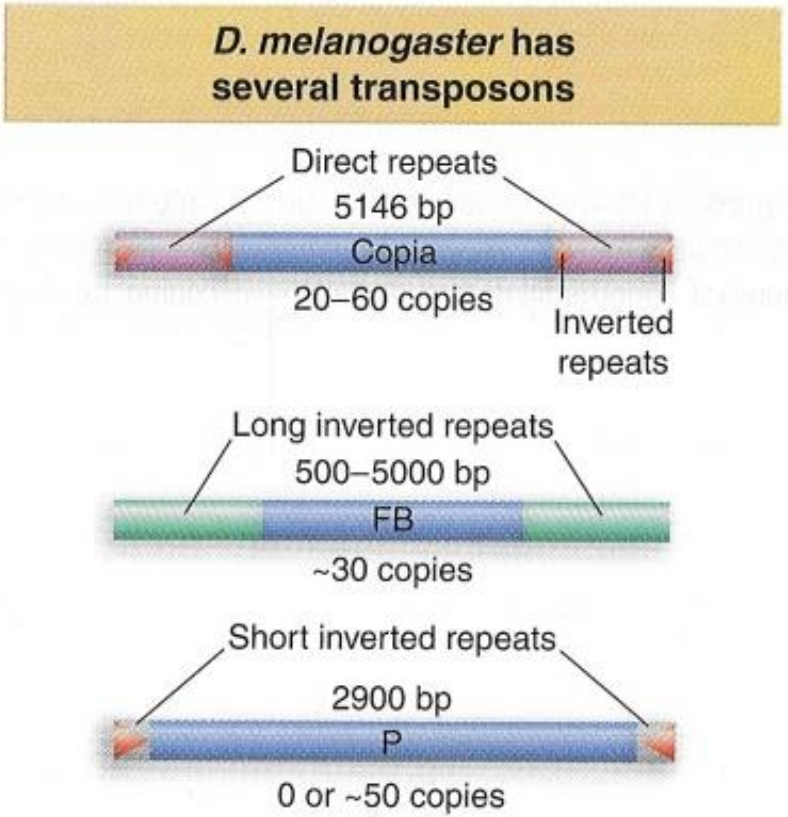


Figure 22.14 Three types of transposable element in *D. melanogaster* have different structures.

Taken from: B.Lewin, „Essential Genes“, Pearson Education International

Retroviruses and transposons constitute half the human genome

Element	Organization	Length (kb)	Human genome Number	Human genome Fraction
Retrovirus/ retroposon	LTR *gag pol (env)* LTR	1–11	450,000	8%
LINES (autonomous) e.g., L1	ORF1 *(pol)* $(A)_n$	6–8	850,000	17%
SINES (nonautonomous) e.g., Alu	$(A)_n$	<0.3	1,500,000	15%
DNA transposon	Transposase	2–3	300,000	3%

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

Taken from: B.Lewin, „Essential Genes“, Pearson Education International

Reverse Transcription → Tool for Genetic Variation

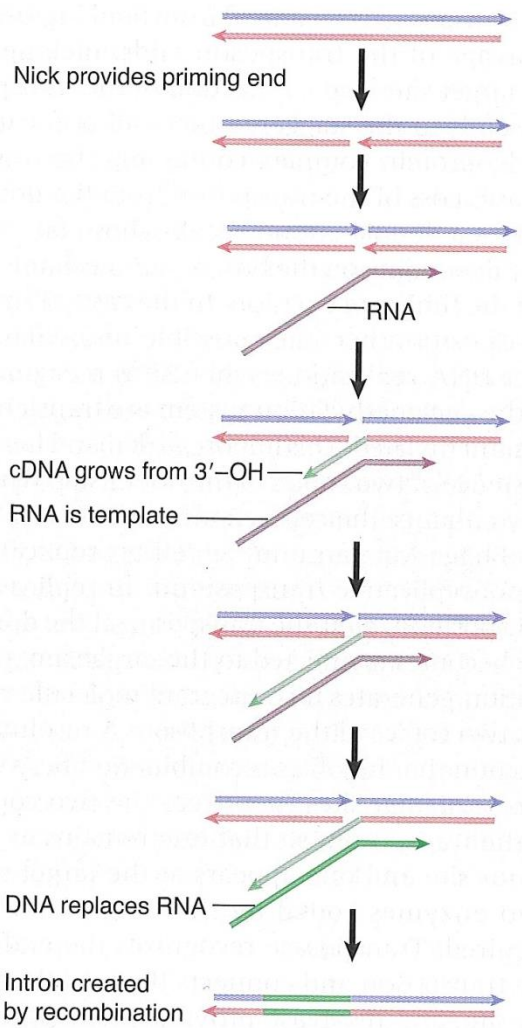
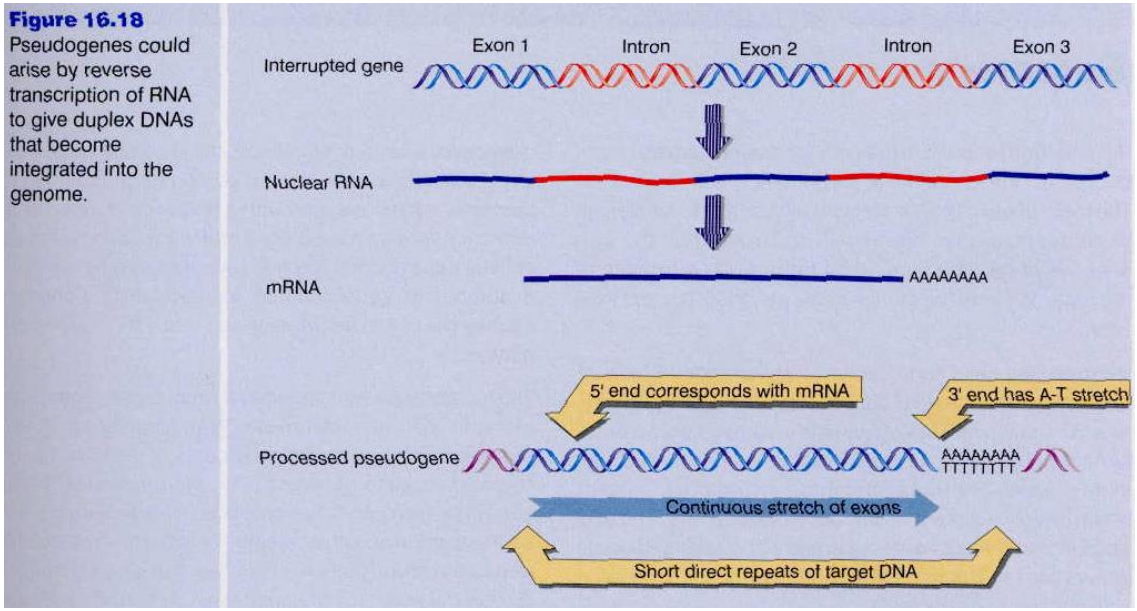


FIGURE 17.36 Retrotransposition of non-LTR retroposons occurs by nicking the target to provide a primer for cDNA synthesis on an RNA template. The arrowheads indicate 3′ ends.



Taken from: J.E. Krebs, E.S. Goldstein, S.T. Kilpatrick; „Lewin´s Genes XI"; Jones&Bsrtlett Learning