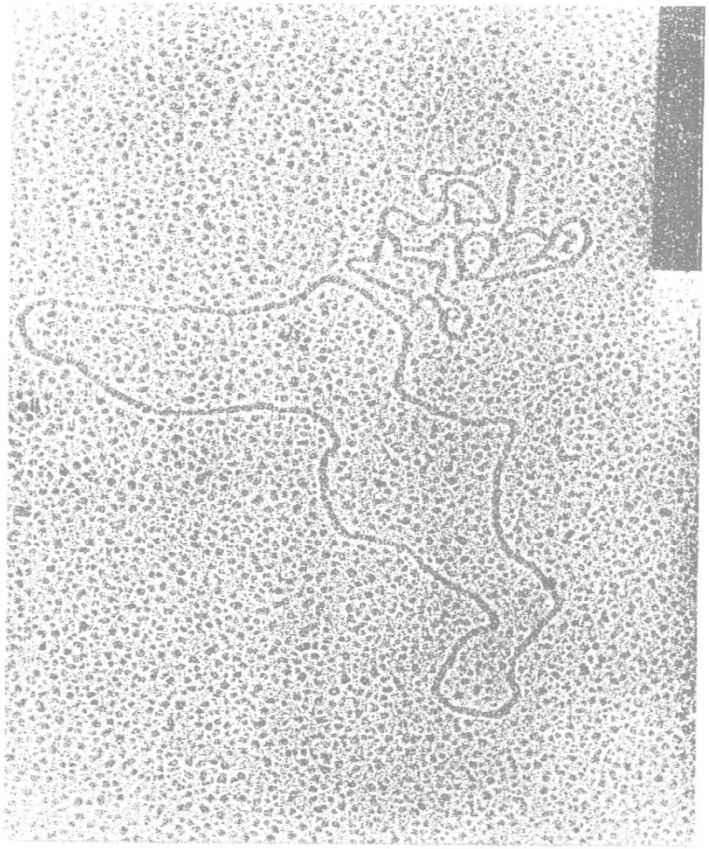
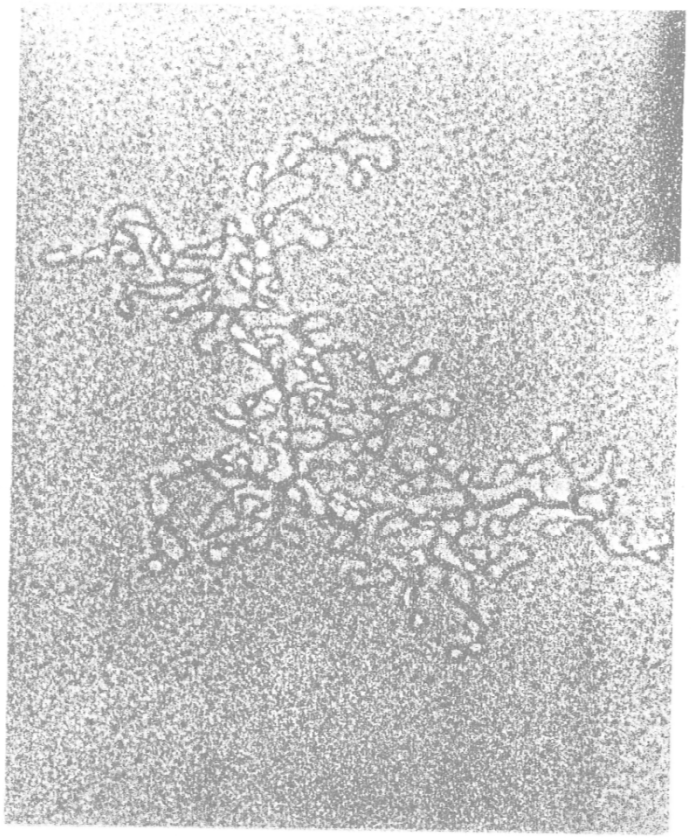
Bacterial Plasmids

Open circular form



Covalently closed circles (ccc-form)



Bacterial Plasmids

Copy Number

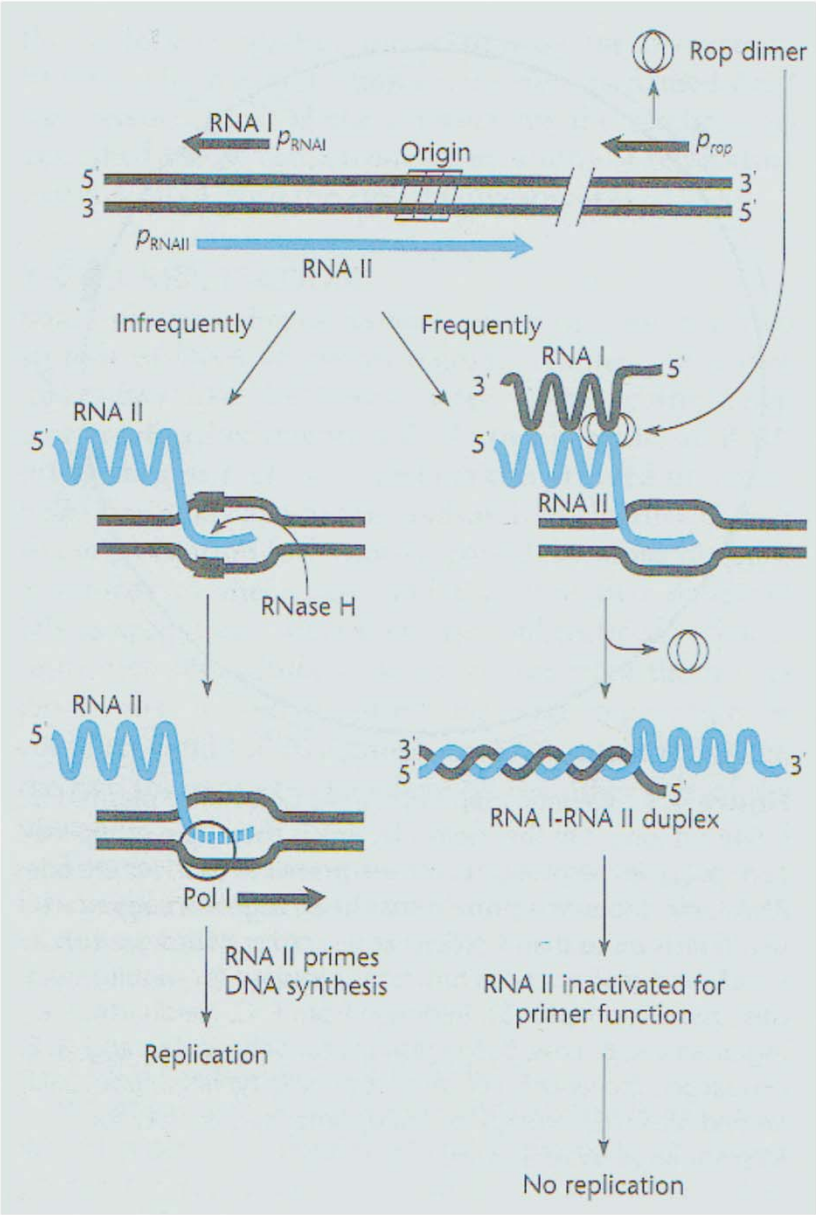
Replication & its control

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

Relaxed Control: high copy number
ColE1, pBR322, pUC18

Incompatibility:
Replication / Control
Partitioning

Genetik



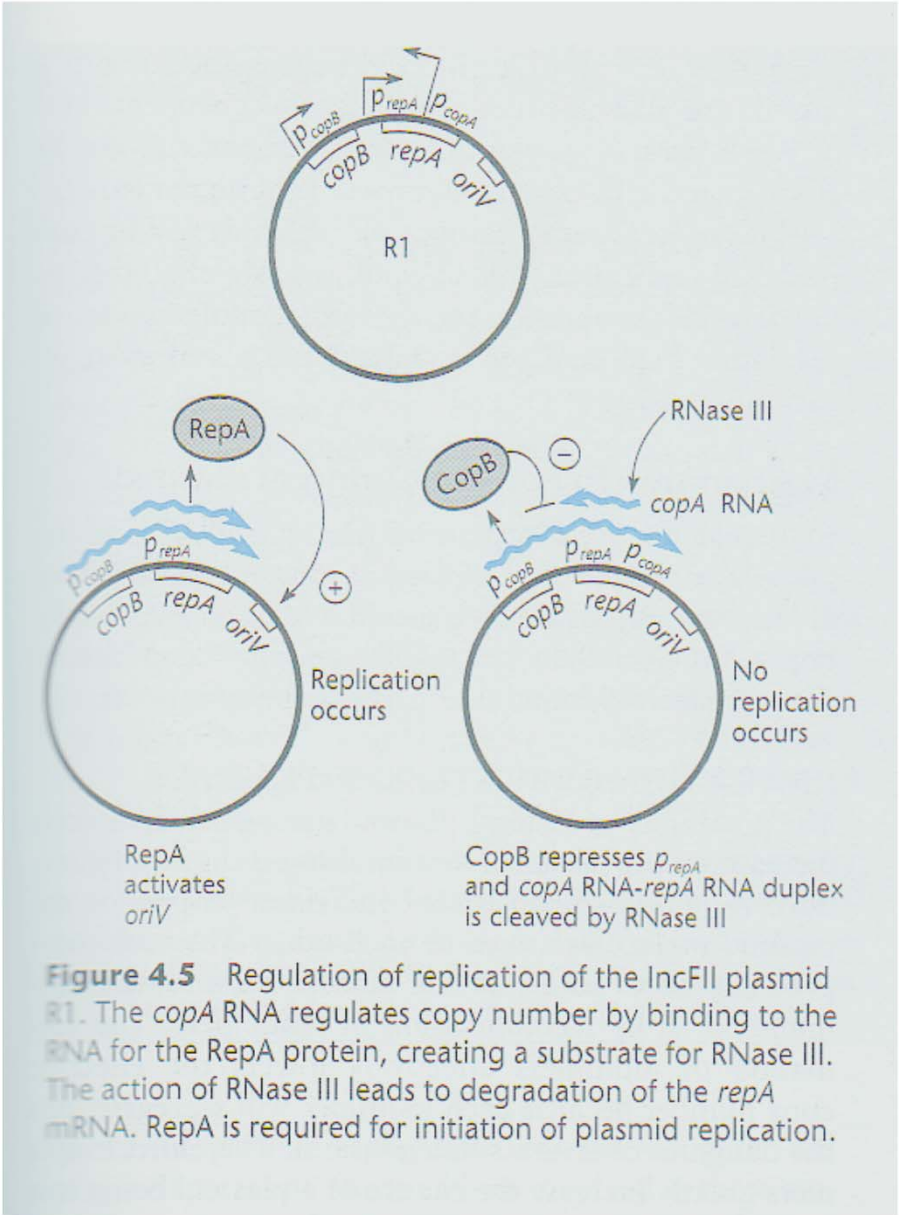


Figure 4.5 Regulation of replication of the IncFII plasmid R1. The *copA* RNA regulates copy number by binding to the RNA for the RepA protein, creating a substrate for RNase III. The action of RNase III leads to degradation of the *repA* mRNA. RepA is required for initiation of plasmid replication.

Iteron Model

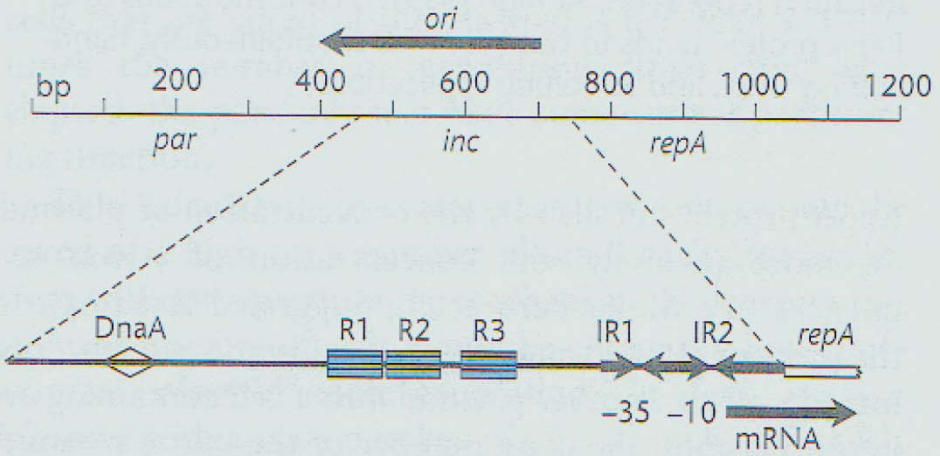


Figure 4.6 The *ori* region of pSC101. R1, R2, and R3 are the three iteron sequences (CAAAGGTCTAGCAGCAGAATT-TACAGA for R3) to which RepA binds to handcuff two plasmids. RepA autoregulates its own synthesis by binding to the inverted repeats IR1 and IR2. The location of the partitioning site *par* (see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

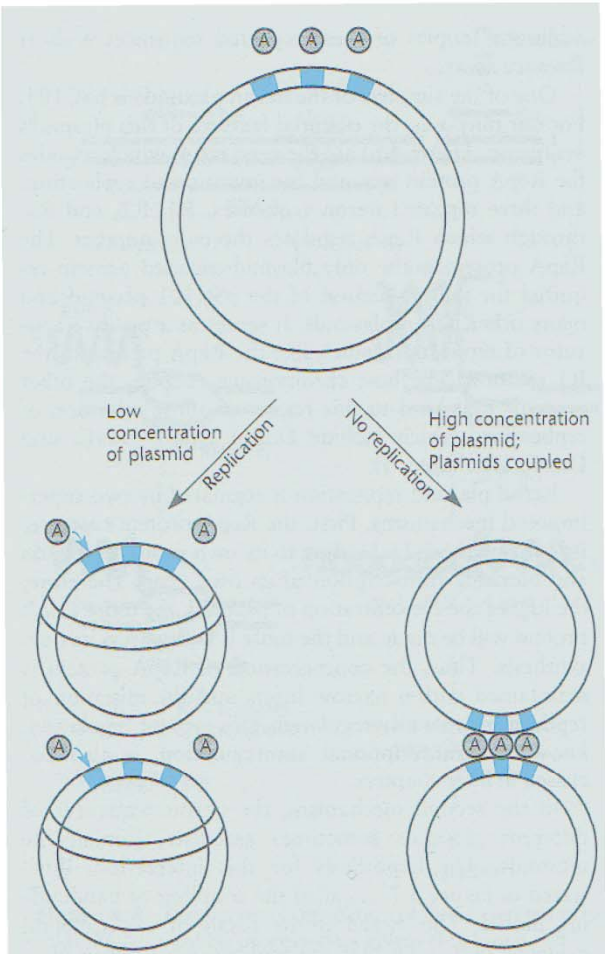


Figure 4.7 The "handcuffing" or "coupling" model for regulation of iteron plasmids. At low concentrations of plasmids, the RepA protein only binds to one plasmid at a time, initiating replication. At high plasmid concentrations, the RepA protein binds to two plasmids simultaneously, handcuffing them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

Plasmids

Conjugative Transfer:	Gram-negative:	F, RP4/RK2,
	Gram-positive:	pAMß1, SCP2*
	Plants	Ti

Bacteriocin-/ Microcin-Production:

Antibiotika-Resistence:	ß-Lactam Antibiotics:	ß-Lactamases
	Chloramphenicol:	Acetyltransferases
	Aminoglycoside-Ab.:	Phosphotransferases
	Tetracycline:	Membrane transfer
	Sulfonamide:	Bypass
	Trimethoprim	

Heavy metal resistence:	mercury, Hg-organic compounds, Tellurium
	Arsenic, Antimony, Cadmium, Copper, Silver

Plasmids

Degradive 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

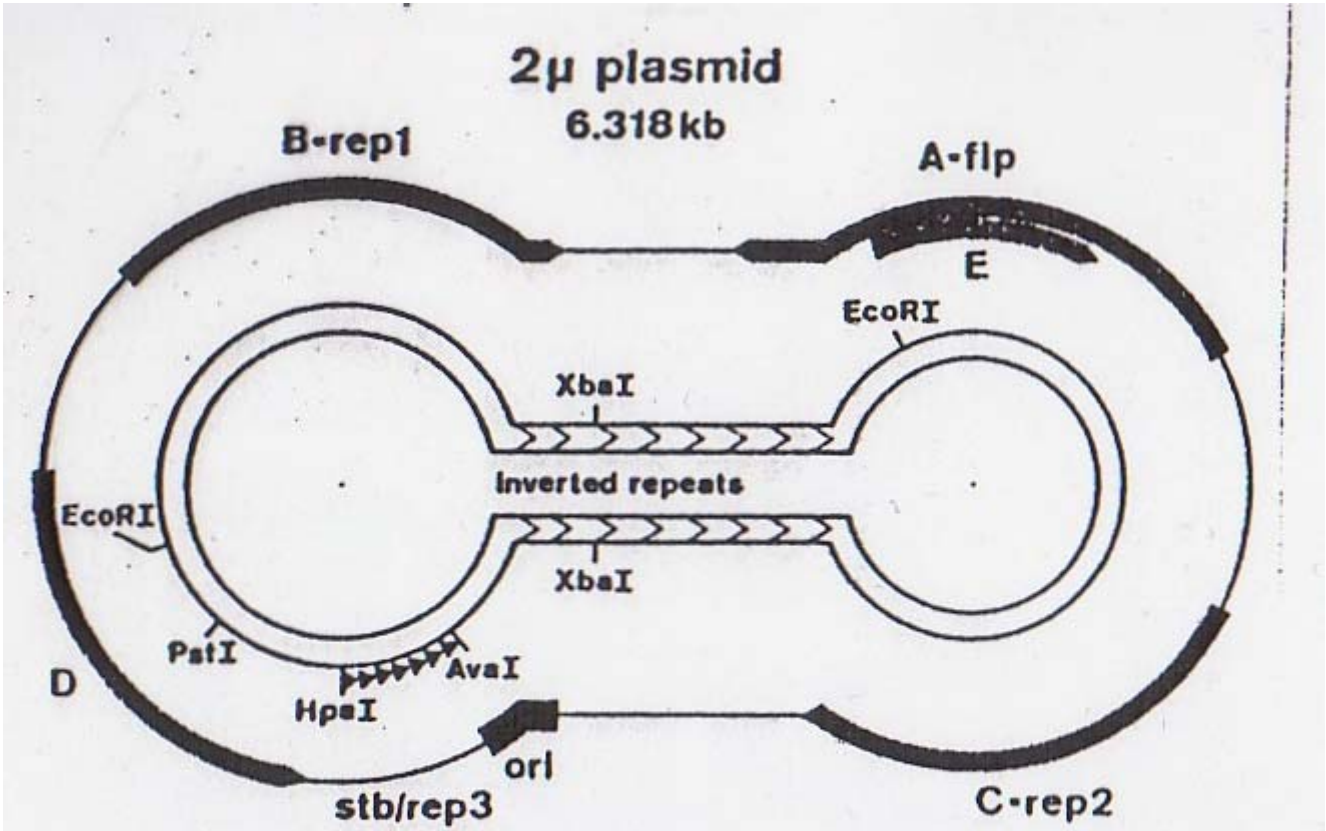


Fig. 4. *Saccharomyces cerevisiae:* Physical map of the 2 μm plasmid (form A) showing restriction sites, the putative origin of replication (*ori*), the inverted repeats, direct repeats (black arrows) and five major open reading frames (*A, B, C, D, E*). (After Hartley and Donelson 1980; Veit and Fangman 1985)

Genetik

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)

Ge

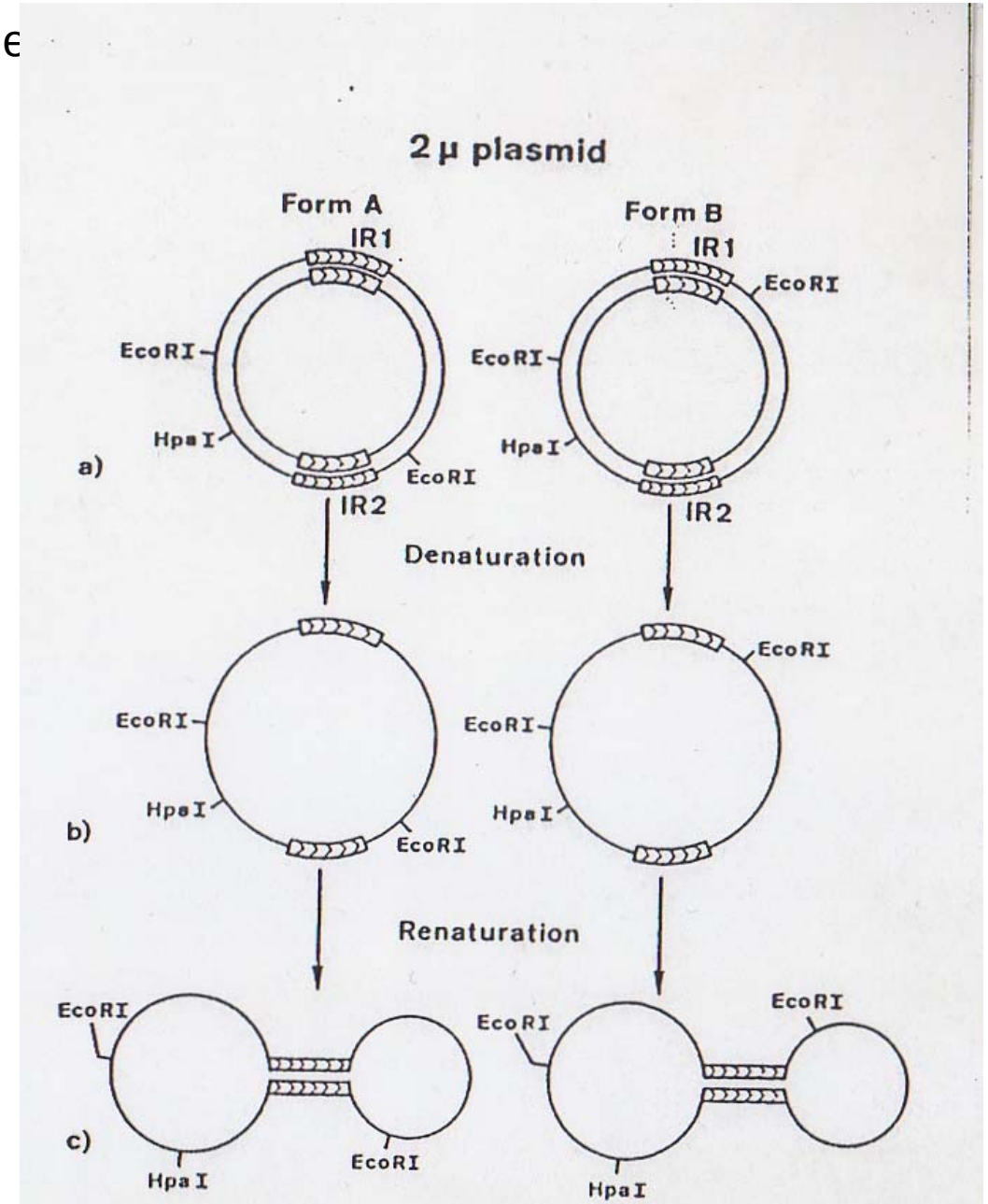


Fig. 3. *Saccharomyces cerevisiae:* Scheme for the formation of two types of homoduplex molecules following denaturation and renaturation of the ds 2 µm plasmid DNA

Genetik

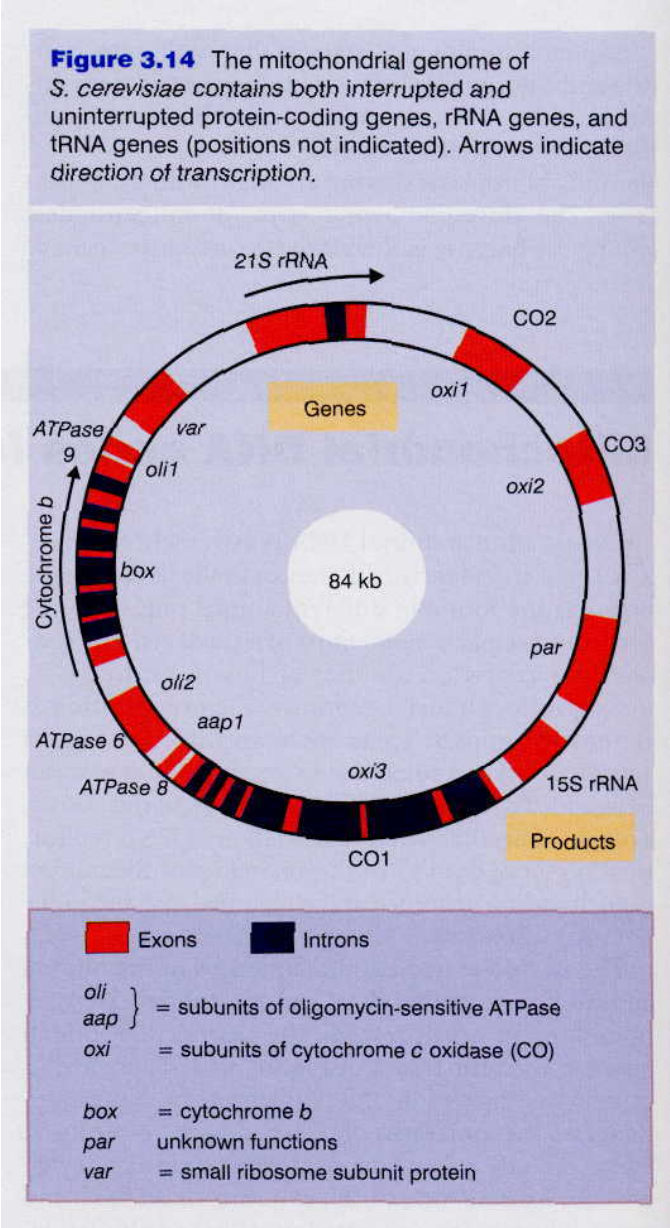


Figure 3.14 The mitochondrial genome of *S. cerevisiae* contains both interrupted and uninterrupted protein-coding genes, rRNA genes, and tRNA genes (positions not indicated). Arrows indicate direction of transcription.

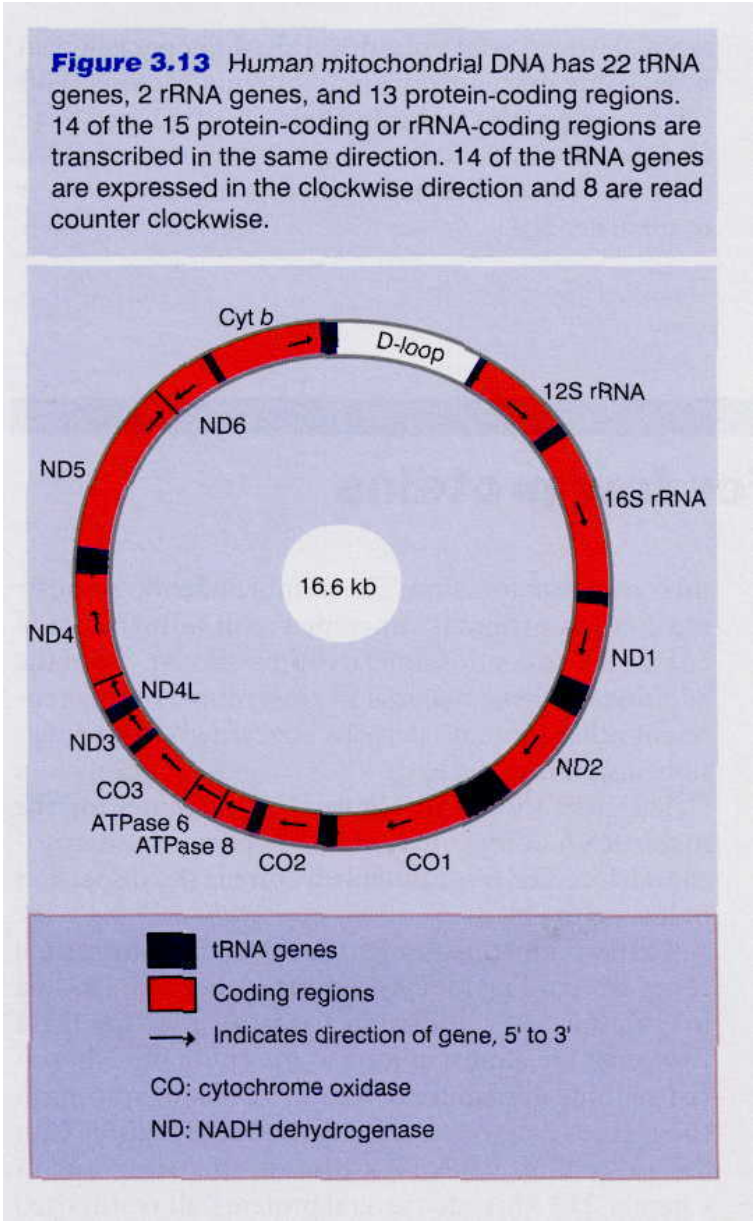


Figure 3.13 Human mitochondrial DNA has 22 tRNA genes, 2 rRNA genes, and 13 protein-coding regions. 14 of the 15 protein-coding or rRNA-coding regions are transcribed in the same direction. 14 of the tRNA genes are expressed in the clockwise direction and 8 are read counter clockwise.

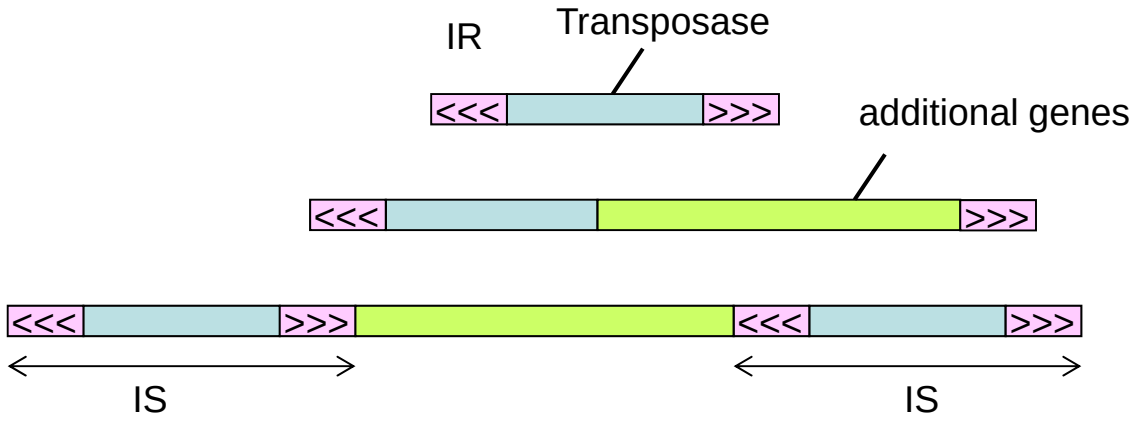
Transposable Elements - Insertion Sequences and Transposons

DNA – DNA Transposition

IS Elements

Simple Transposons

Composite Transposons



DNA – RNA – DNA Transposition

Retrotransposons

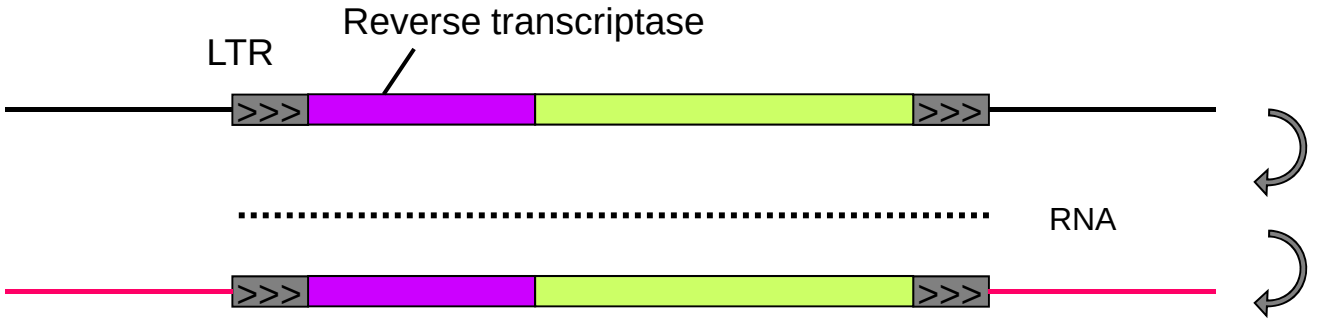
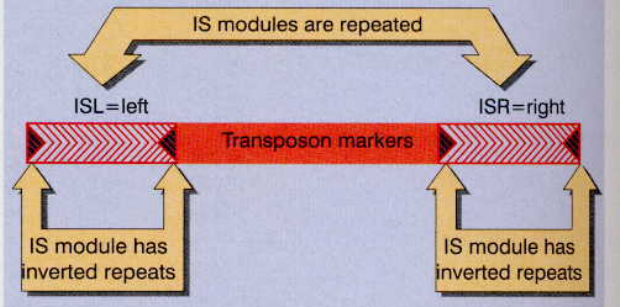
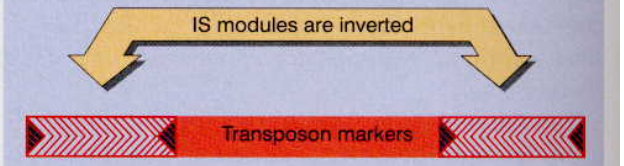


Figure 15.2 A composite transposon has a central region carrying markers (such as drug resistance) flanked by IS modules. The modules have short inverted terminal repeats. If the modules themselves are in inverted orientation (as drawn), the short inverted terminal repeats at the ends of the transposon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

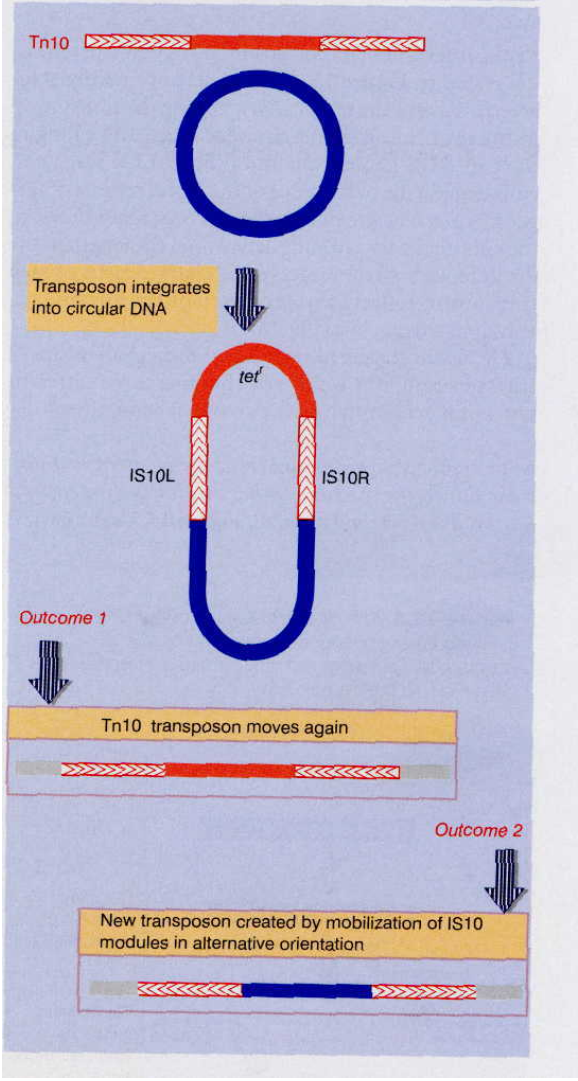


Example			
Tn9	IS1	*cam*^{R}	IS modules identical both functional



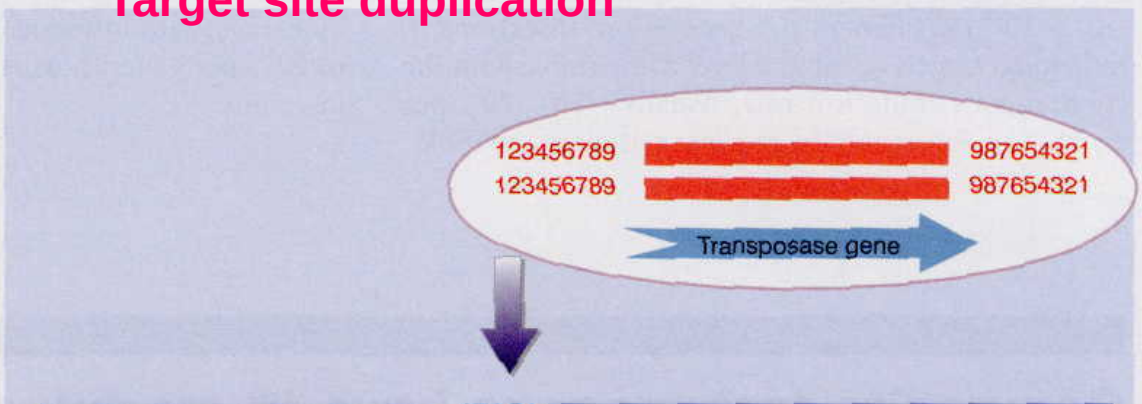
Transposon	Left end	Markers	Right end
Tn903	IS903	*kan*^{R}	both IS ends functional
Tn10	IS10L nonfunctional	*tet*^{R}	IS10R functional
Tn5	IS50L nonfunctional	*kan*^{R}	IS50R functional

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.



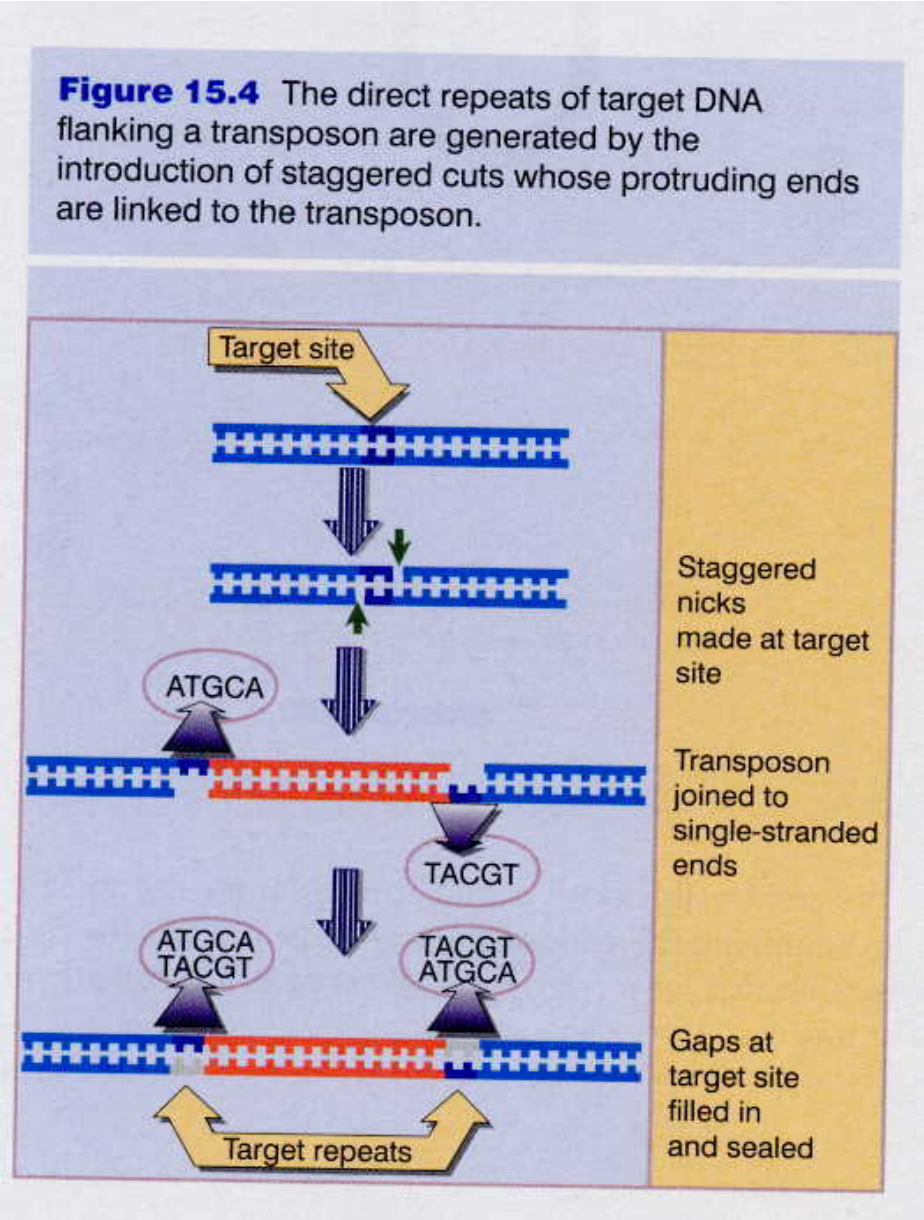
Target site duplication

Figure 15.1 Overview: 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.



			Overall length	Target selection
IS1	9 bp	23 bp	768 bp	random
IS2	5 bp	41 bp	1327 bp	hotspots
IS4	11-13 bp	18 bp	1428 bp	$AAAN_{20}TTT$
IS5	4 bp	16 bp	1195 bp	hotspots
IS10R	9 bp	22 bp	1329 bp	NGCTNAGCN
IS50R	9 bp	9 bp	1531 bp	hotspots
IS903	9 bp	18 bp	1057 bp	random

sertion, the IS DNA is always flanked by

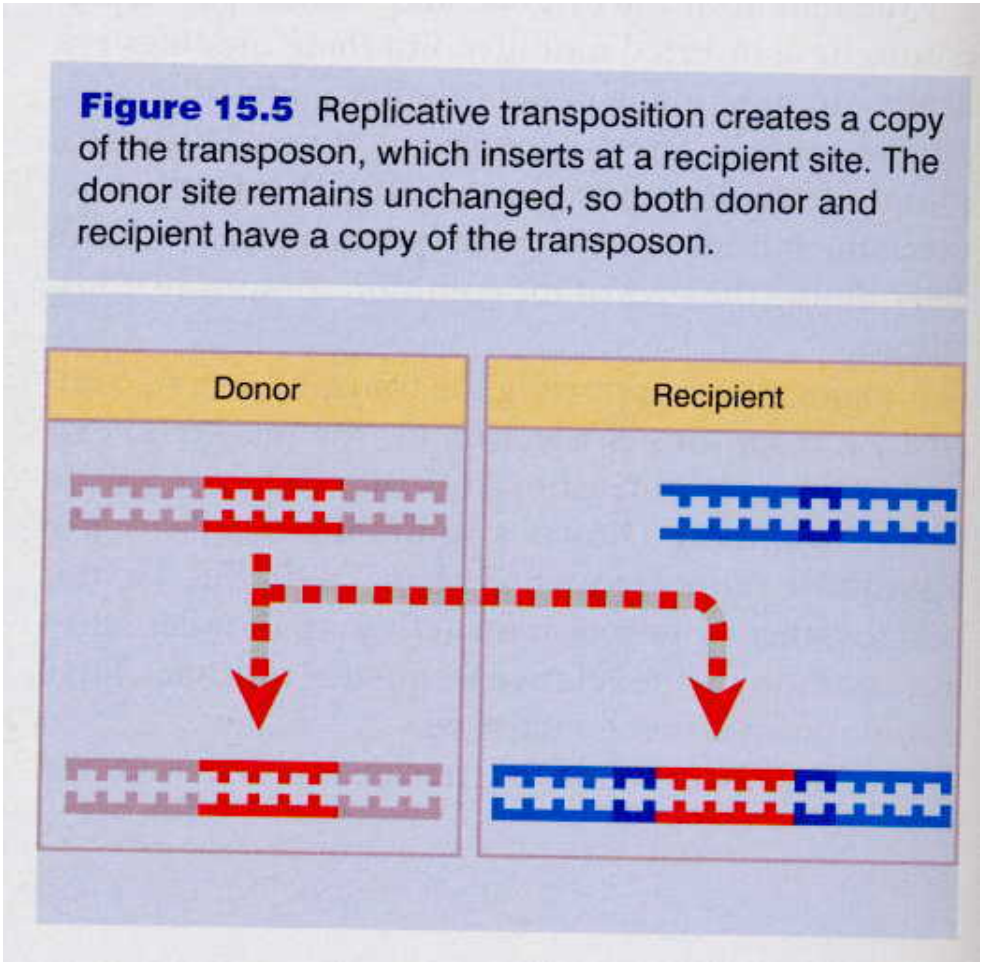


Target site duplication

Target site duplication
caused by staggered cutting

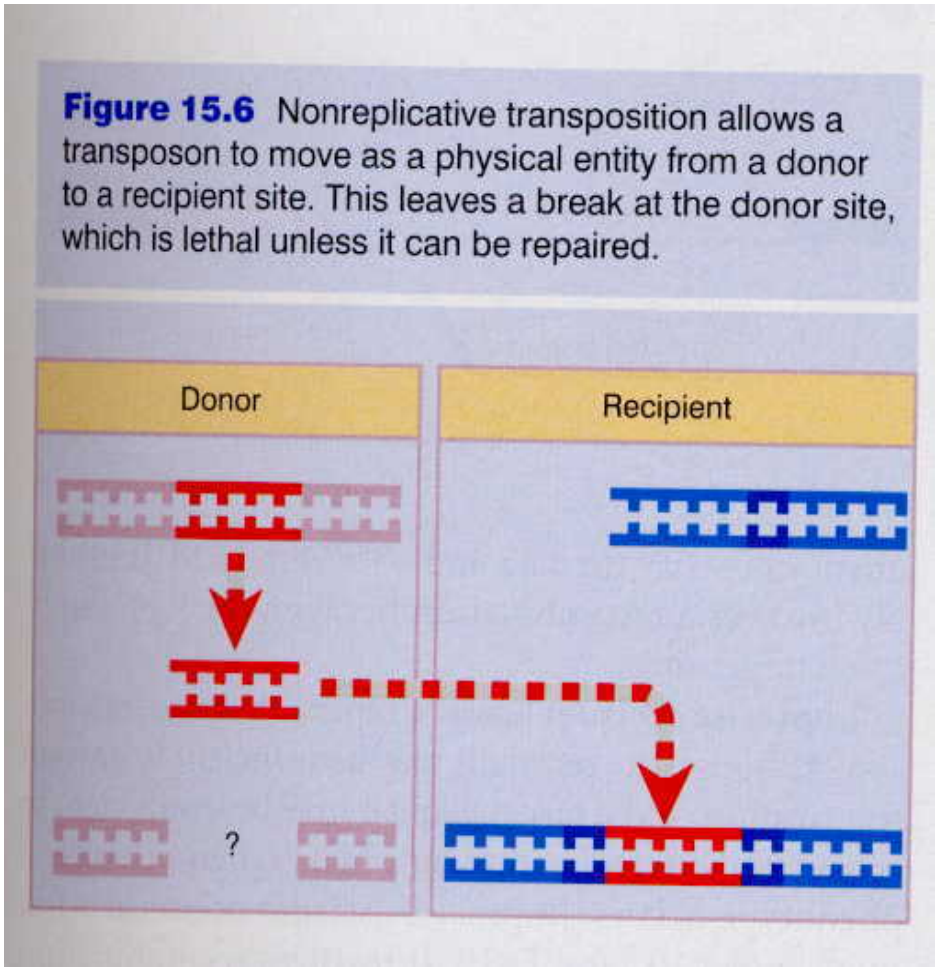
Replicative Transposition

Recipient and donor contain Tn

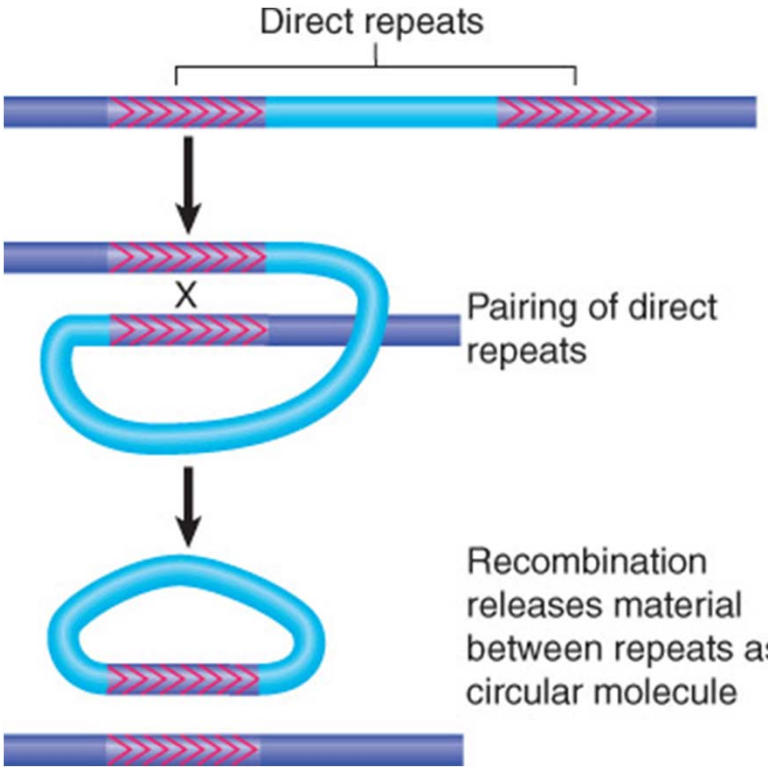


Non-replicative Transposition

Only recipient contains Tn,
Donor looses Tn

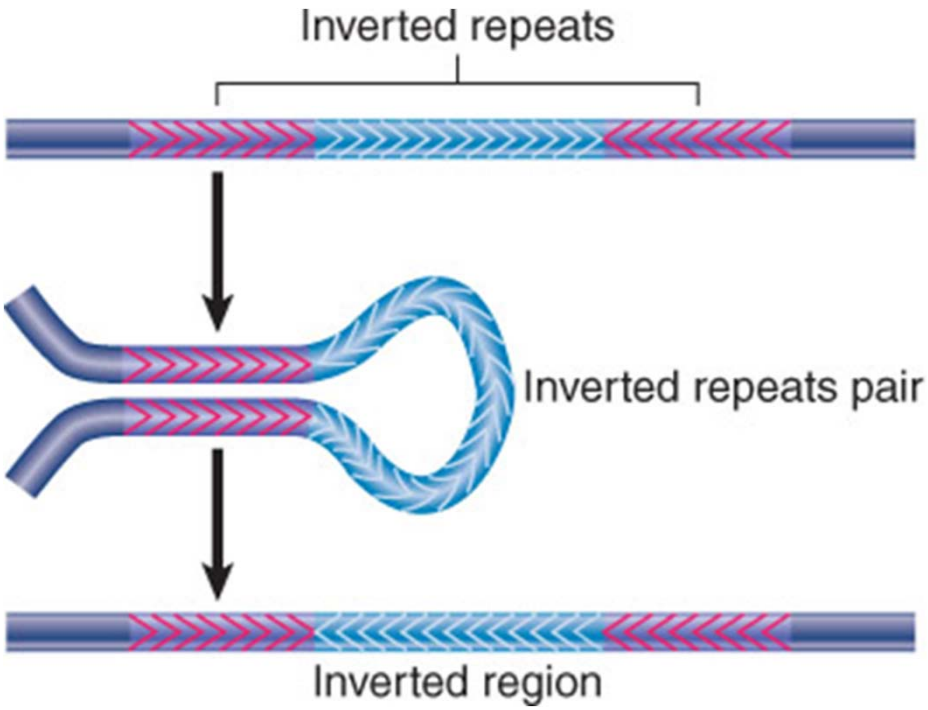


Deletion



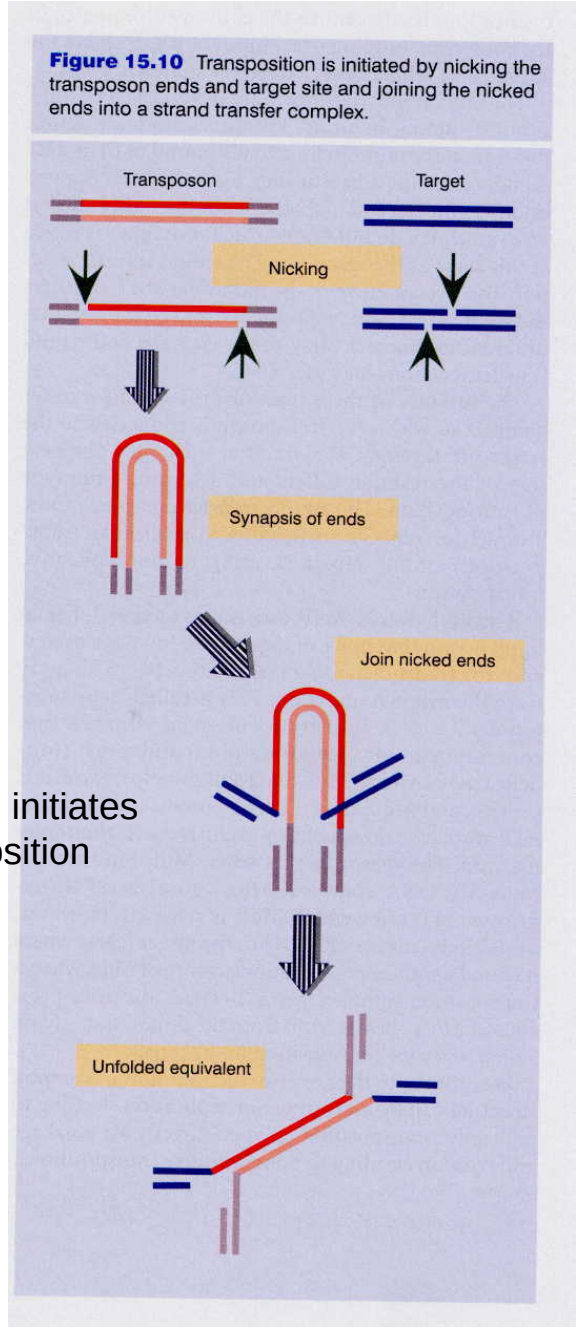
Reciprocal recombination between direct repeats excises the material between them.

Inversion

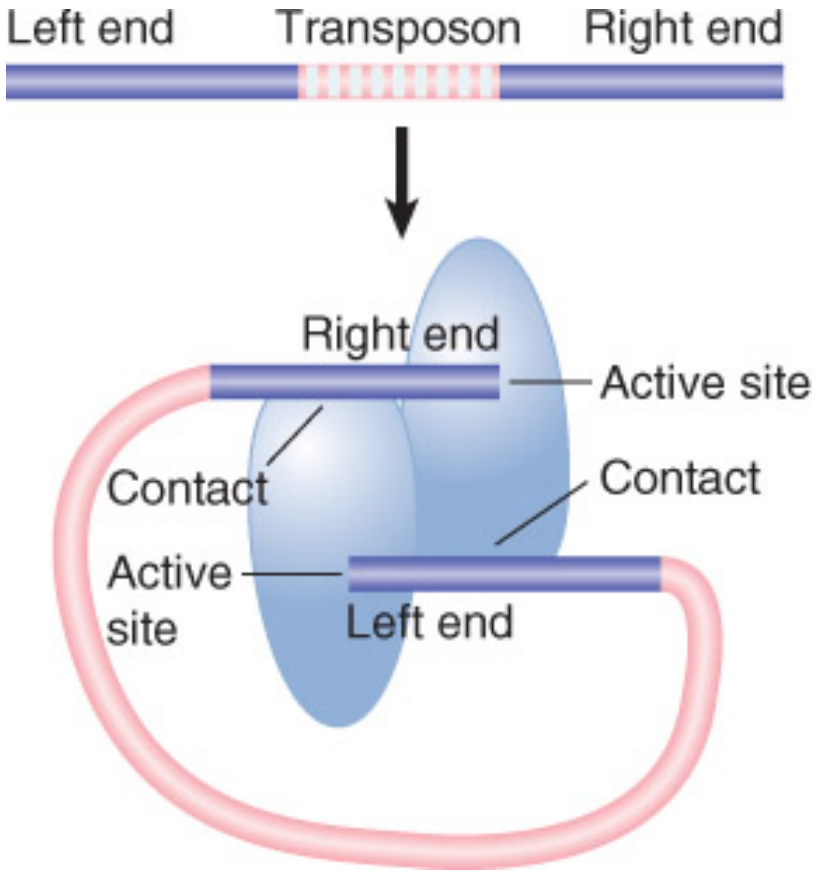


Reciprocal recombination between inverted repeats inverts the region between them.

Genetik



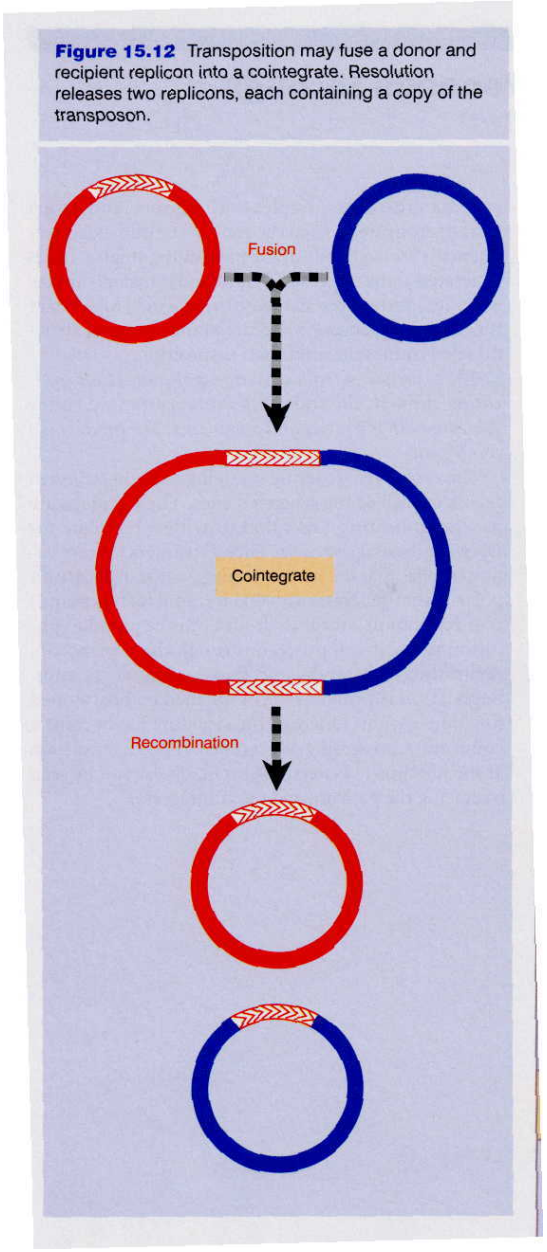
Nicking initiates transposition



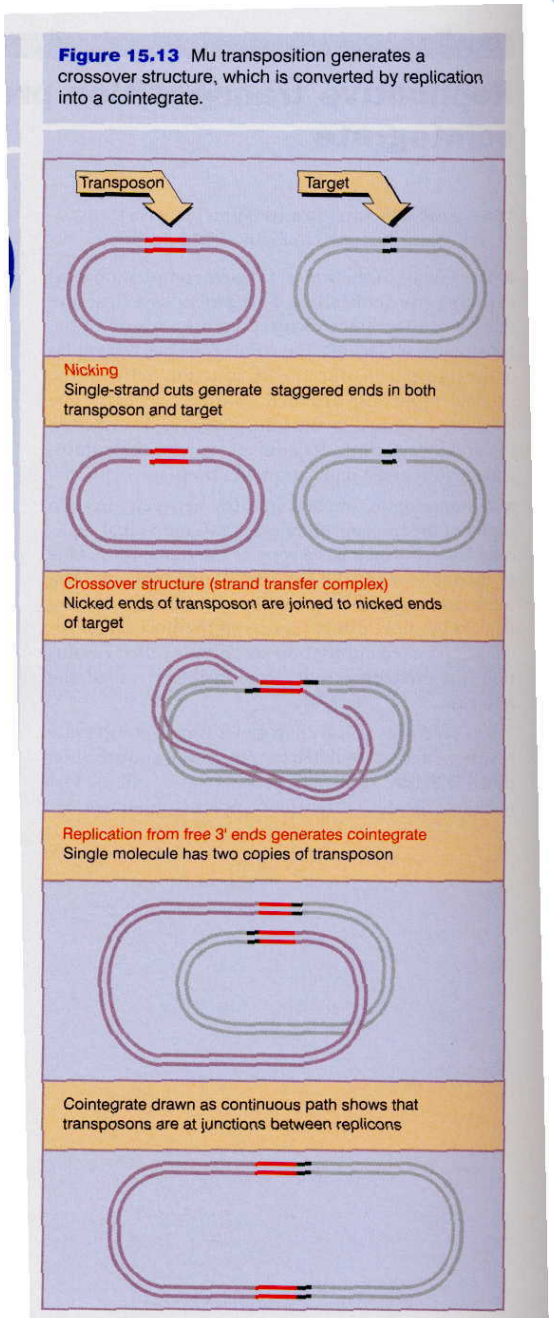
Each subunit of the Tn5 transposase has one end of the transposon in its active site and makes contact elsewhere with the other end.

Genetik

Replicative Transposition



Cointegrate formation and resolution



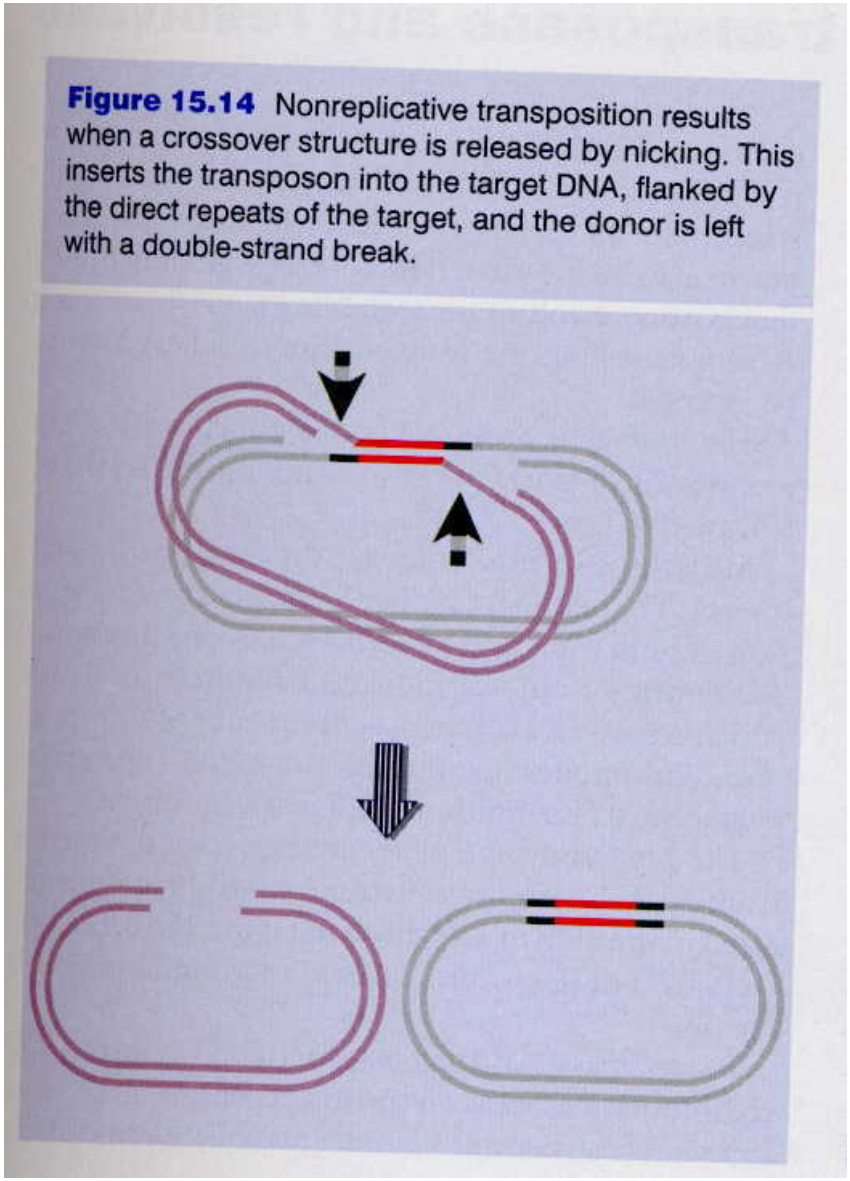


Figure 15.14 Nonreplicative transposition results when a crossover structure is released by nicking. This inserts the transposon into the target DNA, flanked by the direct repeats of the target, and the donor is left with a double-strand break.

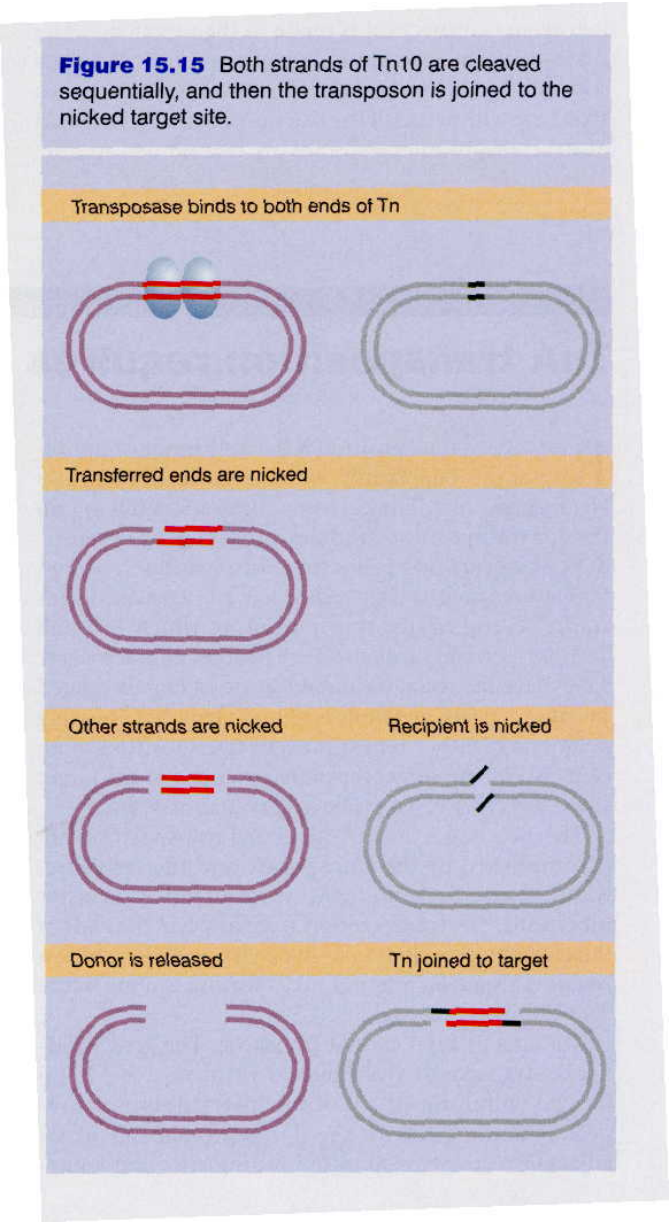
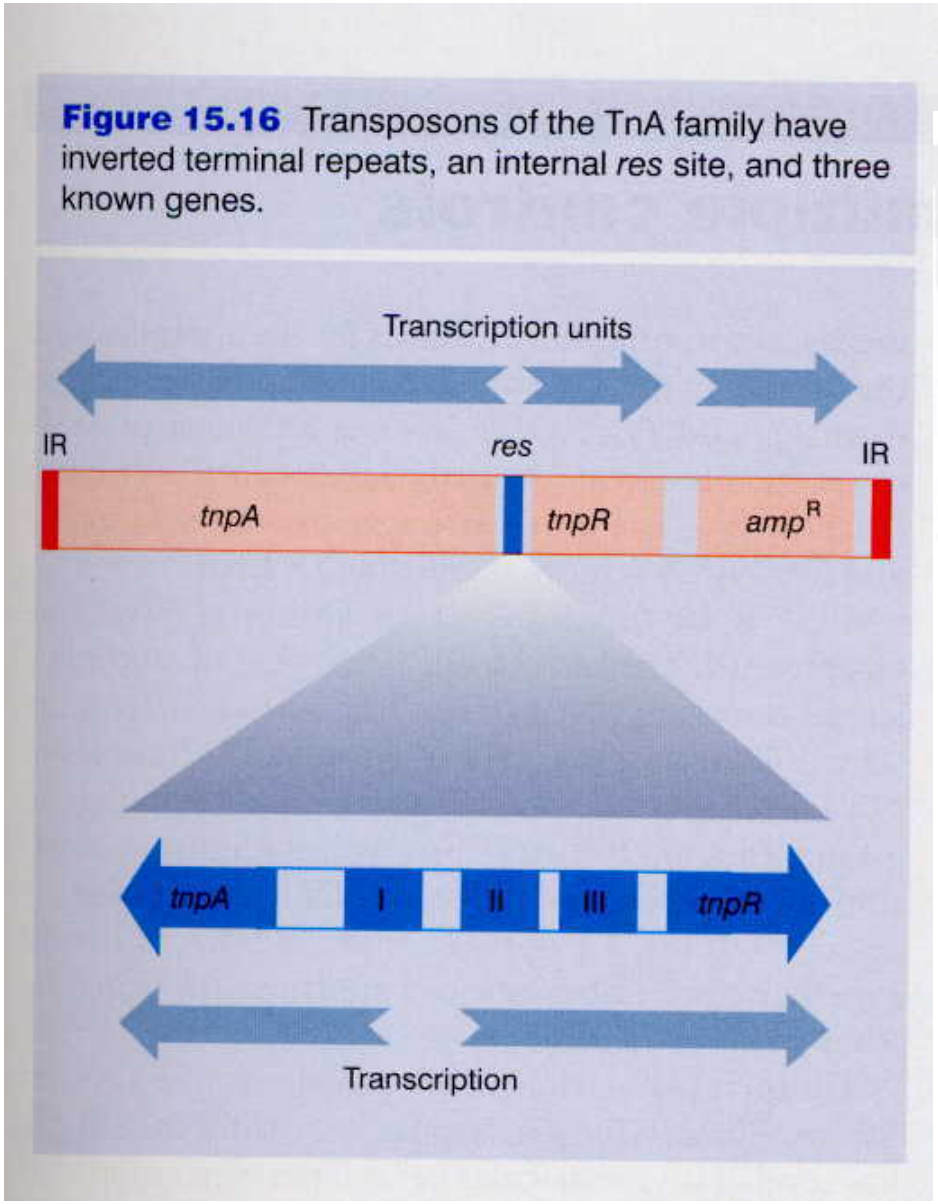
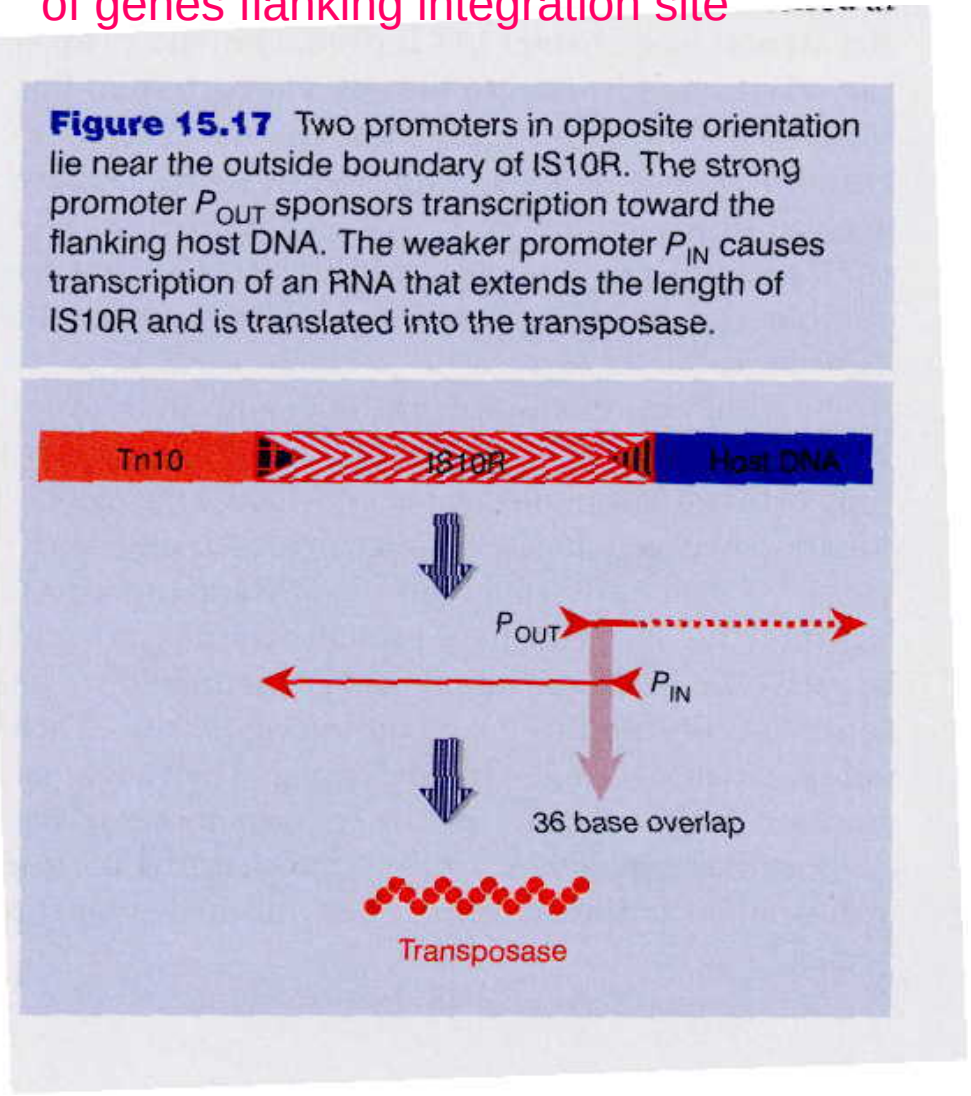


Figure 15.15 Both strands of Tn10 are cleaved sequentially, and then the transposon is joined to the nicked target site.



Transposons can influence expression of genes flanking integration site



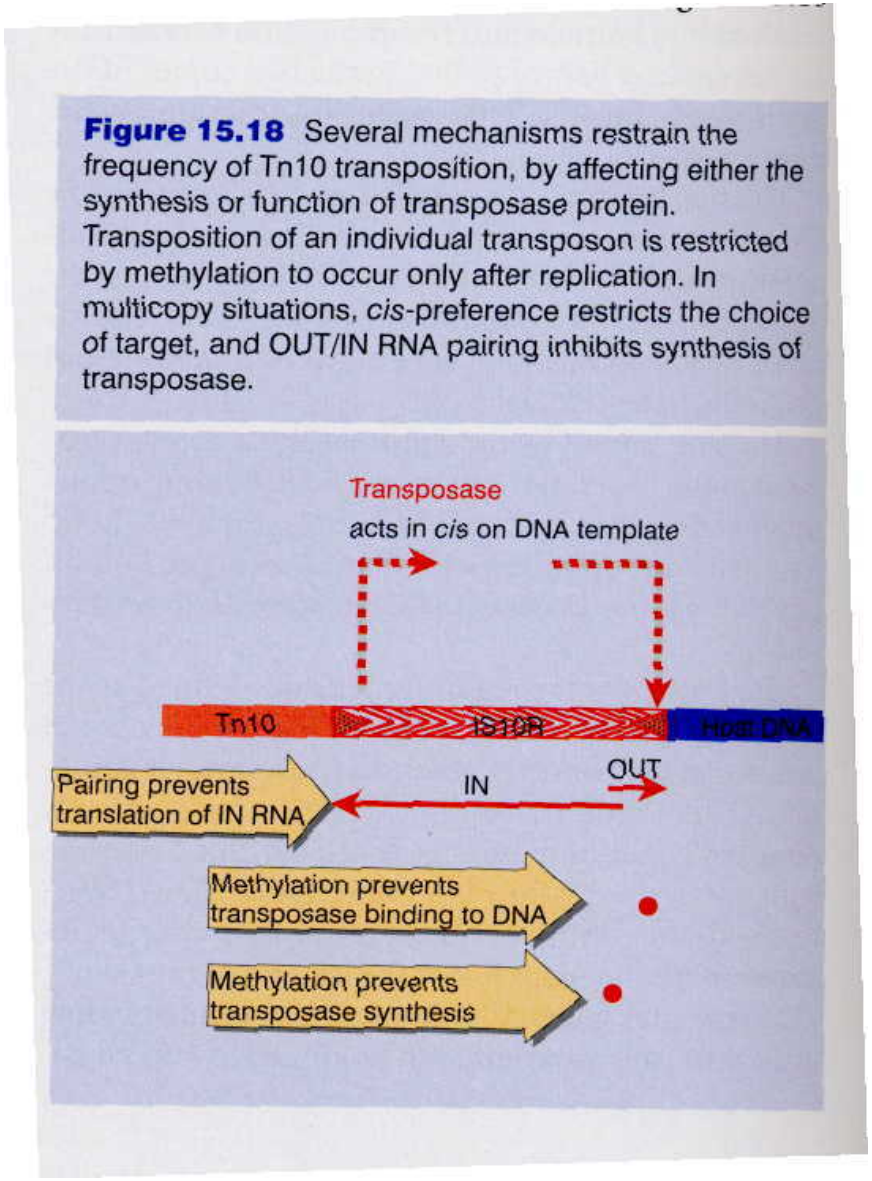
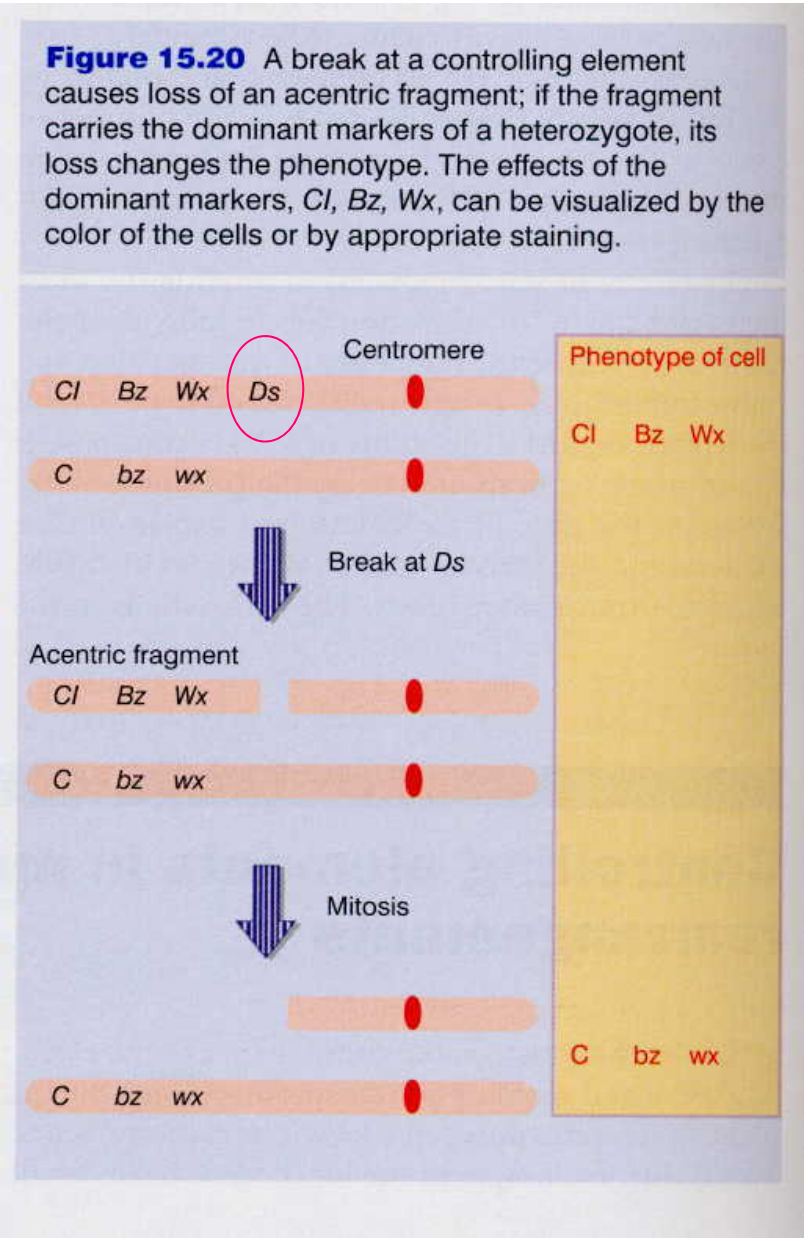
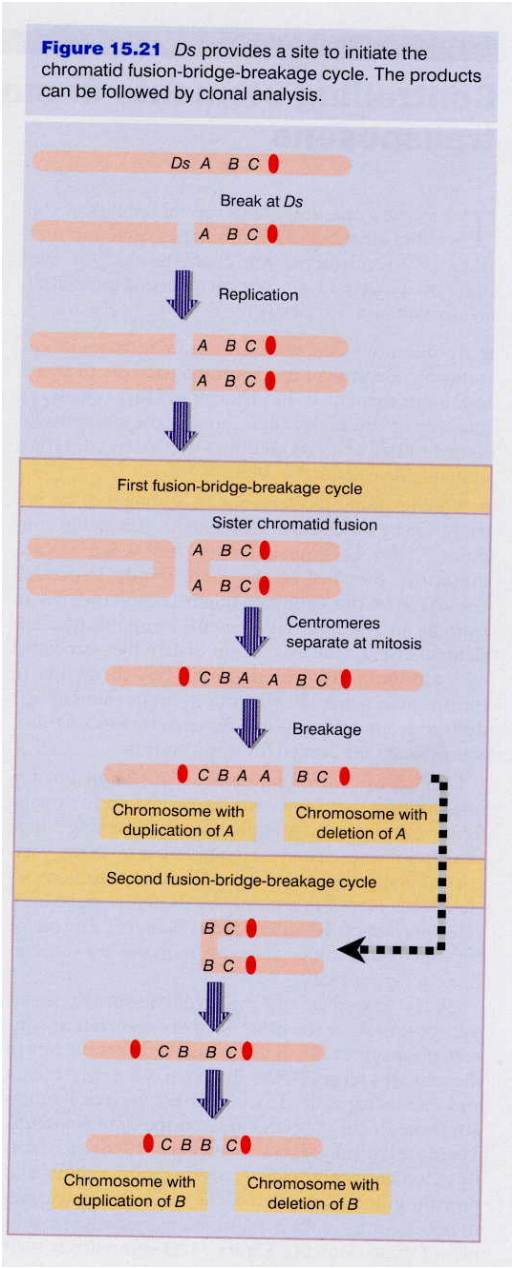


Figure 15.18 Several mechanisms restrain the frequency of Tn10 transposition, by affecting either the synthesis or function of transposase protein. Transposition of an individual transposon is restricted by methylation to occur only after replication. In multicopy situations, *cis*-preference restricts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

Genetik

Transposons of Eukaryotes



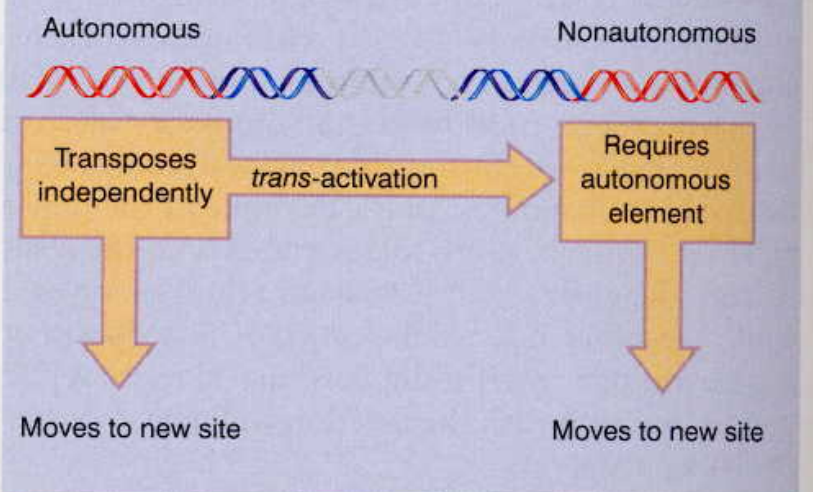


Controlling elements in maize

Genetik

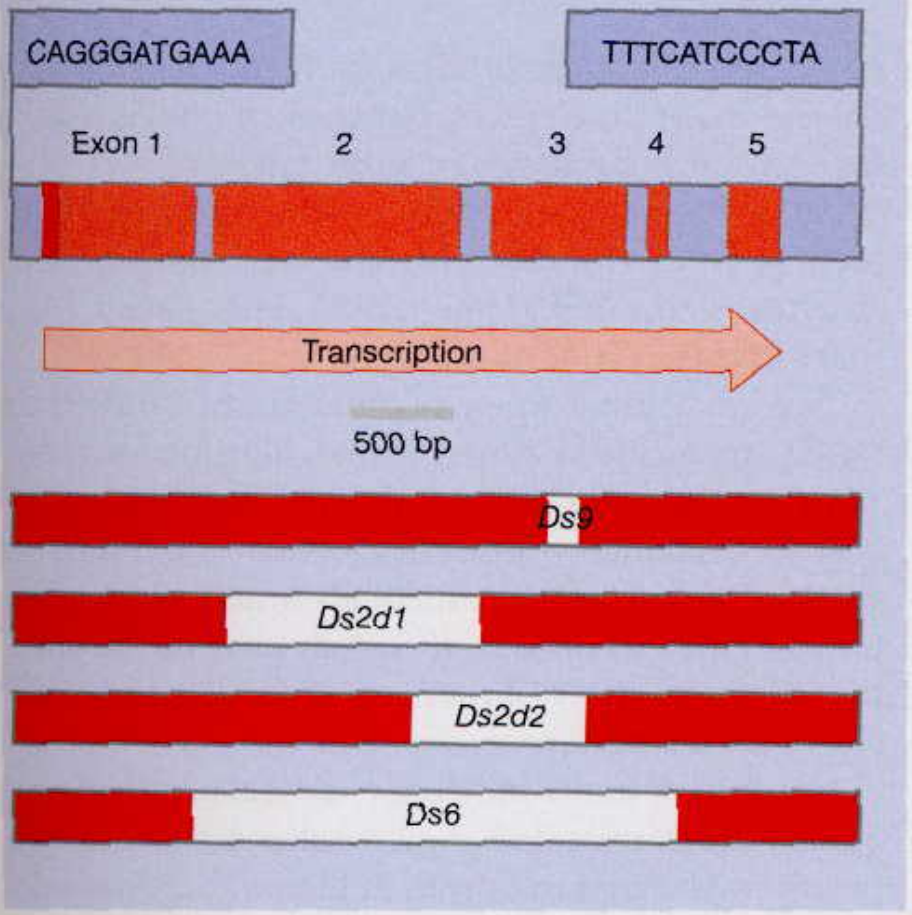
Transposons of Eukaryotes

Figure 15.22 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.



Ac (activator) *Mp* (modulator)	*Ds* (dissociation)
Spm (suppressor-mutator) *En* (enhancer)	*dSpm* (defective *Spm*) *I* (inhibitor)
Dotted	Unnamed
Mu (mutator)	Not known

Figure 15.23 The *Ac* element has two open reading frames; *Ds* elements have internal deletions.



Genetik

21.10-14

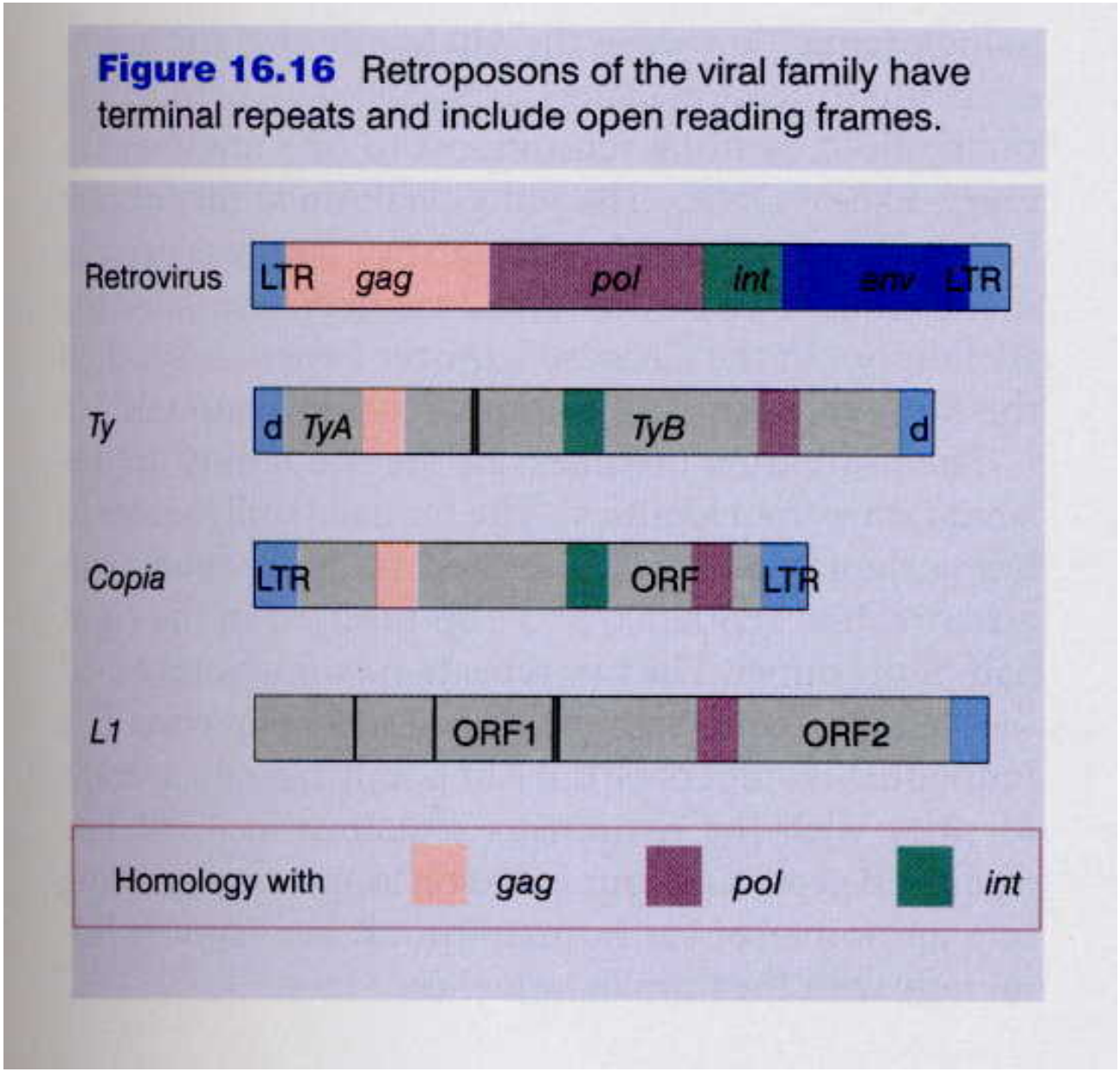


Figure 16.16 Retroposons of the viral family have terminal repeats and include open reading frames.
Retrovirus
LTR
gag
pol
int
env
LTR
Ty
d
TyA
TyB
d
Copia
LTR
ORF
LTR
L1
ORF1
ORF2
Homology with
gag
pol
int

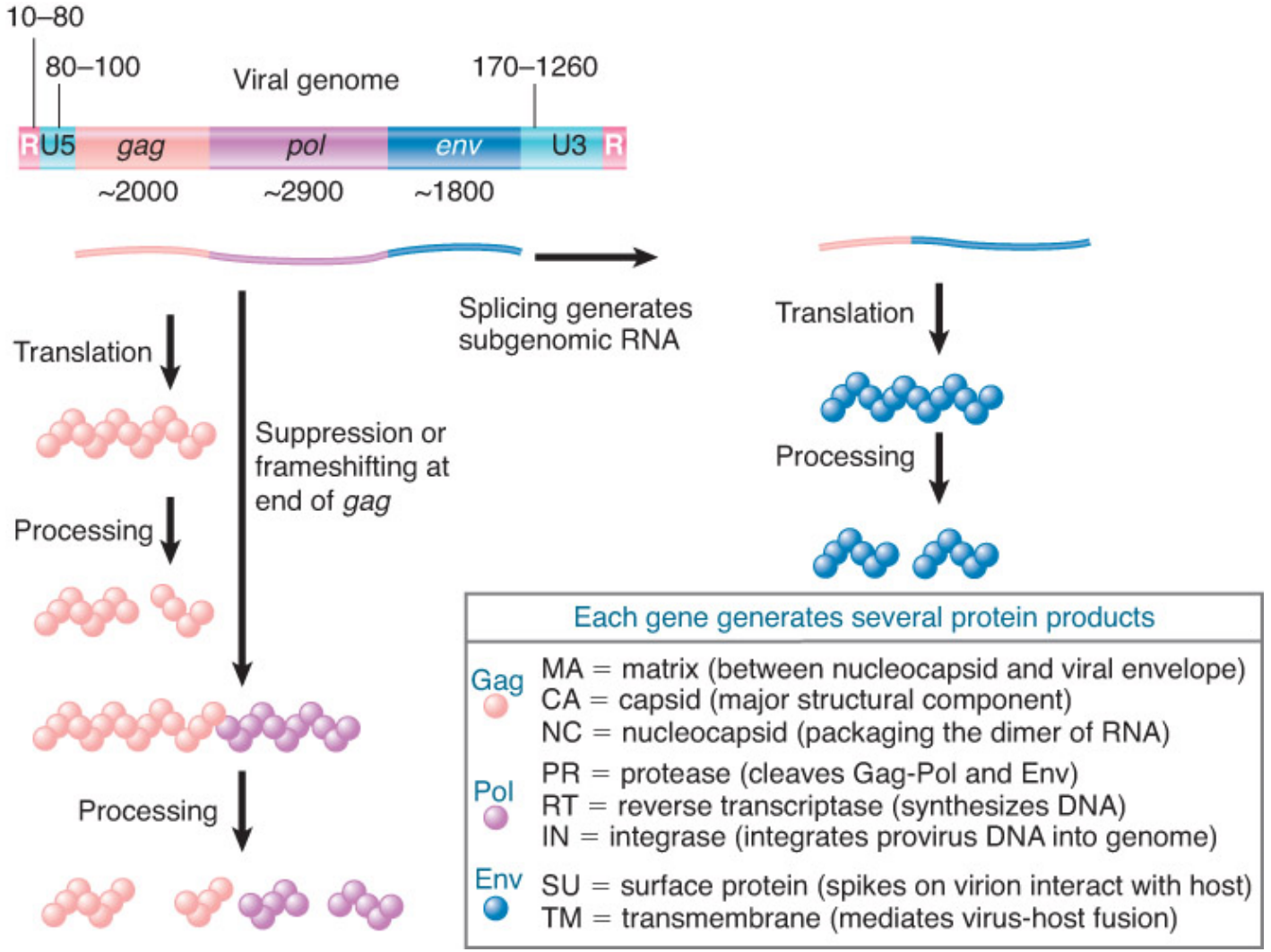


Figure 17.F21: The genes of the retrovirus are expressed as polyproteins that are processed into individual products.

Genetik

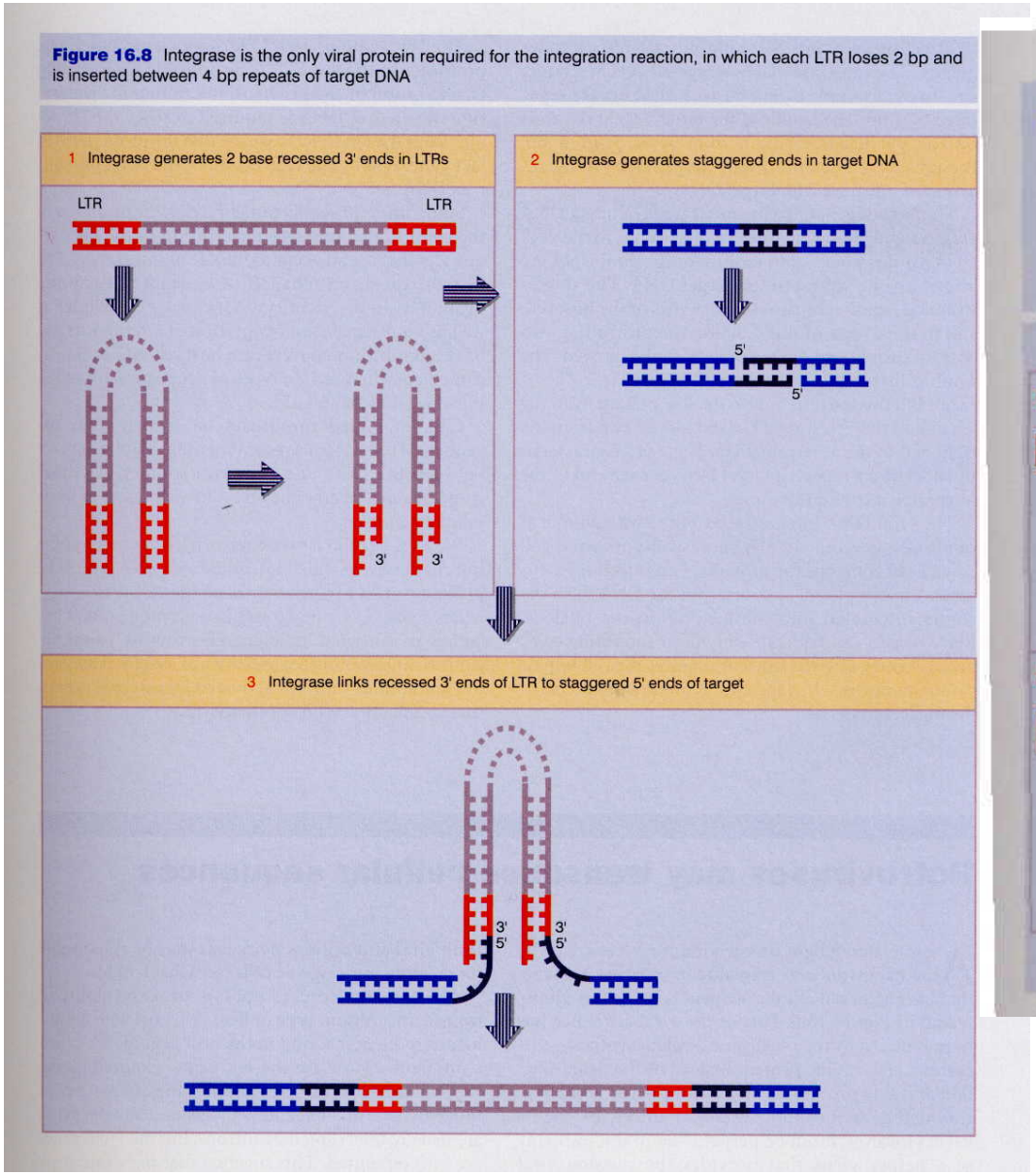


Figure 16.8 Integrase is the only viral protein required for the integration reaction, in which each LTR loses 2 bp and is inserted between 4 bp repeats of target DNA
1 Integrase generates 2 base recessed 3' ends in LTRs
LTR
LTR
3'
3'
2 Integrase generates staggered ends in target DNA
5'
5'
3 Integrase links recessed 3' ends of LTR to staggered 5' ends of target
3'
5'
3'
5'

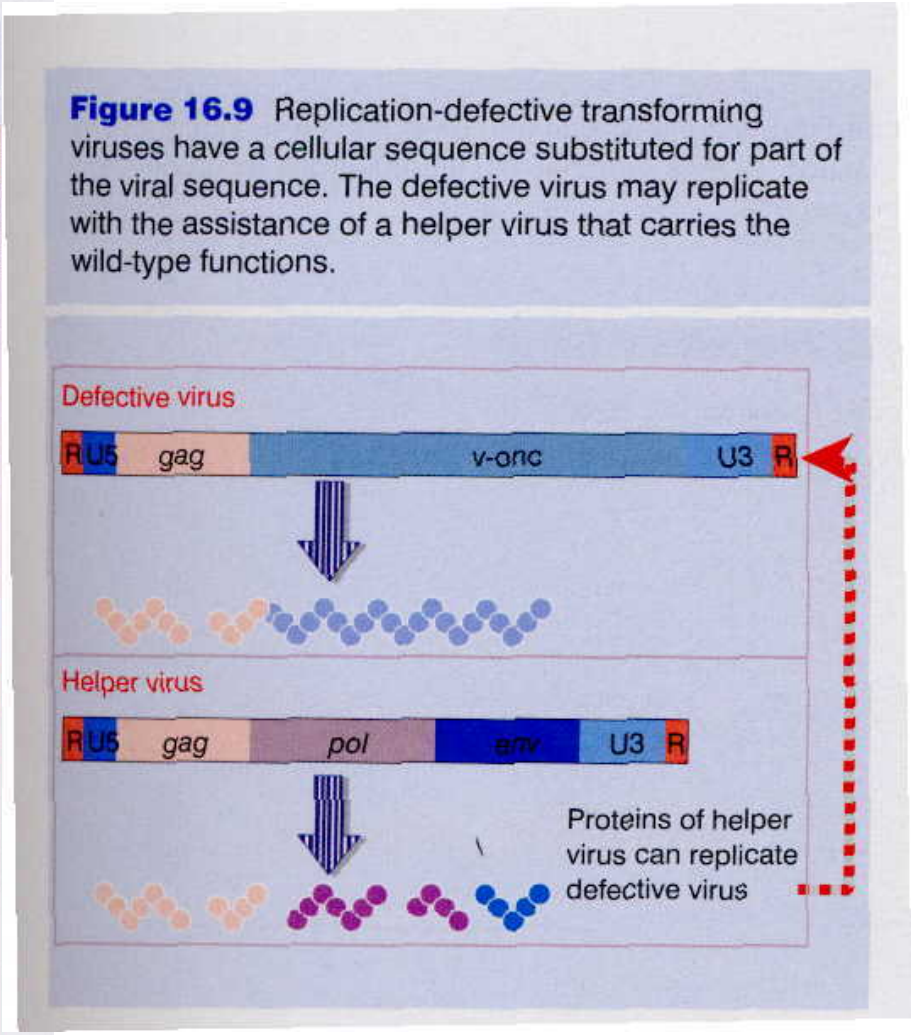


Figure 16.9 Replication-defective transforming viruses have a cellular sequence substituted for part of the viral sequence. The defective virus may replicate with the assistance of a helper virus that carries the wild-type functions.
Defective virus
R U5
gag
v-onc
U3
R
Helper virus
R U5
gag
pol
env
U3
R
Proteins of helper virus can replicate defective virus

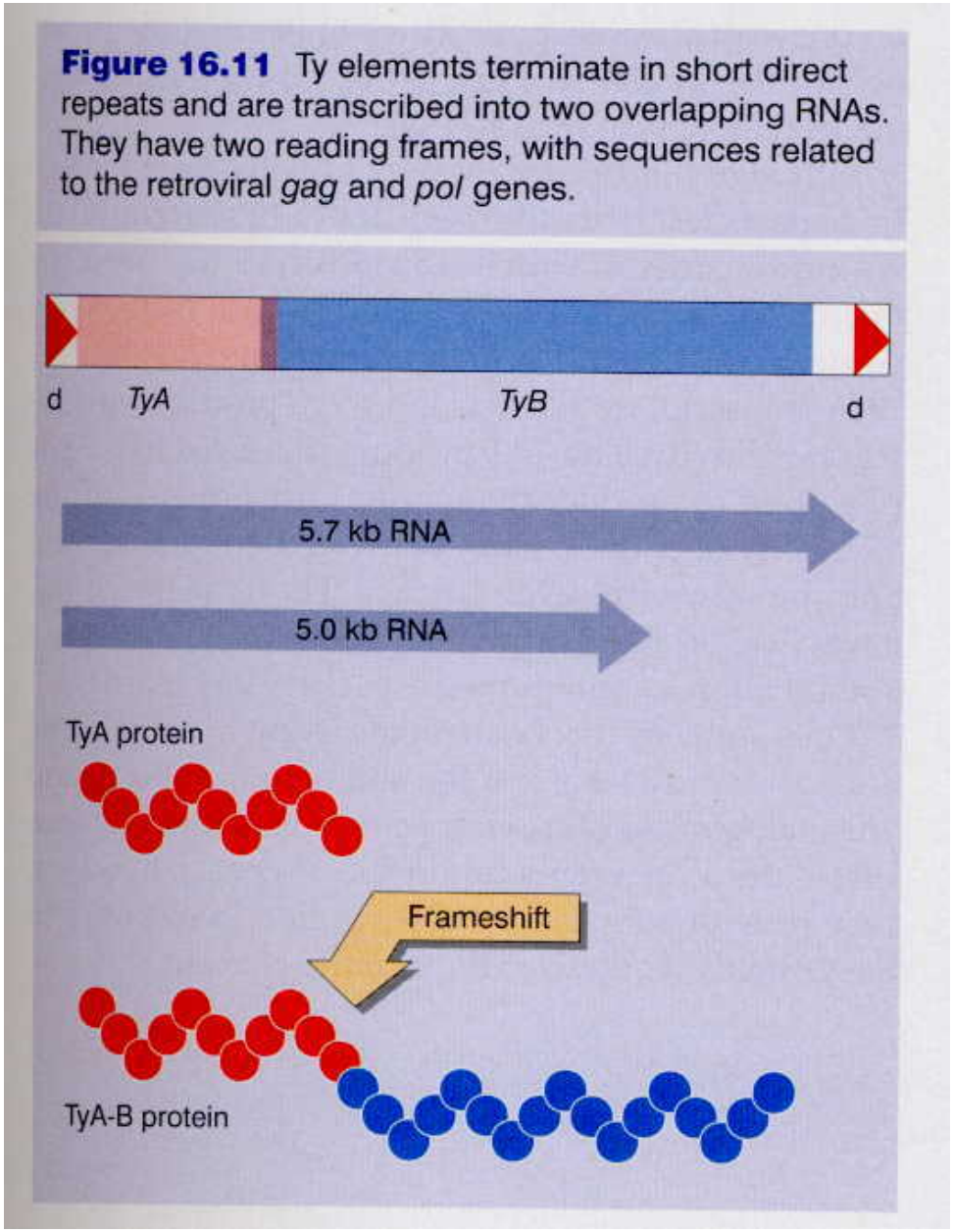


Figure 16.11 Ty elements terminate in short direct repeats and are transcribed into two overlapping RNAs. They have two reading frames, with sequences related to the retroviral *gag* and *pol* genes.

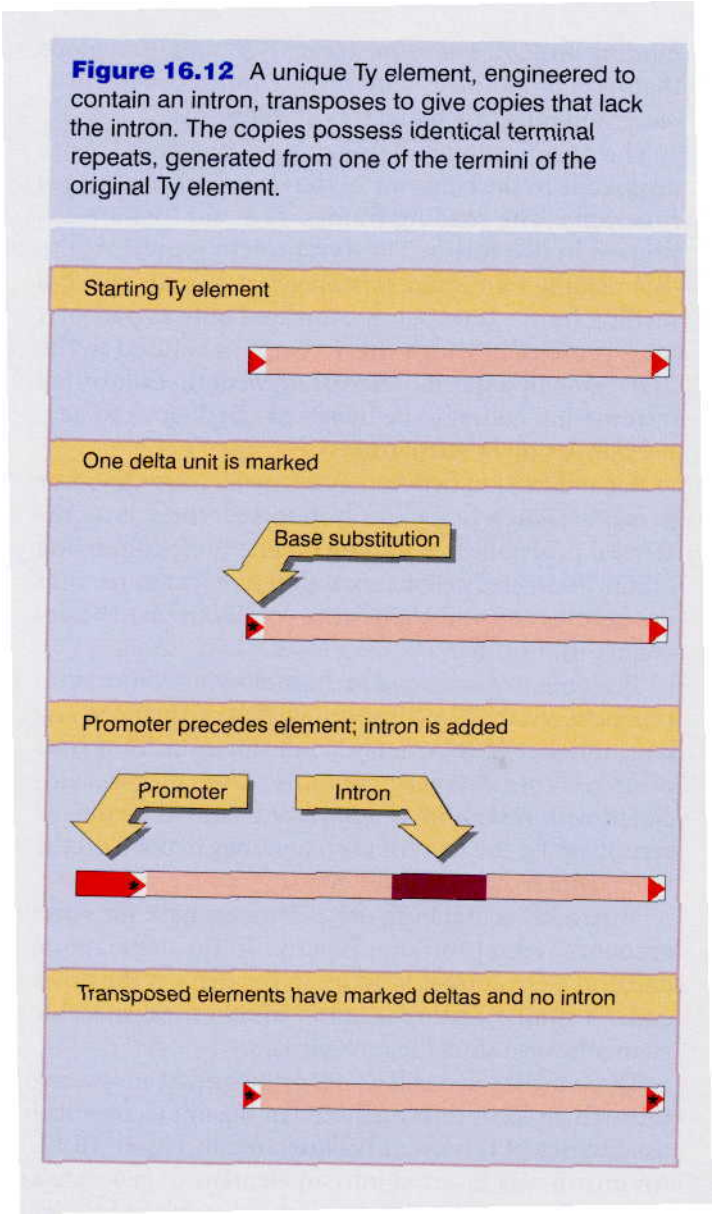


Figure 16.12 A unique Ty element, engineered to contain an intron, transposes to give copies that lack the intron. The copies possess identical terminal repeats, generated from one of the termini of the original Ty element.

Figure 16.13 Ty elements generate virus-like particles. Photograph kindly provided by Alan Kingsman.

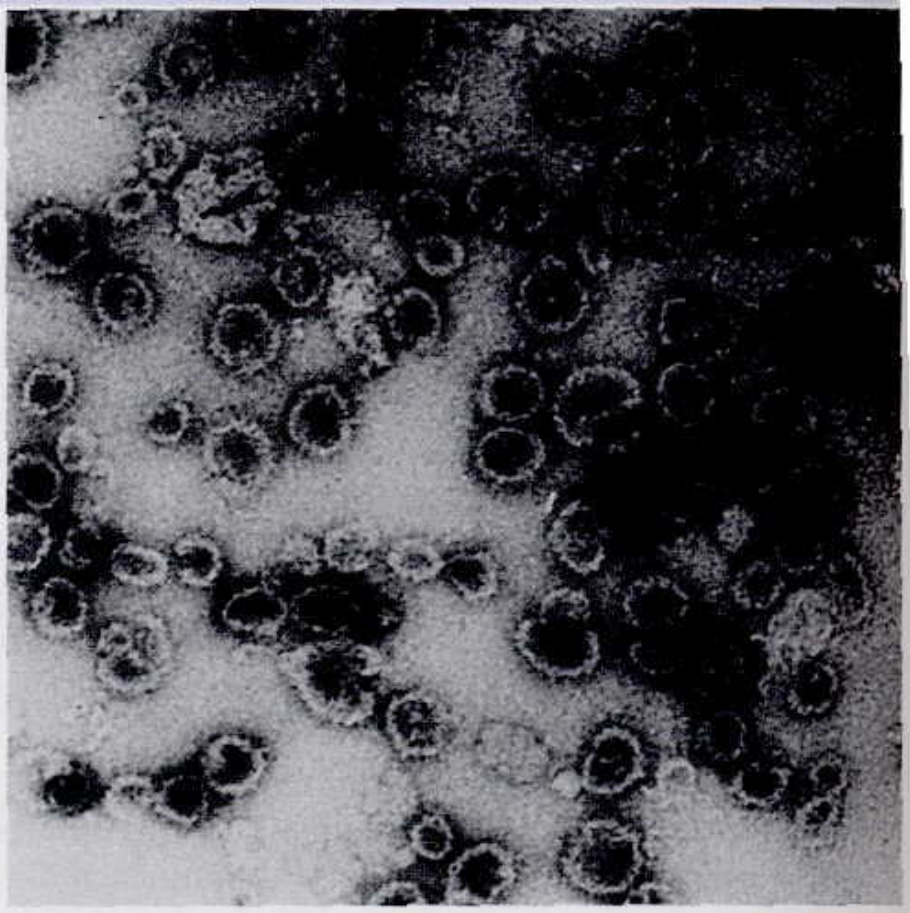
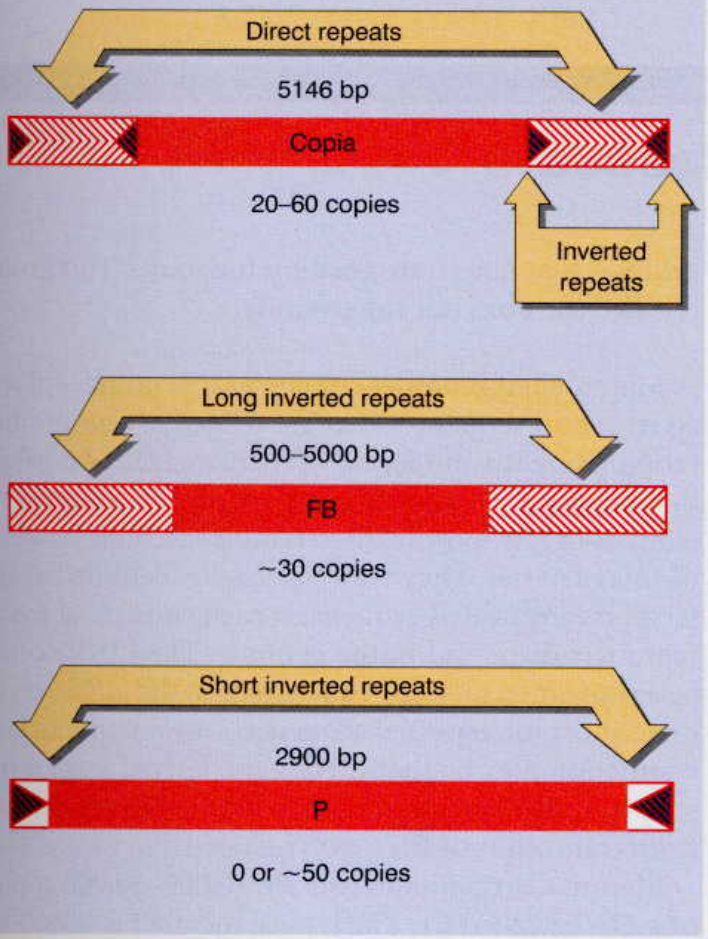
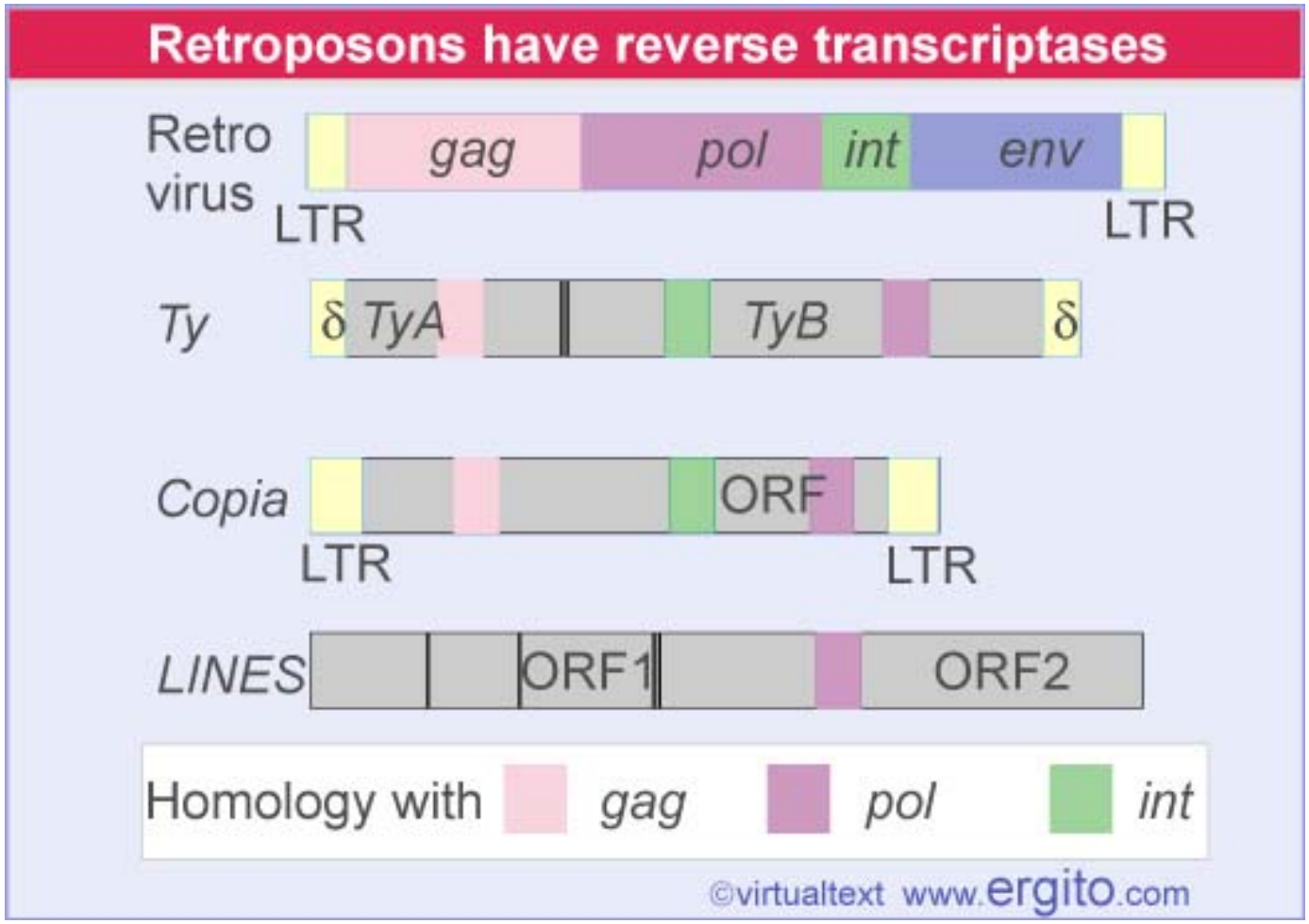


Figure 16.14 Three types of transposable element in *D. melanogaster* have different structures.



Mammalian 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


Retroposons have reverse transcriptases
Retro virus
gag
pol
int
env
LTR
LTR
Ty
δ TyA
TyB
δ
Copia
ORF
LTR
LTR
LINES
ORF1
ORF2
Homology with
gag
pol
int
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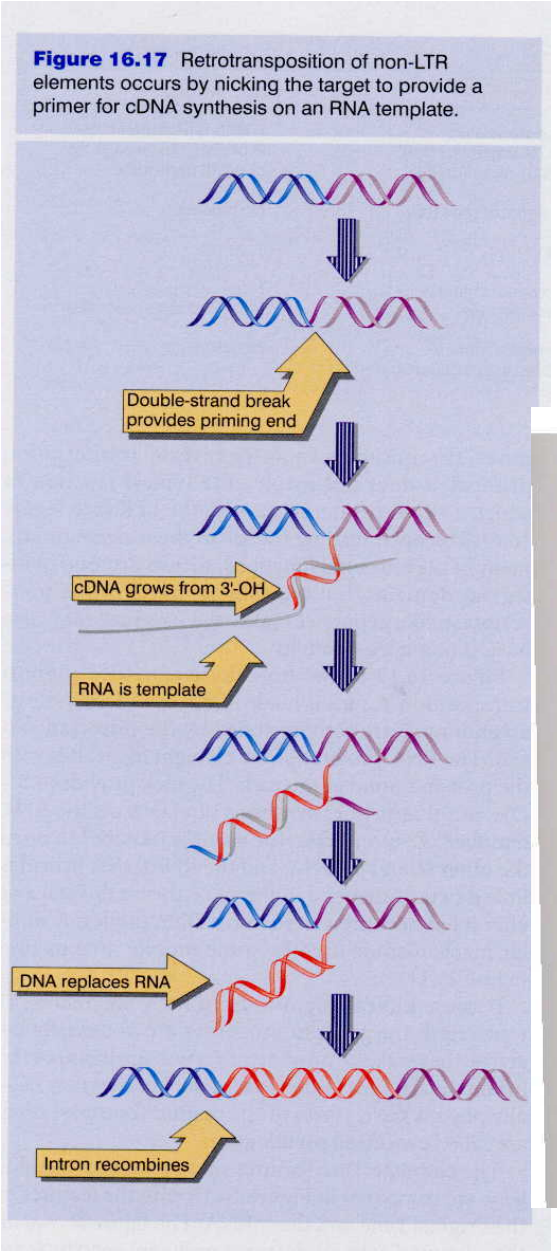
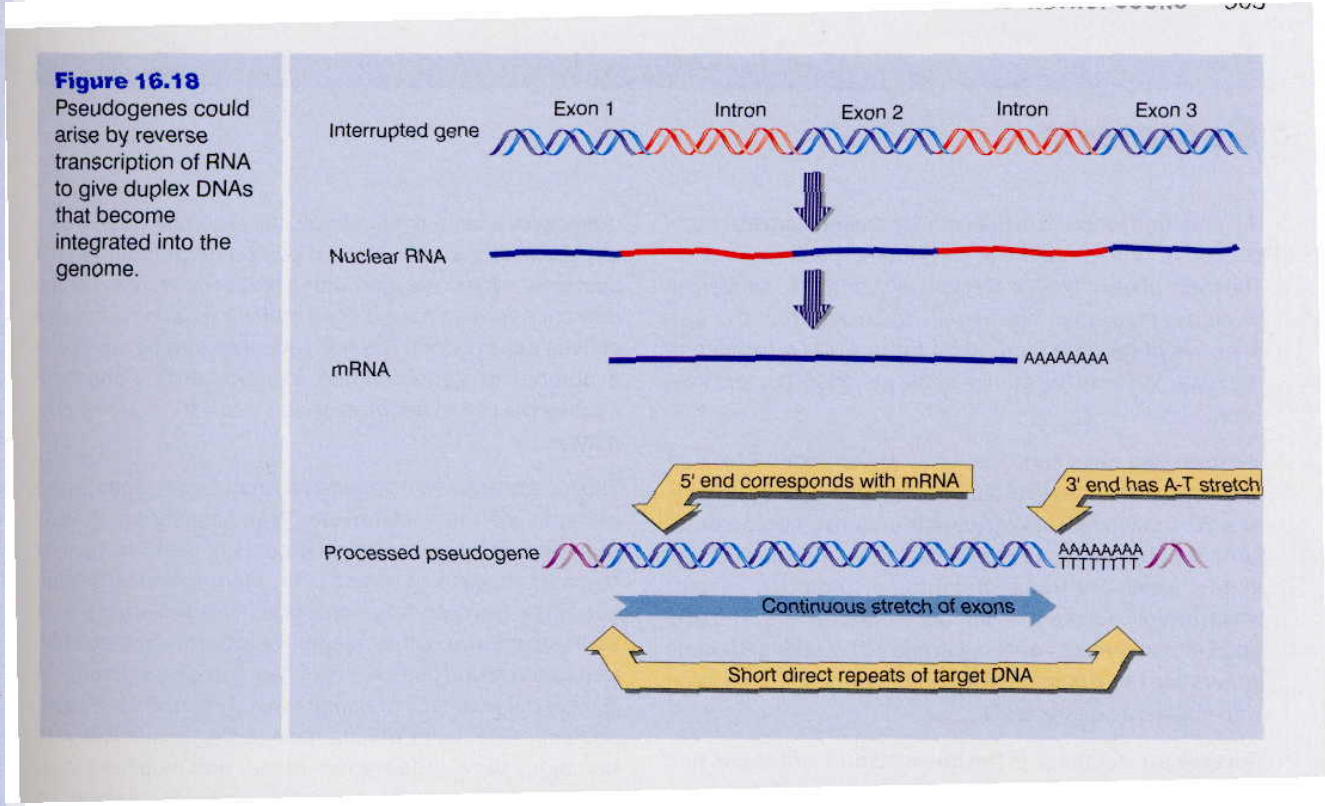
Retroviruses and transposons constitute half the human genome

Element	Organization	Length (Kb)	Human genome Number	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%

Genetik



Reverse Transcription → Tool for Genetic Variation



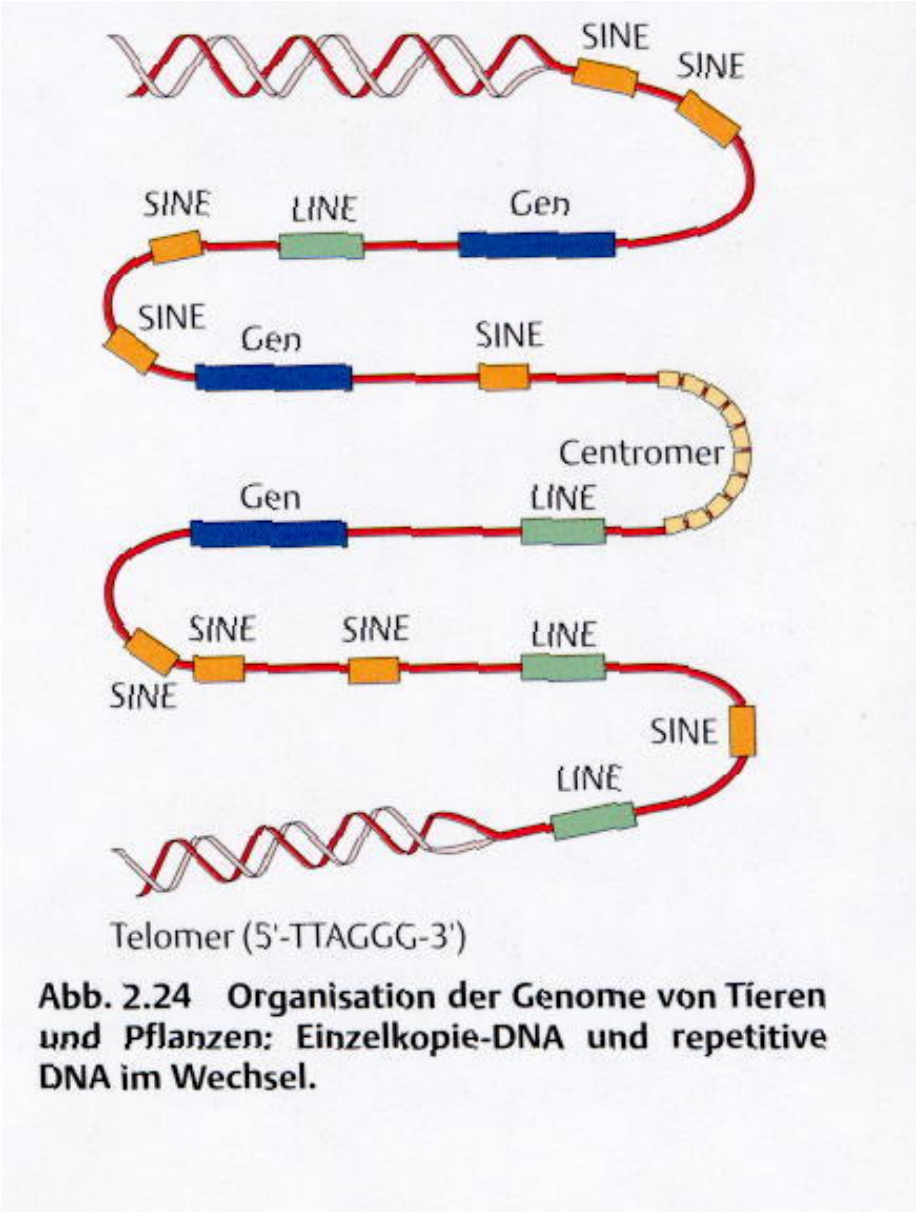


Abb. 2.24 Organisation der Genome von Tieren und Pflanzen: Einzelkopie-DNA und repetitive DNA im Wechsel.