

# **Bakteriální transpozony**

**Transpozon = sekvence DNA schopná transpozice, tj. přemístění z jednoho místa v genomu do jiného místa**

**Transpozice = proces přemístění transpozonu**

**Transponáza (transpozáza) = enzym zprostředkující transpozici**

**IS = inzerční sekvence (IS-elementy)**

**Tn = transpozony**

**Poprvé byly IS popsány v r. 1967 u *E. coli* analýzou mutant s těmito vlastnostmi:**

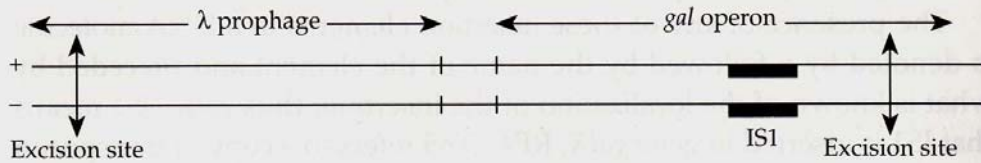
- 1. Mutace byly vysoce polární - každá se mapovala v prvním genu operonu, ale nebyly syntetizovány proteiny genů po směru transkripce. Polarita byla důsledkem přítomnosti transkripčně-terminační sekvence inzerčního elementu.**
- 2. Tyto mutace nebylo možné revertovat analogy bází nebo frame-shift mutageny, takže podstatou mutací nemohly být substituce ani adice nebo delece bází.**
- 3. Jestliže byly do kmenů s mutacemi přeneseny plazmidy, podobné polární mutace (i když v jiných genech) se na nich občas objevovaly. Např. F<sup>+</sup>lac<sup>+</sup> se stal lac<sup>-</sup>.**
- 4. Fyzikální studium plazmidů ukázalo, že plazmid s mutací je delší díky inzerci elementu.**

## **Specifické rysy transpozice:**

- **cílová místa nejsou homologická s místy donorovými**
- **obvykle dochází k duplikaci přenášené sekvence, tj. transpozon zůstává i v původním donorovém místě**
- **v místě inzerce se zdvojují ve stejném směru sekvence DNA - transpozon je na obou koncích ohraničen přímými repeticemi, což je důsledek mechanismu transpozice**
- **po inzerci transpozonu do cílového místa dochází k inaktivaci genů, po excizi transpozonu se funkce obnovuje.**

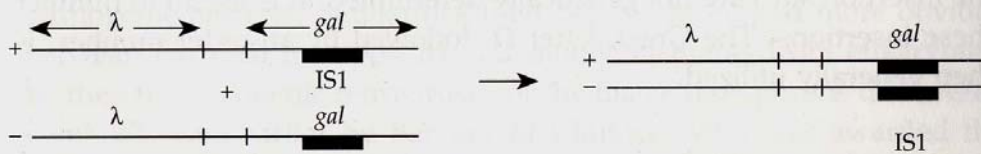
# Důkaz přítomnosti IS-sekvencí u *E. coli*

(a)



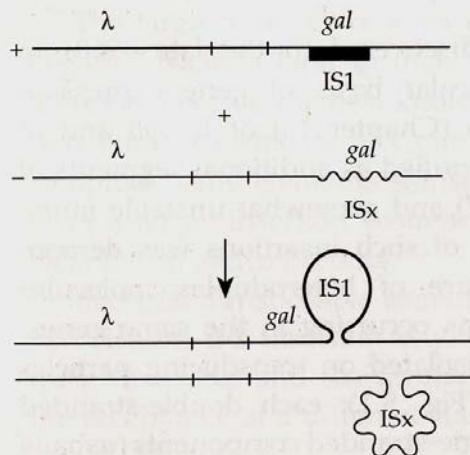
Vznik specificky  
transdukujících fágů

(b)



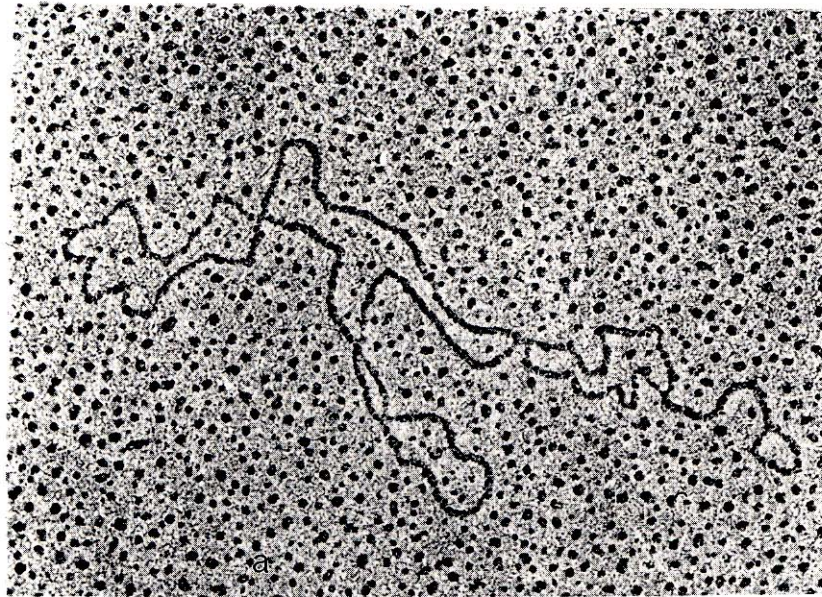
Vznik heteroduplexů

(c)

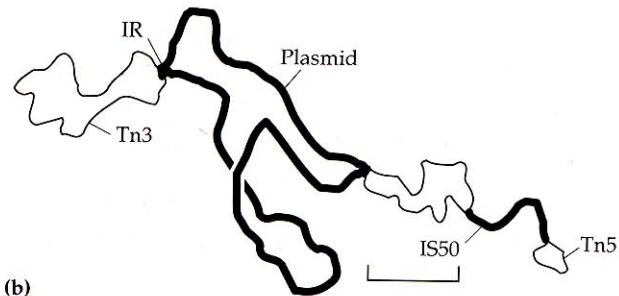


Mapování neznámých  
IS v *gal* operonu  
heteroduplexní  
analýzou

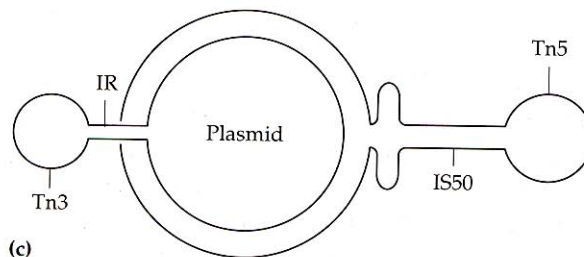
# Znázornění přítomnosti transpozonů v EM - heteroduplexní analýza



(a)



(b)



(c)

# Struktura IS sekvencí a složených transpozonů

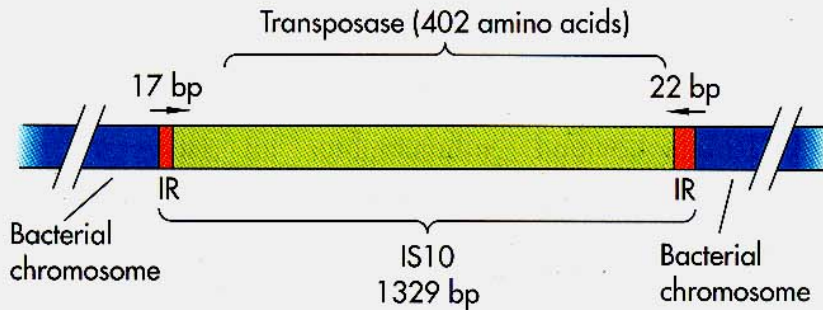


FIGURE 10-1

Structure of IS10, a simple bacterial insertion sequence. IS10 is a 1329-bp transposable element found in *E. coli*. The element consists of a gene that encodes a 402 amino acid protein, thought to be the transposase enzyme required for IS movement, flanked by short inverted repeats (IR). The IRs are blocks of similar (but not identical) sequence in opposite orientation to one another. The IRs are recognized by the transposase enzyme in the first steps of transposition and therefore define the ends of the sequence to be transposed.

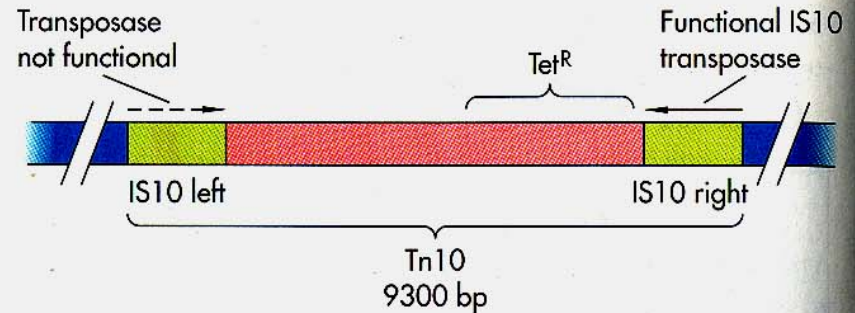
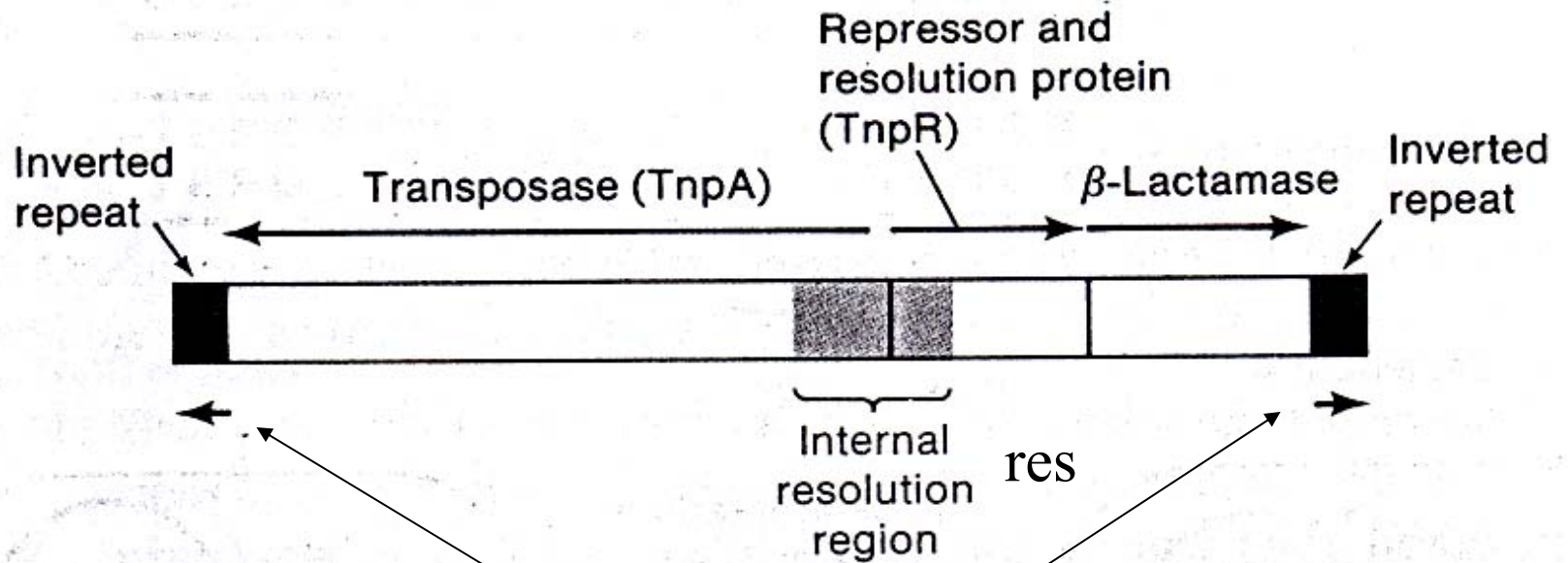


FIGURE 10-2

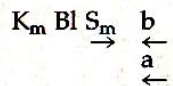
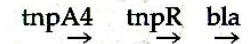

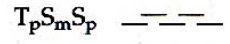
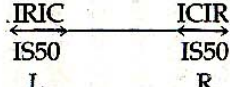
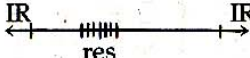
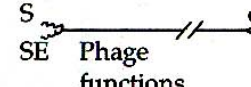
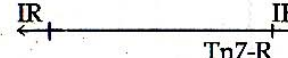
Complex transposons are genes flanked by two IS elements. Tn10 is a 9300-bp-long movable element that encodes genes for resistance to the antibiotic tetracycline ( $Tet^R$ ). Each end contains an IS10 insertion element (oriented in opposite directions). The right IS encodes a functional transposase that is required for movement of the transposon. The left IS10 element has accumulated mutations so that it no longer encodes an active transposase protein. Between the IS elements are genes required for tetracycline resistance. The IS elements can transpose individually or in tandem; in the latter case they carry the intervening DNA with them.

# Struktura transpozonu Tn3



38 bp obrácená opakování

# Čtyři hlavní třídy transpozonů u G- bakterií

	A Class I	B Class II	C Class III	D Class IV
Structure of a representative example	Tn5	Tn3	Phage Mu	Tn7
mRNAs				
DNA				
Size of Tn	5.7 kbp	5 kbp	39 kbp	14 kbp
Size of target duplication	9 bp	5 bp	None	5 bp
Markers <sup>a</sup>	Km, Sm, Bl	Ap	Phage functions	Tp, Sm, Sp
Transposition functions	ISR: a = active transposase; b = transposase inhibitor ISL: inactive	<i>tnpA</i> transposase <i>tnpR</i> resolvase	Two proteins: A and B	Five proteins necessary
Comments	Composite Tn, with two distal, nearly identical, ISs, of which the left one is inactive	IR 39 bp	Largest known transposon	IR 28 bp
Other well-studied elements	Tn9 (IS1) Cm Tn10 (IS10) Tc	Tn1 Ap Tn501 Hg Tn21 Hg, Sm, Ap, Su Tn1000 ( $\gamma^8$ ) IS101 (209 bp)	Phage D108	

IR, IC, inverted repeats; res, site of co-integrate resolution; SE, c, striped ends. a, See Table 3.5 (p. 76) for explanation of the symbols.



# IS u gramnegativních bakterií

Designation	Host DNA and copy number	Size (in bp)	Inverted repeat <sup>a</sup> (in bp)	Target duplication (in bp)	Open reading frame (no.)	Special properties
IS1	Enterobacterial chromosomes, phages and plasmids (5–8 copies per strain)	768	20/23	9 (8–11)	8	Several Class I transposons are formed by inverted or direct repeats of IS1 (Tn9, Tn2350, Tn1681)
IS2	<i>E. coli</i> chromosomes, plasmids (F)	1327	32/41	5	2	Inverted repeats of Tn951
IS3	<i>E. coli</i> chromosomes (4–5 copies) Plasmid F (2 copies)	1258	39/39	3	3	Behaves as a mobile promoter
IS4	Chromosomes of <i>E. coli</i> K12 (1 copy at a single location)	1426	16/18	11, 12 or 14	2	1 specific insertion site
IS5	<i>E. coli</i> <i>Shigella</i> Phages $\lambda$ , Mu	1195	15/16	4	3	The most abundant IS in <i>E. coli</i> ; has a promoter activity by creation of a promoter
IS10	Tn10	1329	17/22	9	1	Tn10 inverted repeats
IS15	<i>E. coli</i> <i>Salmonella</i> Several plasmids					Tn1525 direct repeats

pokračování

IS21	Inc P1 plasmids	2100					Mobile promoter active only when in tandem repeats
IS26	Tn2680	820	14/24	8	2		Tn2680 direct repeats
IS30	Phage P1 <i>E. coli</i>	1221	23/26	2	3		Insertion site quite specific
IS46	IncN plasmids	810					Mainly forms cointegrates
IS50R	Tn5	1534	8/9	9	2		Tn5 inverted repeats; only IS50R is active
IS52	<i>Pseudomonas savastanoi</i>	1209	9/10	4			
IS66	<i>Agrobacterium</i> plasmid pTiA66	2548					
IS102	Plasmid pSC102	1057	18	9	3		
IS136 (IS426)	<i>Agrobacterium tumefaciens</i> (pTC137)	1313	32/30	9	3		
IS200	<i>Salmonella typhimurium</i> (6–10 copies)						Only found in <i>Salmonella</i> species
IS222	<i>Pseudomonas aeruginosa</i> chromosome and phage D3	1350	40				
IS476	<i>Xanthomonas campestris</i>	1225	13	4			
IS4400	<i>Bacteroides fragilis</i>	1150					
ISRm2	<i>Rhizobium meliloti</i>	2700	24/25	8			

a, The two figures refer to the sizes of the two inverted repeats, when these are not identical.

# IS a transpozony u G+ bakterií

Element	Hosts	Phenotype <sup>a</sup>	Size (kbp)	Terminal repeats	Target duplication	Class <sup>b</sup>
IS231	<i>Bacillus thuringiensis</i>	None	1.65	20	11	
ISS1	<i>Streptococcus lactis</i>	None	0.82	18	8	I (IS15)
IS110	<i>Streptomyces coelicolor</i>	None	1.55	10/15	ND	
IS257	<i>Streptococcus lactis</i>	None				I (IS15)
IS861	<i>Streptococcus</i>					I (IS50, IS3)
Tn4001	<i>Staphylococcus aureus</i>	Gm, Tm, Km	4.7	IS256	ND	I
Tn551	<i>Staphylococcus aureus</i>	Em	5.3	40	5	II
Tn917	<i>Streptococcus faecalis</i>	Em	5.27	38	5	II
Tn4430	<i>Bacillus thuringiensis</i>	None	4.194	38	5	II
Tn4451	<i>Clostridium perfringens</i>	Cm	6.2	12	ND	II
Tn4556	<i>Streptomyces fradiae</i>		6.62			II
Tn916	<i>Streptococcus faecalis</i>	Tc conjugative	16.4	Imperfect	0	V
Tn918	<i>Streptococcus faecalis</i>	conjugative	16	ND	ND	V
Tn919	<i>Streptococcus sanguis</i>	conjugative	16	ND	ND	V
Tn1545	<i>Streptococcus pneumoniae</i>	Tc, Em, Km conjugative	25.3	Imperfect	0	V
Minicircle	<i>Streptomyces coelicolor</i>	None	2.6	Imperfect	0	V
Tn554	<i>Staphylococcus aureus</i>	Em, Sp	6.69	0	0	V

**Nová třída V**

**- nemají IR**

**- netvoří TD**

a, See Table 3.5, p. 76 for explanation of symbols; Gm, gentamycin; ND, not determined; Tm, tobramycin.

b, The IS in brackets indicates Gram-negative elements having homologies with those described.

**Konjugativní transpozony**

## IS u archeí

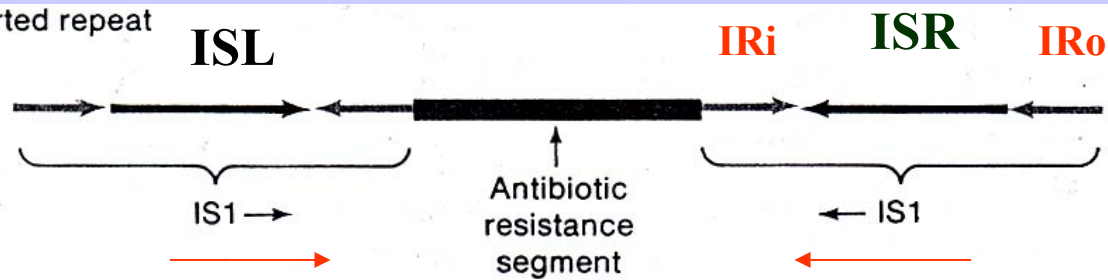
Element	Hosts	Size bp	Inverted repeats	Target duplication (bp)	ORFs
ISH1	<i>Halobacterium halobium</i>	1118	8/9	8	1
ISH2	<i>Halobacterium halobium</i>	520	19	10, 11 or 20	3
ISH25	<i>Halobacterium halobium</i>	588	none	none	
ISH50	<i>Halobacterium halobium</i>	996	23/29	none	2
ISHS1	<i>Halobacterium halobium</i>	1700	26/27	8	
ISH51	<i>Haloferax volcanii</i>	1371	15/16	3	

**Table 12-1** Properties of some *E. coli* insertion elements

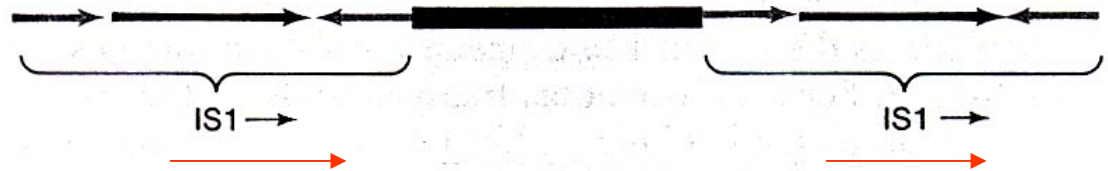
Element	Number of copies and location*	Number of base pairs
IS1	5–8 in chromosome	768
IS2	5 in chromosome; 1 in F	1327
IS3	5 in chromosome, 2 in F	1258
IS4	1 or 2 in chromosome	1426
IS5	Unknown	1195
Tn 1000 ( $\gamma\delta$ )	1 or more in chromosome; 1 in F	5980

# Struktura složených transpozonů

Inverted repeat



Direct repeat

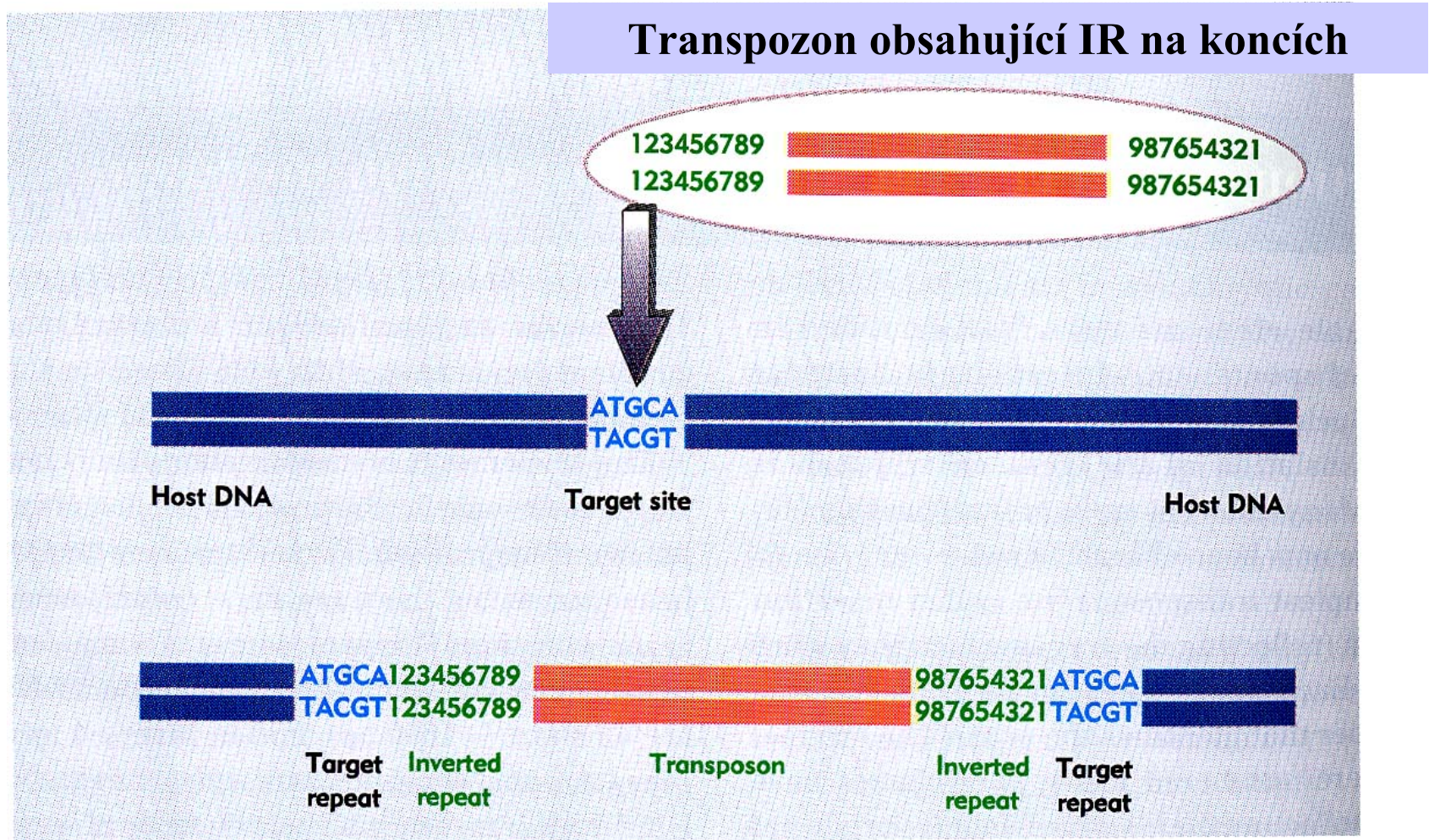


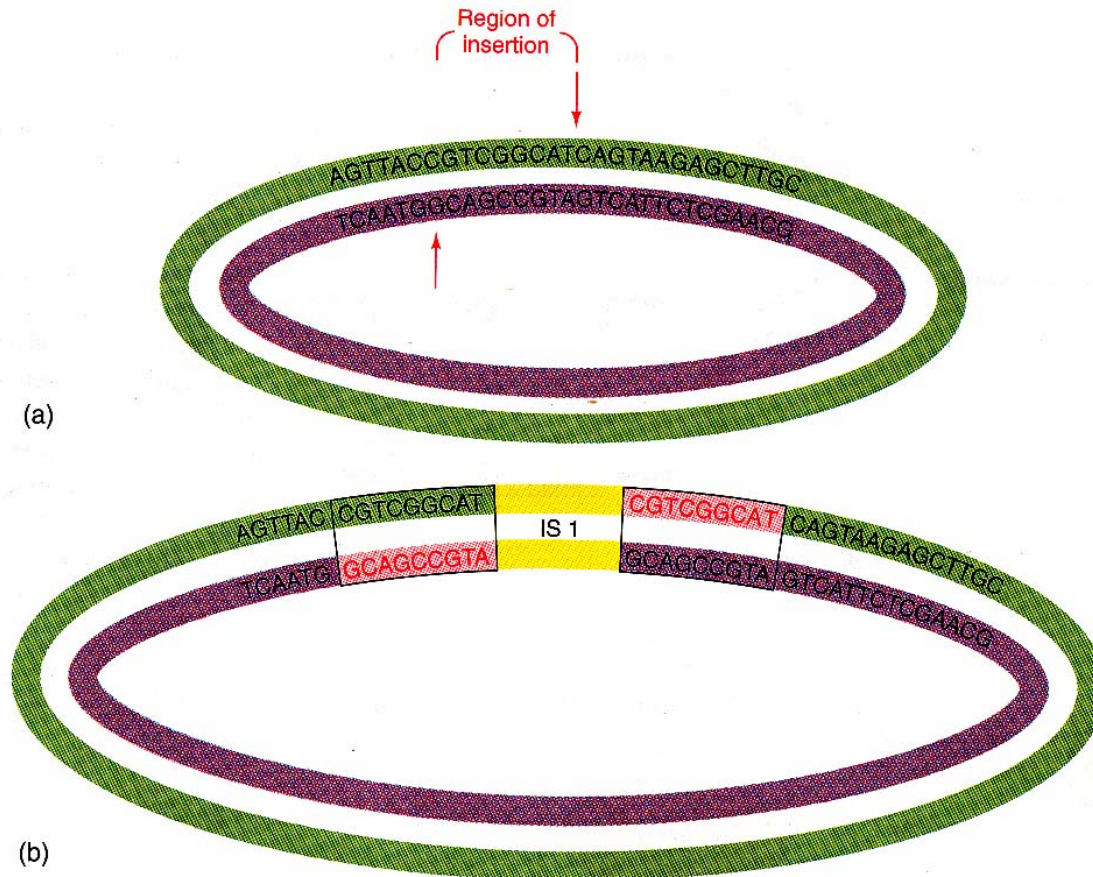
**Table 12-2** Properties of selected composite type I transposons of *E. coli*

Element	Genes carried*	Size in base pairs	Terminal IS element and size in base pairs	Relative directions of terminal IS Elements
Tn5	<i>kan</i>	5818	IS50 (1533)	Inverted
Tn9	<i>cam</i>	2638	IS1 (768)	Direct
Tn10	<i>tet</i>	9300	IS10 (1329)	Inverted
Tn204	<i>cam, fus</i>	2457	IS1 (768)	Direct
Tn903	<i>kan</i>	3094	IS903 (1057)	Inverted
Tn1681	<i>ent</i>	2088	IS1 (768)	Inverted

\**cam*, chloramphenicol; *ent*, enterotoxin; *fus*, fusidic acid; *kan*, kanamycin; *tet*, tetracycline.

# Vznik přímých repetíc v cílovém místě po začlenění transpozonu

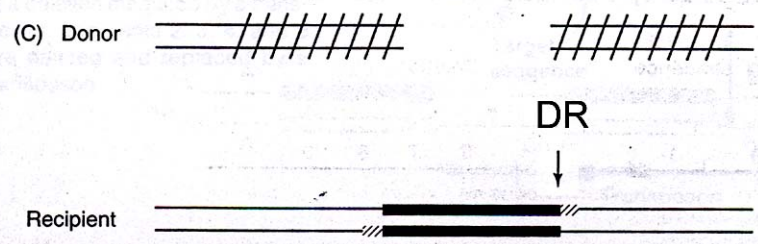
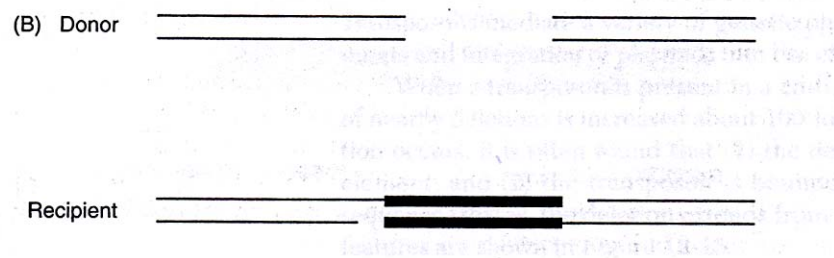
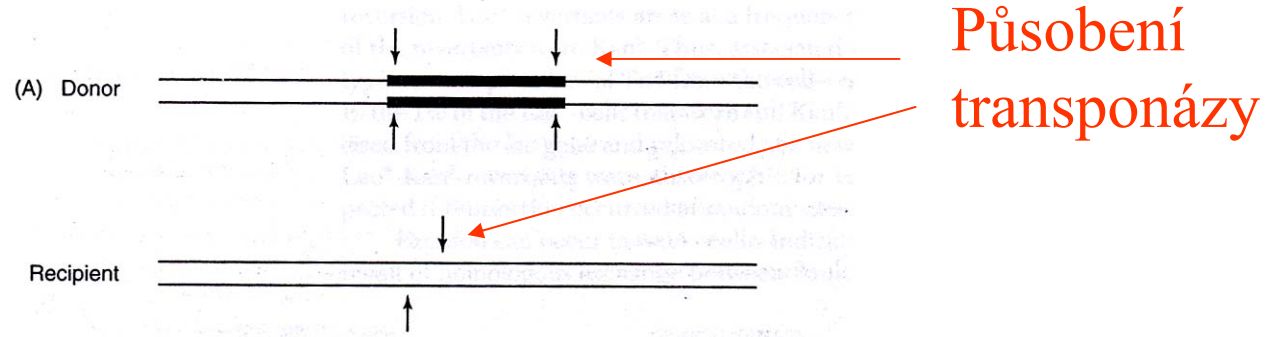




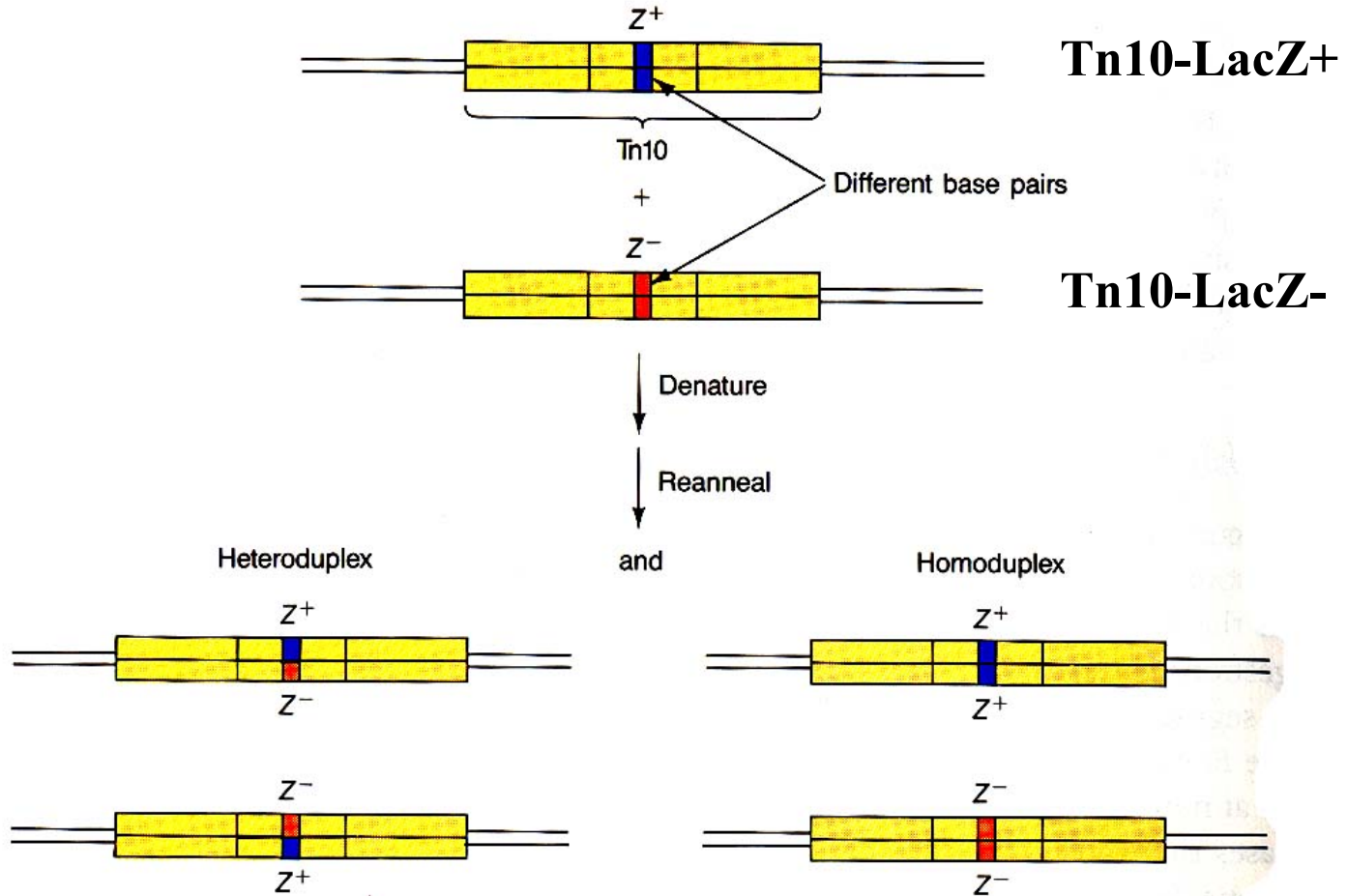
**Figure 21-22** Duplication of a short sequence of nucleotides in the recipient DNA is associated with the insertion of a transposable element; the two copies bracket the inserted element. Here the duplication that attends the insertion of IS1 is illustrated in a way that indicates how the duplication may come about. IS1 insertion causes a nine-nucleotide duplication. If the two strands of the recipient DNA are cleaved (arrow) at staggered sites that are nine nucleotides apart, as shown in (a), followed by insertion of IS1 between the resulting single-stranded ends, then the subsequent filling in of single strands on each side of the newly inserted element, indicated by red letters in (b), with the right complementary nucleotides could account for the duplicated sequences (boxes). (From S. N. Cohen and J. A. Shapiro, "Transposable Genetic Elements." Copyright © 1980 by Scientific American, Inc. All rights reserved.)



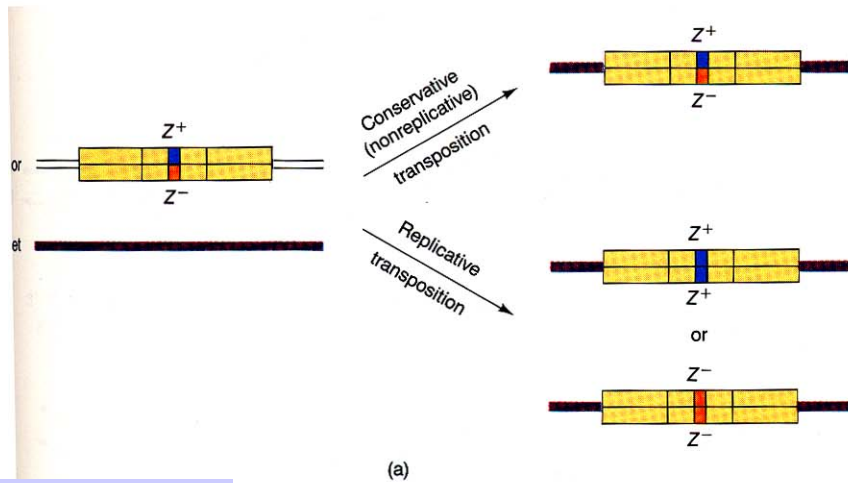
# Model nereplikativní (konzervativní) transpozice



# Důkaz konzervativní transpozice Tn10

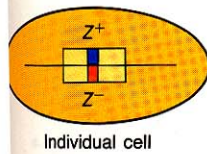


Vytváření směsi heteroduplexů a homoduplexů z transpozonů Tn10, které nesou alely genu lacZ lišící se toliko 3 bázemi. Tn10 je přítomen v transdukujících fágách lambda.

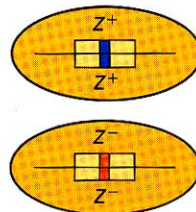


**Figure 21-20** Consequences of conservative and replicative transposition. (a) The heteroduplex of homoduplex nature of DNA (see Figure 21-19) is transposed into a target gene. If the starting DNA is heteroduplex, then the resulting DNA will still be heteroduplex only in a conservative, or nonreplicative, pathway. (b) Because the heteroduplex results in a transposed cell that maintains the heteroduplex nature of the DNA during conservative transposition, colonies will arise that are part  $Z^+$  and part  $Z^-$ . However, in a replicative pathway, transposition results in individual cells that are either all  $Z^+$  or all  $Z^-$ , and all the colonies will either be  $Z^+$  or  $Z^-$ .

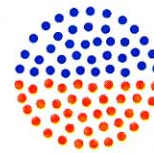
Konzervativní transpozice



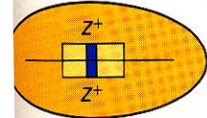
First division



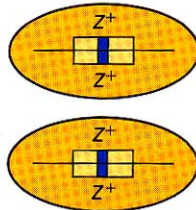
Growth of subsequent colony



Replikativní transpozice



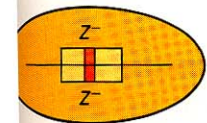
First division



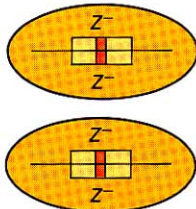
Colony



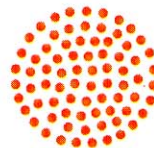
or



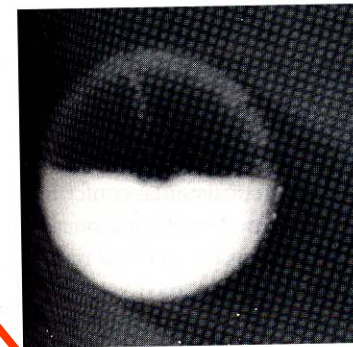
First division



Colony

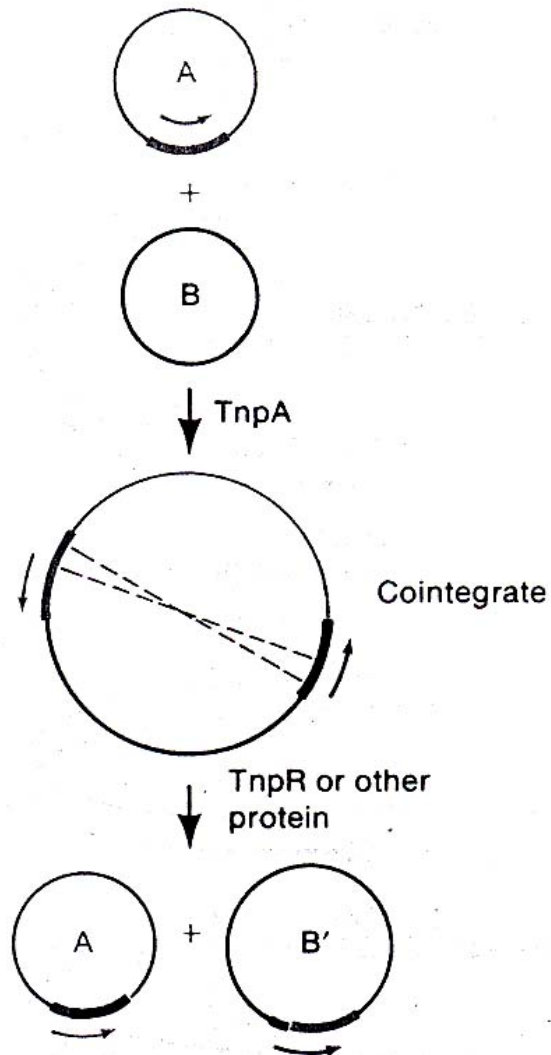


(b)

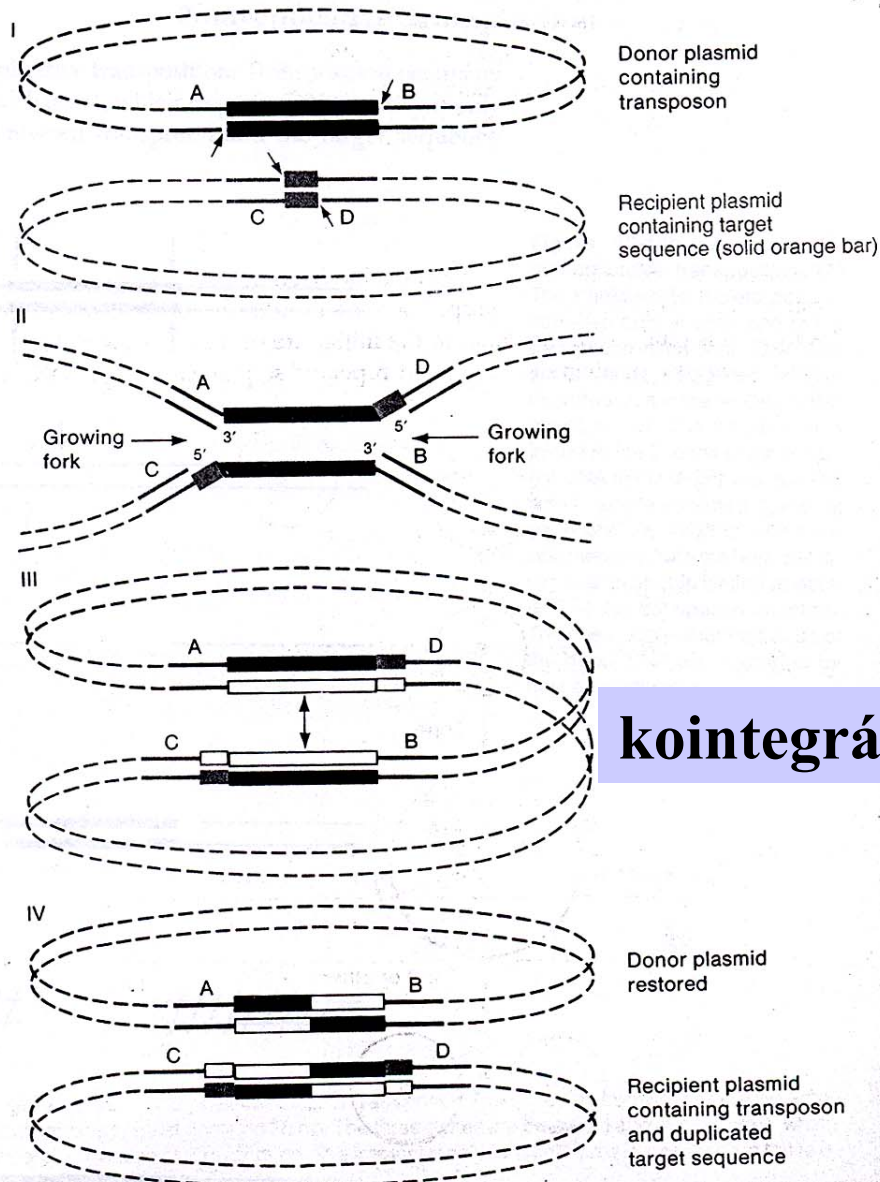


Většina případů

# Model transpozice prostřednictvím tvorby kointegrátu



# Model replikativní transpozice



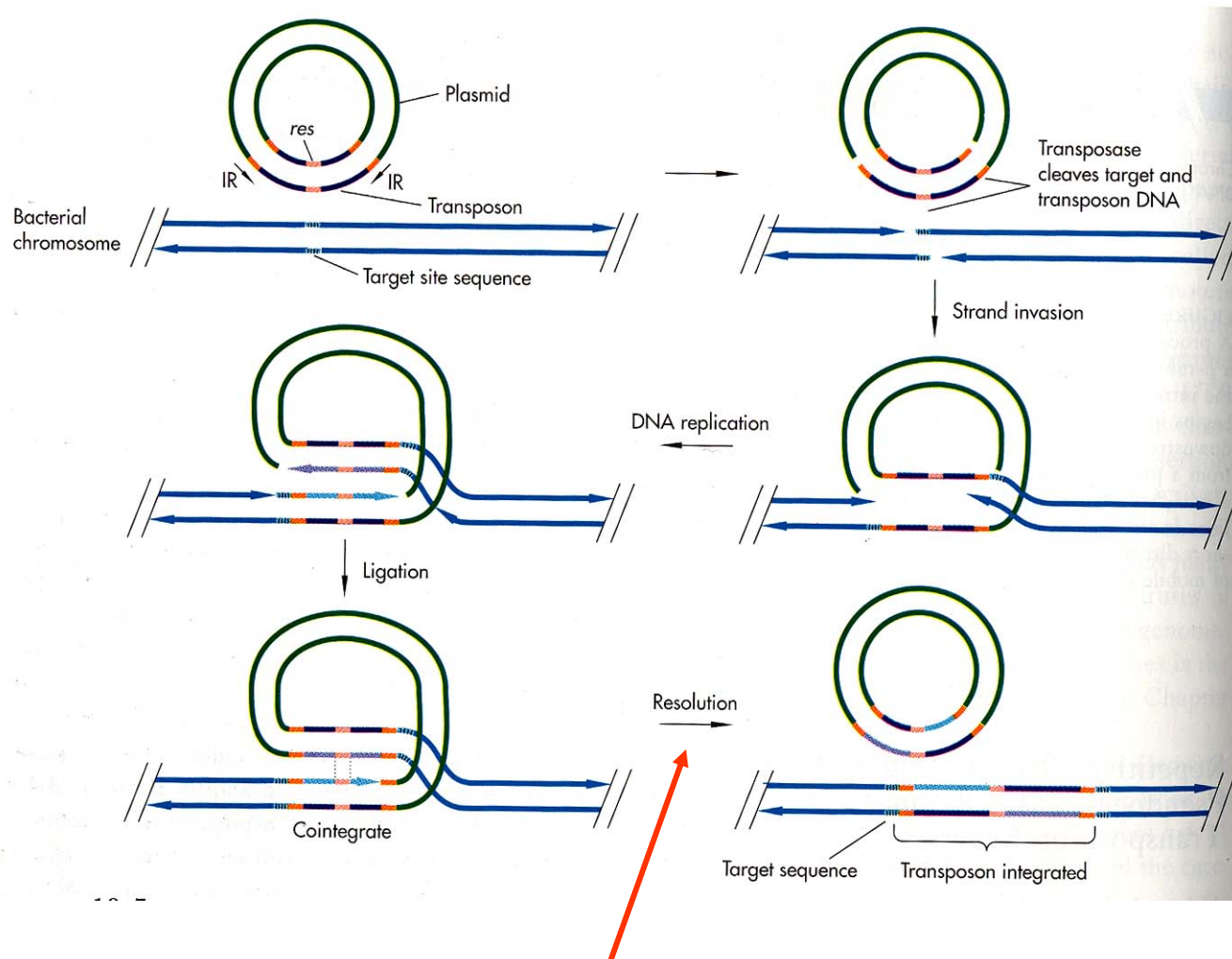
1. Vytvoření zlomů na DNA transponázou, replikace transpozonu a vznik kointegrátu

2. Rozklad kointegrátu:

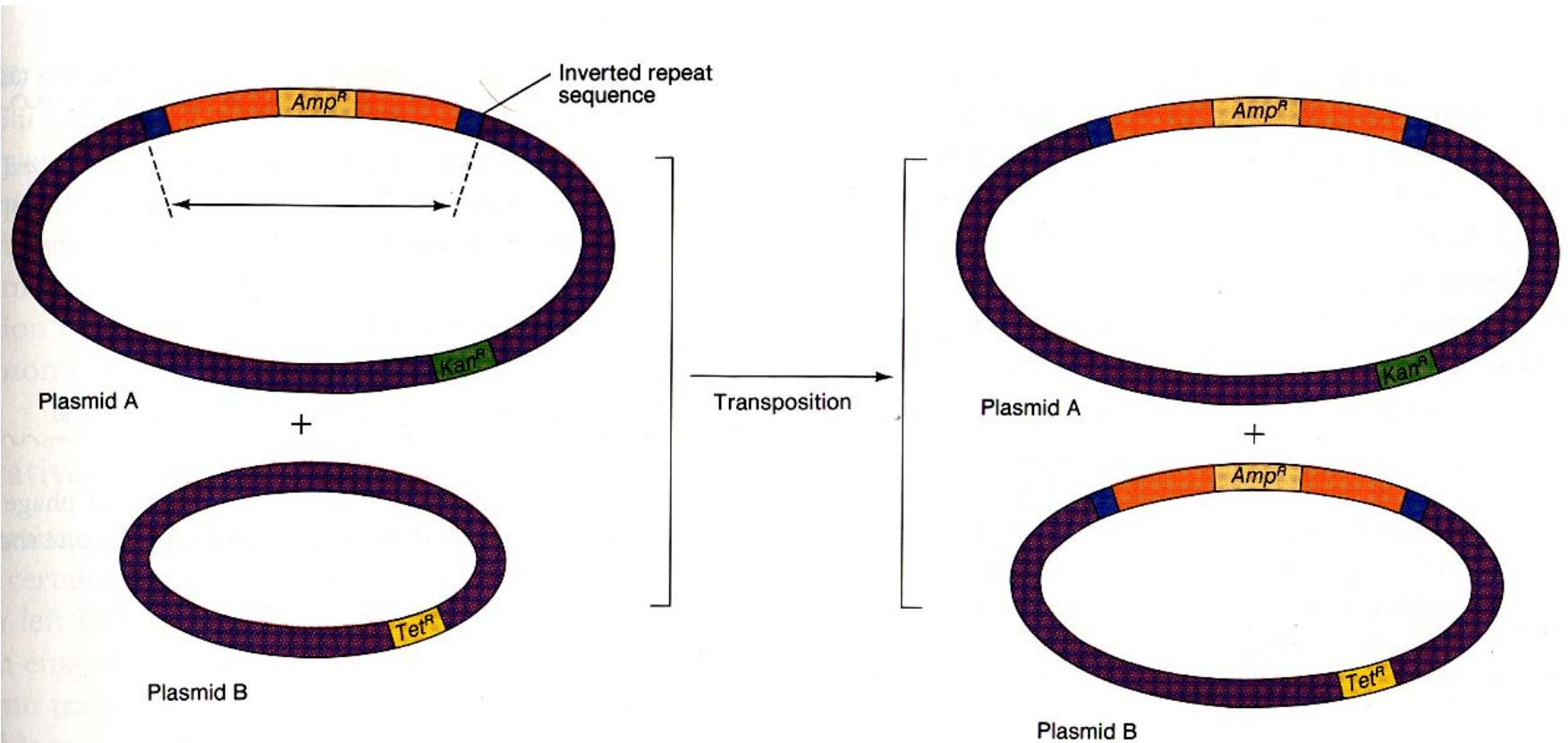
a) homologní rekombinací v  $recA^+$

b) místně specifickou rekombinací působením resolvázy

# Mechanismus replikativní transpozice

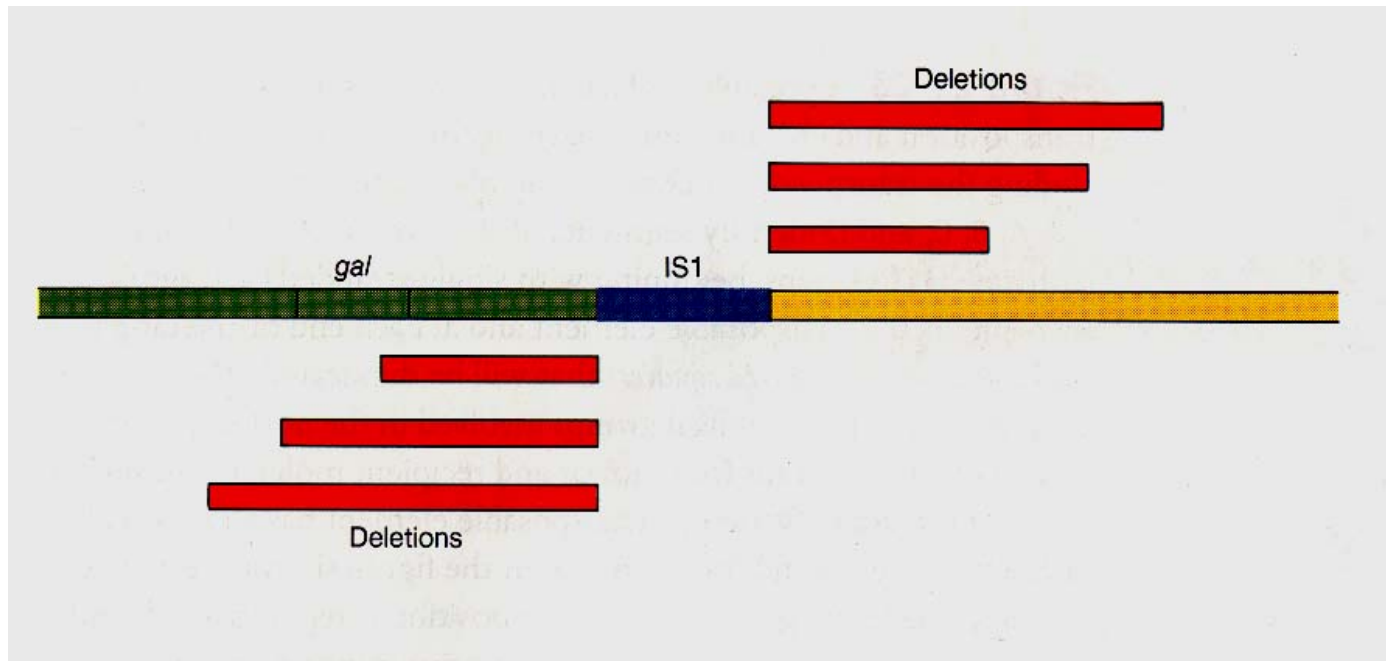


Rozklad kointegrátu zprostředkovaný místně-specifickým enzymem kódovaným transpozonem (resolváza u Tn3) nebo rekombinačním aparátem hostitelské buňky (RecA)

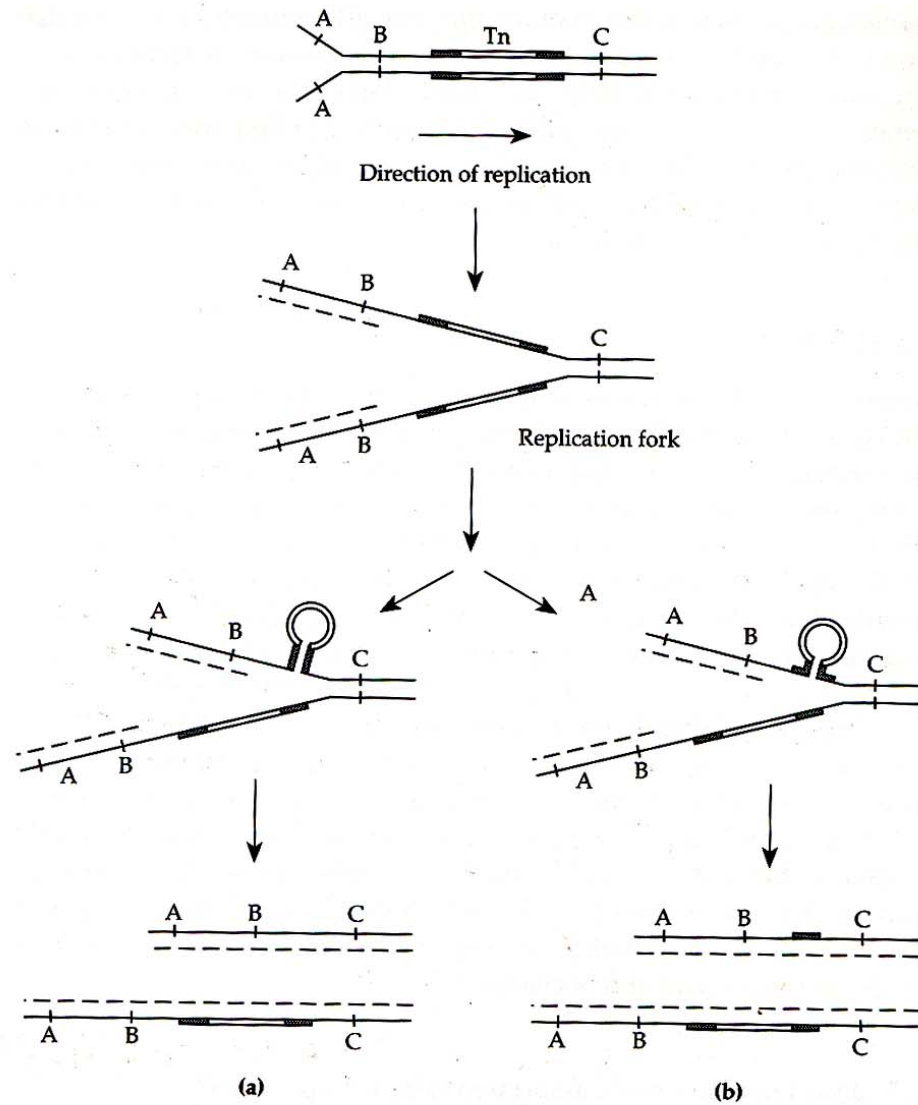


**Figure 21-11** Transposition of the transposon Tn3, which carries a gene conferring resistance to the antibiotic ampicillin ( $Amp^R$ ). It is shown as originally being part of plasmid A, which also includes a gene for resistance to kanamycin ( $Kan^R$ ). A plasmid B, which confers resistance to tetracycline ( $Tet^R$ ), acquires a copy of the transposon. The new plasmid B confers resistance to ampicillin and tetracycline. (From S. N. Cohen and J. A. Shapiro, "Transposable Genetic Elements." Copyright © 1980 by Scientific American, Inc. All rights reserved.)

Delece pozorované v místě začlenění IS1  
v lokusu *gal* *E. coli*

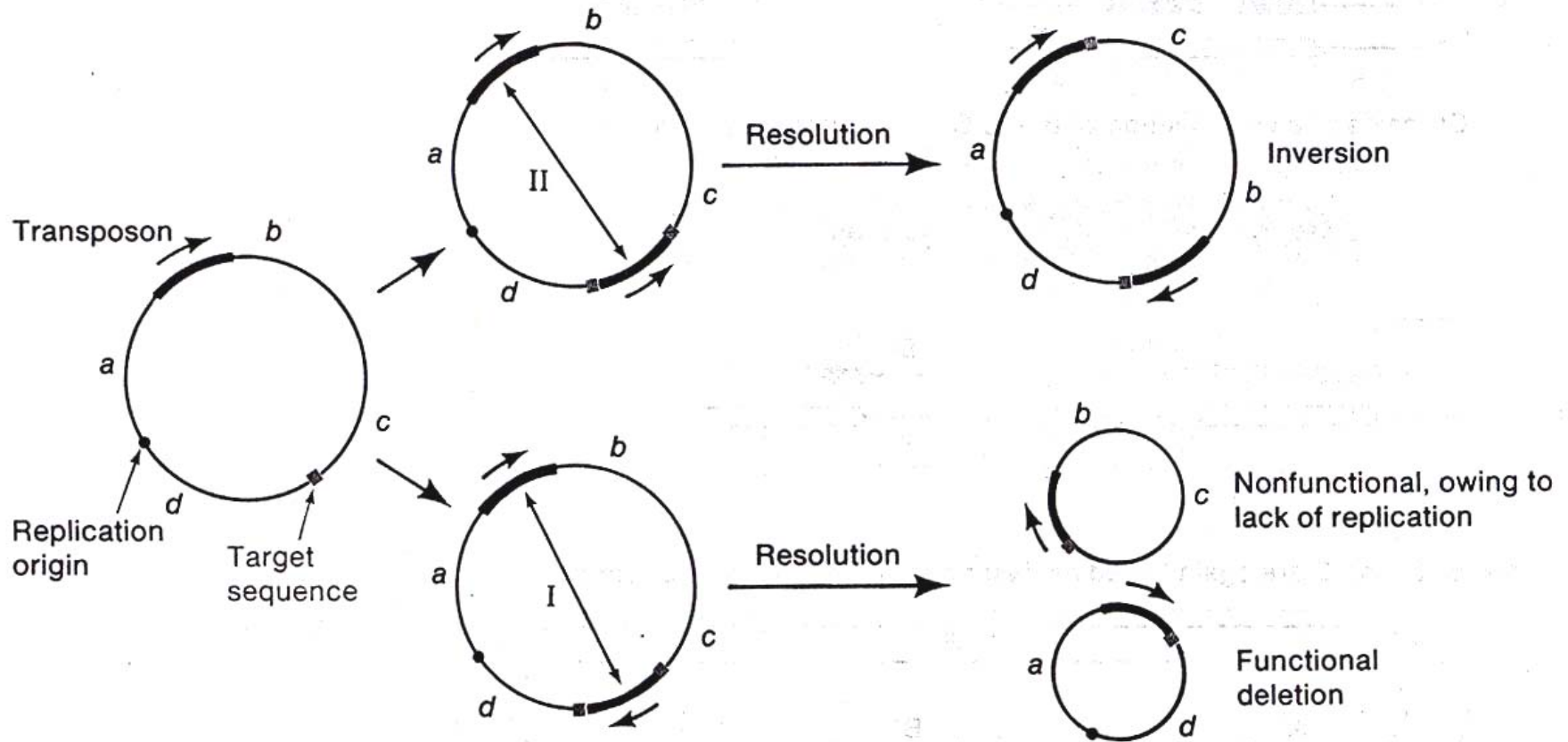






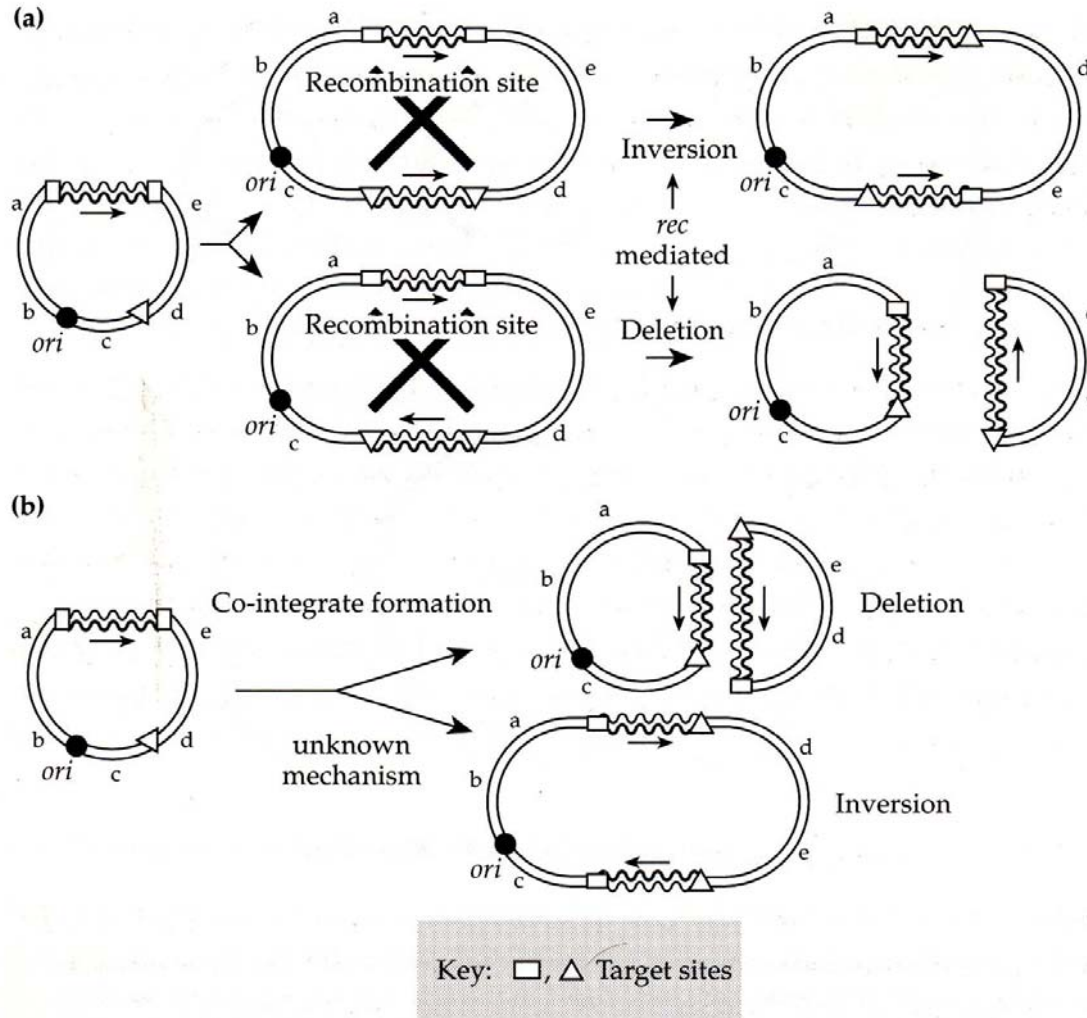
**Fig. 5.5** Excision of a transposable element during replication of host DNA. Excision takes place by formation of a stem-and-loop structure, during the transient single-strand form of the DNA at the replication fork. Excision is shown in one strand only (although it may occur in both strands), involving the whole element (precise excision, a) or part of it (imperfect excision, b).

# Vznik delecí a inverzí po transpozici



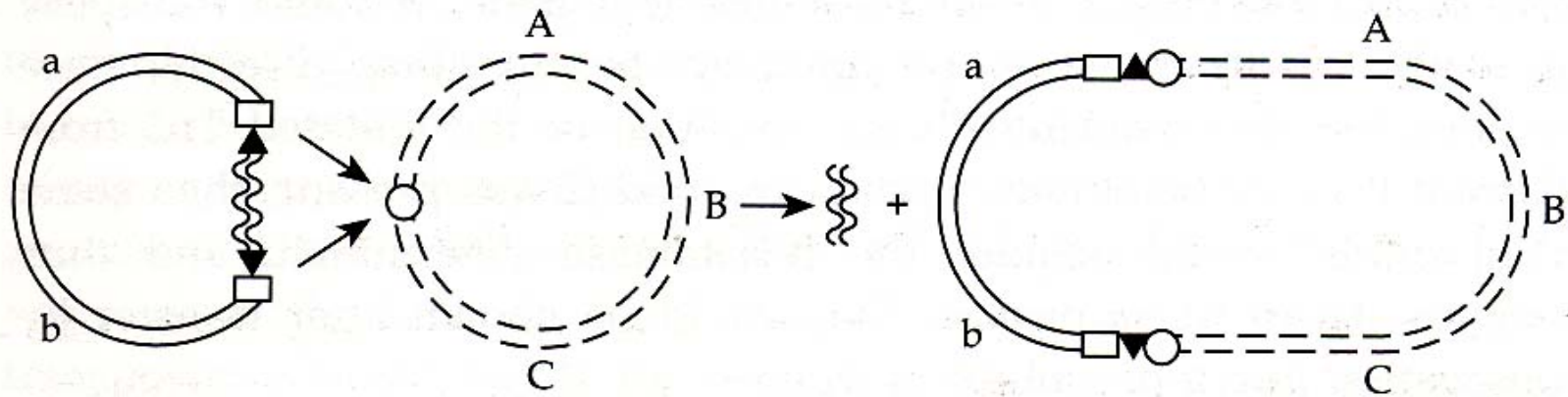
**Figure 12-17.** Model for production of genetic deletions and inversions. The transposon DNA is inserted into the target sequence in orientation I or II. The circle could be a plasmid or the chromosome. Resolution by a site-specific strand

exchange at sites indicated by double-headed arrows or by exchange in homologous sequences yields a deletion from I and an inversion from II.



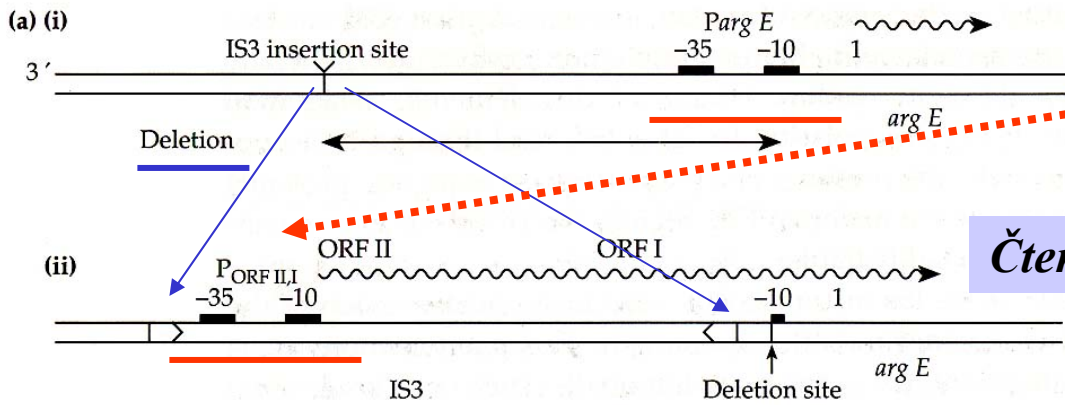
**Fig. 5.7** DNA rearrangements involving transposon replication in the same replicon. (a) Rearrangements may result from a *rec*-dependent recombination between the two copies of the same transposon in the same replicon. This may happen whatever the process which brought the two transposon copies into the replicon. (b) Rearrangements may result from the formation of an intra-replicon co-integrate.

# Inverzní transpozice



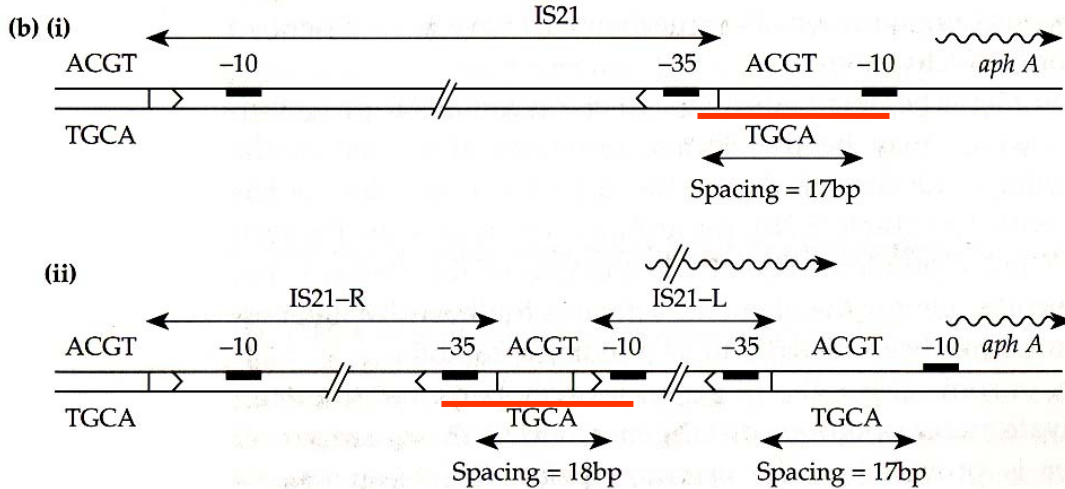
Key: □, ○ Target sites; ▲ IR

**Fig. 5.8** Inverse transposition. The replicon carrying the mobile element is inserted into the second replicon instead of the mobile element.



IS3 působí jako mobilní promotor

Čtení bez zastávky



Vznik kompletního promotoru kombinací promotorových sekvencí -35 a -10

Vznik funkčního promotoru inzercí dvou IS21 (R a L)

**Fig. 5.4** Role of insertion sequences as mobile promoters. (a) IS3 as a mobile promoter for *argE* in *E. coli* (i). IS3 can insert upstream of *argE*. A deletion, as indicated, removes the -35 part of the *argE* promoter (ii). Transcription of *argE* is still performed, but starts from a promoter site inside IS3. (b) IS21 as a mobile -35 promoter region. (i) Insertion of one copy of IS21 in plasmid RP4. Its -35 region can complete the -10 region of *aphA* ( $Km^r$ ), allowing its transcription. (Standard spacing between the two regions for the *E. coli*  $\sigma$  factor of RNA polymerase: 16–18 base pairs). The IS21 is not transcribed. (ii) Two tandem direct copies of IS21 into plasmid R6845. Formation of a complete and functional promoter using the -35 region of IS21-R and the -10 region of IS21-L allows transcription of IS21. Transcription of *aphA* is still possible.

## **Charakteristické rysy transpozice**

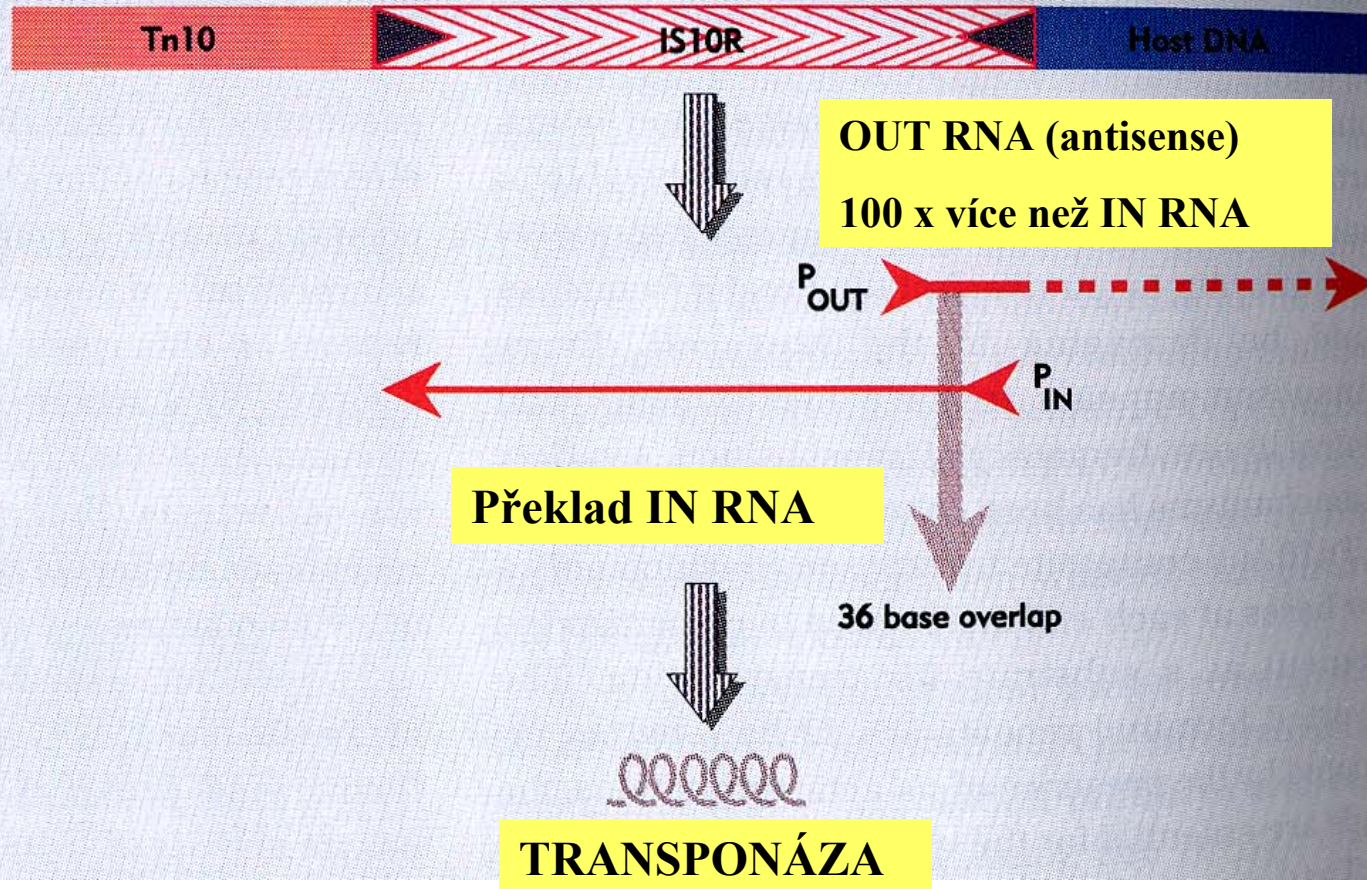
- frekvence transpozice  $10^{-4}$  až  $10^{-7}$  cílový replikon**
- specifita začlenění je pro různé elementy různá, liší se pro různé replikony (chromozom x plazmidy)**
- mutace v genu pro transponázu ovlivňuje specifitu místa začlenění**
- transpozice vyžaduje neporušenost koncových IR**
- u Tn3 je známa imunita k transpozici podmíněná přítomností sekvencemi IR**

## **Frekvence transpozice**

- **transponáza je v buňkách přítomna ve velmi nízkých koncentracích (0,15 molekuly na buňku)**
- **aktivita transponázy se obtížně detekuje**
- **preference působení transponázy v cis: působí přednostně na DNA, z níž byla transkribována**
- **po uvolnění z DNA dochází k rychlému rozkladu transponázy**

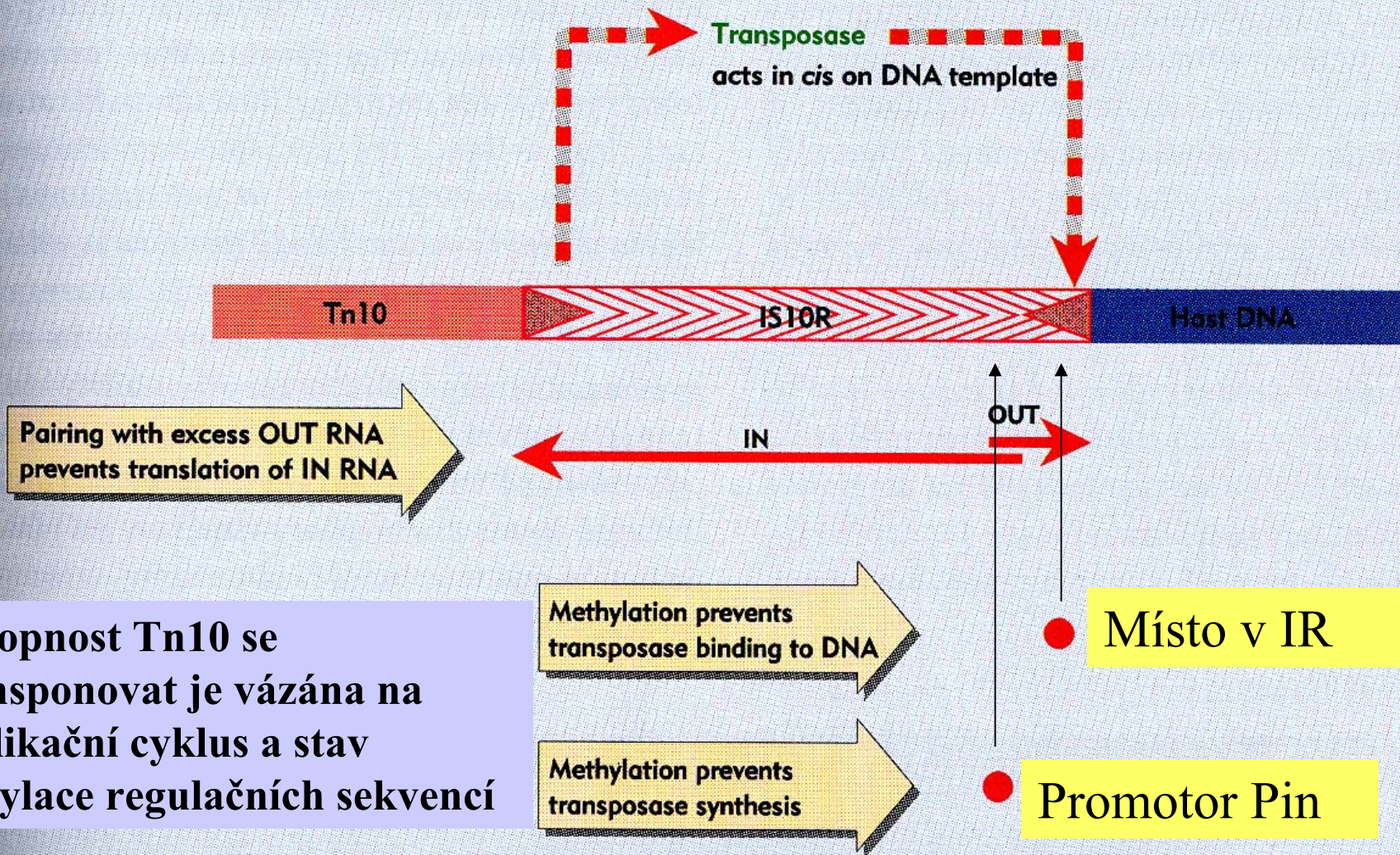
# Regulace transpozice Tn10

Two promoters in opposite orientation lie near the outside boundary of IS10R. The strong promoter  $P_{OUT}$  sponsors transcription that may continue into the flanking host DNA. The weaker promoter  $P_{IN}$  starts transcription of an RNA with a coding region that extends the length of IS10R and represents a 47,000 dalton protein. The "OUT" and "IN" transcripts have a 40-base overlap.



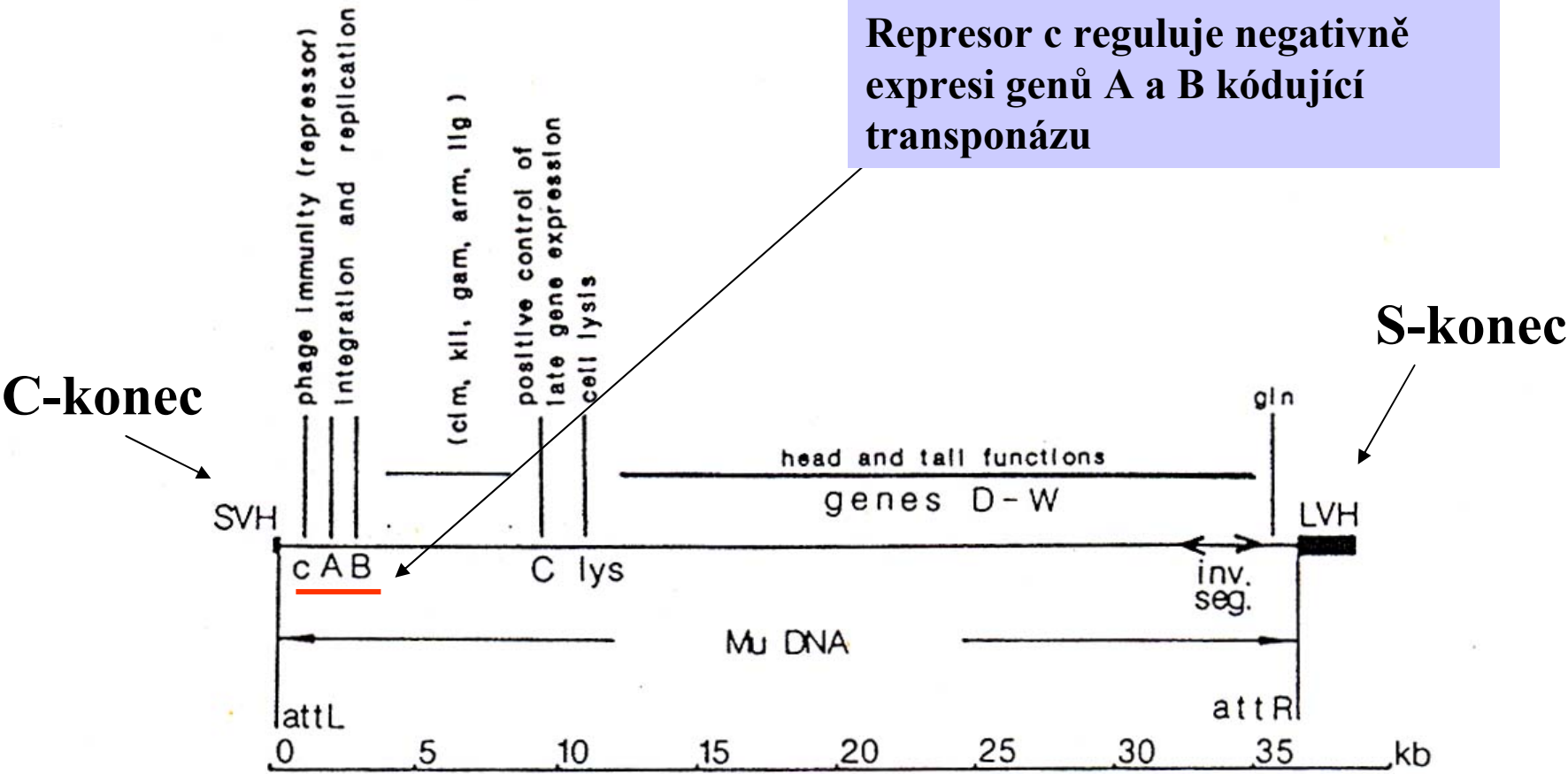


Several mechanisms restrain the frequency of Tn10 transposition, by affecting either the synthesis or function of transposase protein. Transposition of an individual transposon is restricted by methylation to occur only after replication. In multicopy situations, *cis*-preference restricts the choice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



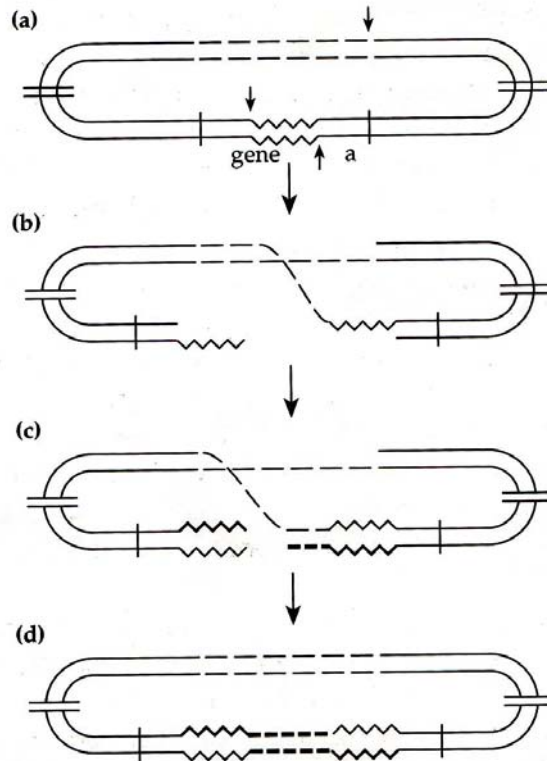
Schopnost Tn10 se transponovat je vázána na replikační cyklus a stav metylace regulačních sekvencí

# Genom bakteriofága Mu (dsDNA, 37 kb)



A protein se váže ke koncům genomu Mu, což stimuluje B protein. Vazba probíhá na 22 bp sekvencích. Vzniklý komplex = transpososom. Na 3' koncích vznikají zlomy, stejně je zlomena DNA v hostitelském chromozomu.

## Replikace fága Mu a jeho mutagenní působení



Key:   
 - - - - Original Mu DNA;   
 - - - - replicated Mu DNA;   
 ———— host DNA;   
 ~~~~~ original target DNA;   
 ~~~~~ replicated target DNA;   
 ↓ or ↑ single-stranded nick

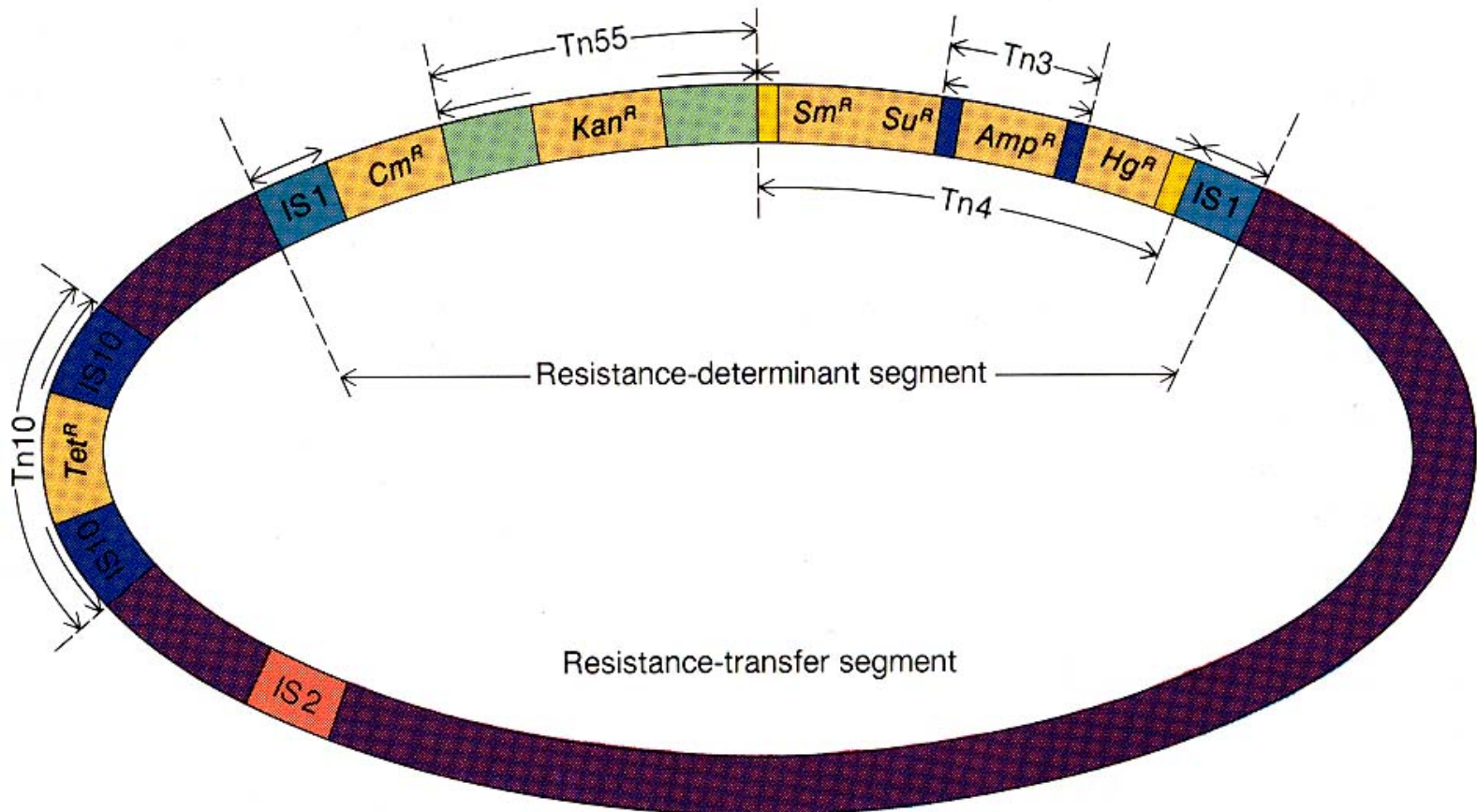
Po infekci buněk se fág začlení do genomu zřejmě konzervativní transpozicí, během lytického cyklu se množí replikativní transpozicí.

V obou případech jsou místa začleňování profága zcela náhodná

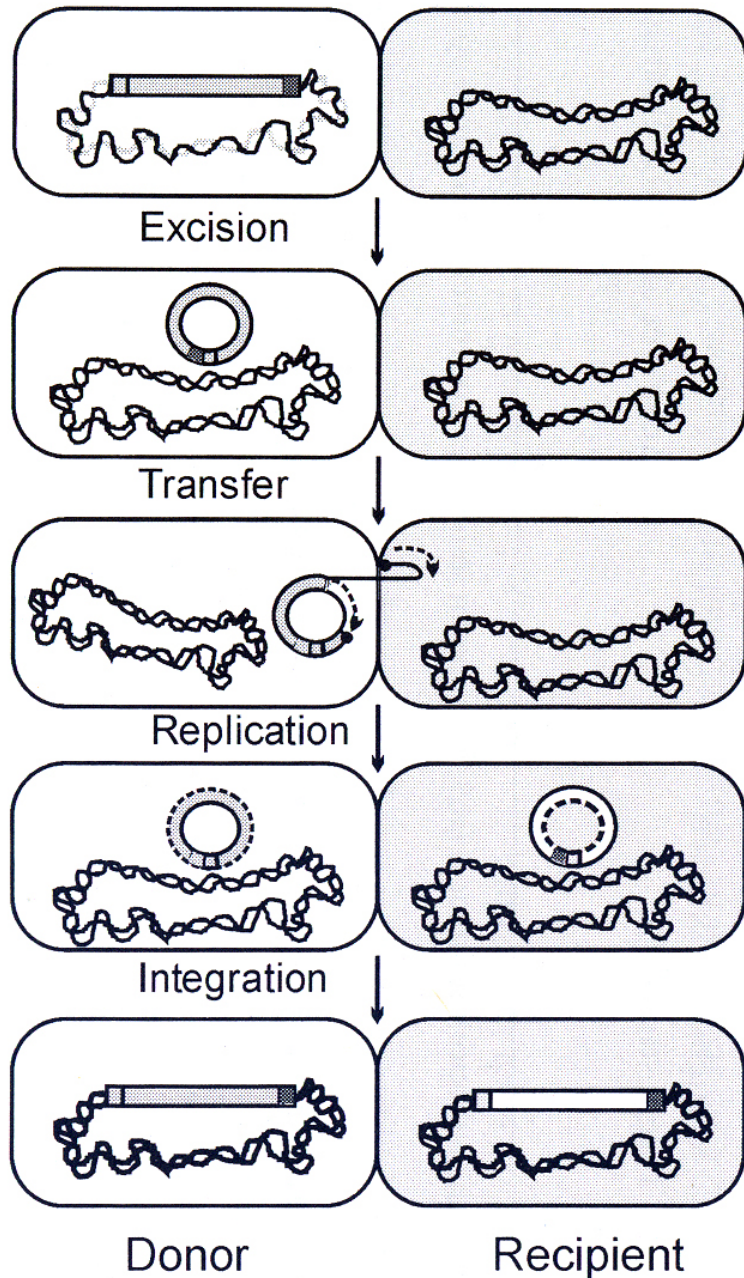
- a) vznik ss zlomů na koncích profága Mu a v cílovém místě
- b) volný konec profága Mu se spojí s řetězcem cílového místa
- c) doplnění jednořetězců replikací
- d) spojení jednořetězcových zlomů vede k duplikaci Mu

# Úloha transpozonů při evoluci R-plazmidů

- každý transpozon může být přenášen nezávisle



# Průběh přenosu konjugativních transpozonů

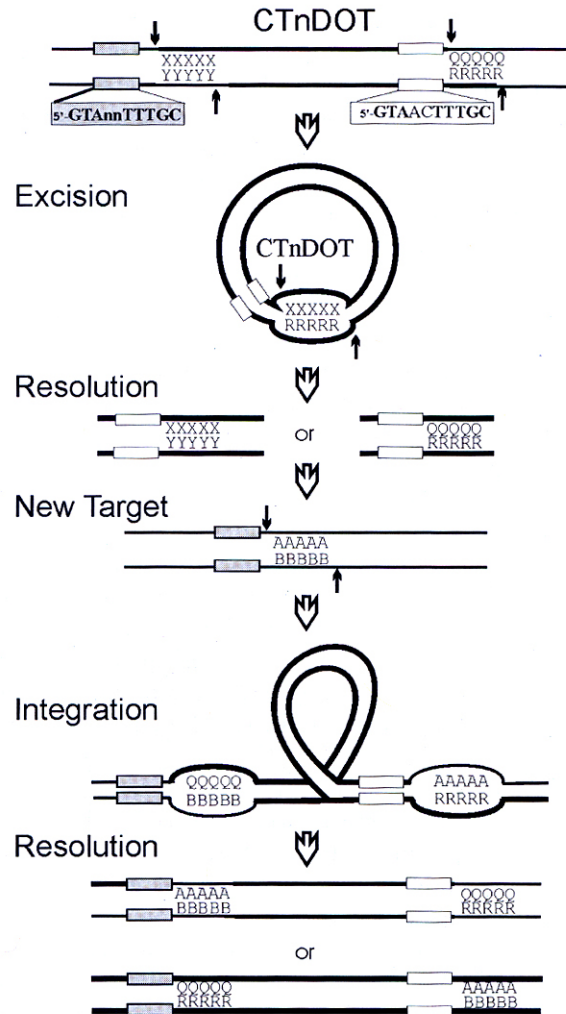
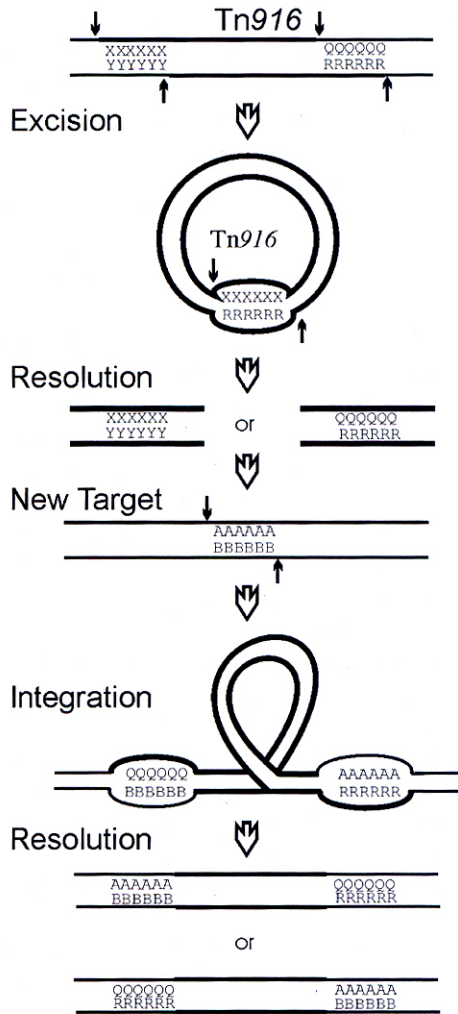


Transpozon začleněný do chromozomu se vyčlení a vytvoří kružnicový intermediát.

Do recipientní buňky se přenáší kopie jednoho z řetězců prostřednictvím multiproteinového párovacího aparátu spojujícího obě buňky.

Přenesená jednořetězcová kopie se změní na dvouřetězcovou formu, která se začlení do chromozomu recipientní buňky

# Model excize a integrace konjugativních transpozonů Tn916 a CTnDOT

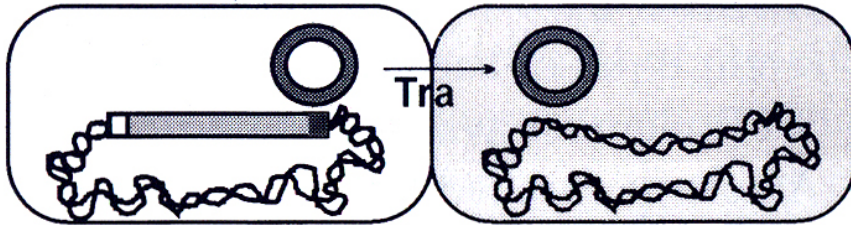


**Spojovací chromozomové sekvence (XXX/YYYY nebo QQQ/RRR jsou původně vzájemně komplementární).**

**Šipky naznačují místa vzniku posunutých zlomů před excizí nebo před integrací**

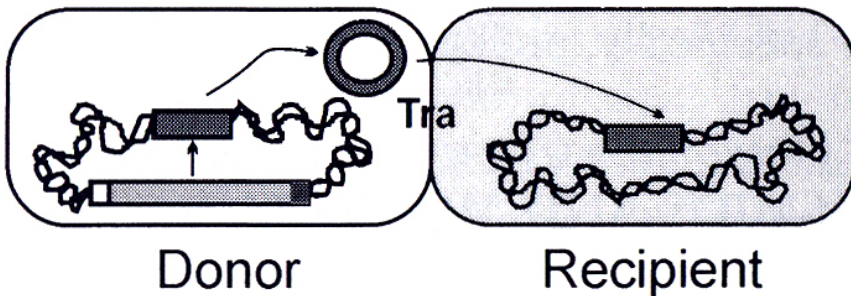
# Mobilizace genetických elementů konjugativními transpozony (působení *in trans*)

Mobilization of co-resident plasmids *in trans*



Mobilizovatelný rezidentní plazmid nese geny kódující proteiny vytvářející zlom v jeho DNA, CTn zajišťuje vytvoření multiproteinového párovacího aparátu

Excision and mobilization of MTns



CTn navozuje excizi rezidentního mobilizovatelného transpozonu (MTn) - CTn poskytuje proteiny pro excizi a cirkularizaci a pro přenos ss-formy MTn do recipienta, kde se MTn již samostatně integruje do chromozomu