

## 411-7 2009 Transposons

PP:

### IV. Transposons

- A. Classes of transposons
- B. Mechanisms-
  - 1.replicative (Tn3)
  - 2.conservative (Tn10)
- C. Genetic Consequences
- D. Regulation

OH:

	RecA+	RecA-
Survival – UV	(100%)	(100%)
Survival + UV	50%	0.1%
Naladixic resistance–UV	10 <sup>-7</sup>	10 <sup>-7</sup>
Naladixic resistance + UV	10 <sup>-5</sup> (of survivors!)	10 <sup>-7</sup> (of survivors!)

Why difference in survival?- no repair of damage what can't be replicated

Why difference in proportion NalR mutants?- no error-prone repair mutations

### I. Fig. 1- Classes of transposons

PP-leave up; mention arrows represent orientation of end repeat sequences “inverted repeats”

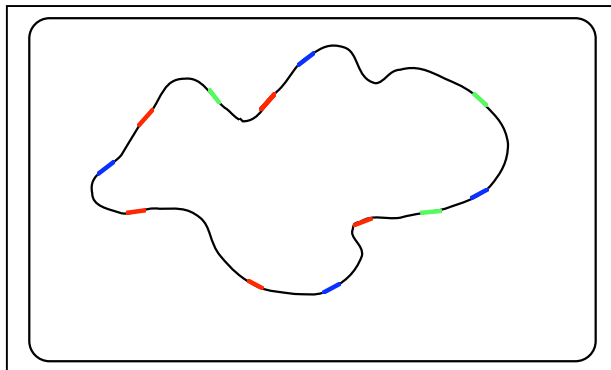
transposons are genetic elements able to move from one place in the genome to another essentially at random- "transposition"

Transposon always inserted in something else- doesn't have own replication origin

Insertion Sequences (IS elements)- 10 types in E. coli: IS1, IS2, ...

1-11 copies of each per xsome (45 total identified= 1% of DNA!)

OH:



In some organisms, number of ISs is much higher. Seen in highly virulent pathogens, *Y. pestis* and plague- 15 in pseudo TB, 10X that many in *Yp*. Rate of transposition to a new site quite low, typically  $10^{-8}$  per cell generation/tn (although rate controlled in complicated ways we'll discuss next time)

tnp=transposase (no other gene needed specifically for transposition)

terminal inverted repeats- typically about 20 bp long- where transposase binds- only acted on by same family- e.g., IS1 transposase won't bind to IS2 ends, only IS2 tase will

Discovered by ability to generate spontaneous mutations- remember *lacI* studies -typically 1-5% of total loss of function

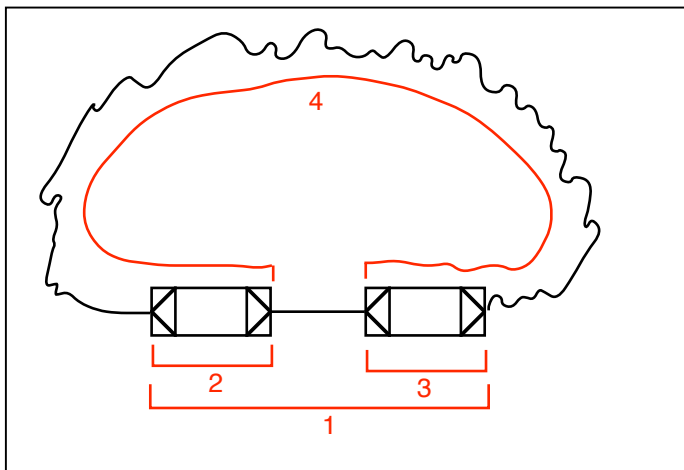
### Composite transposons- Tn10 and Tn5

IS elements flanking central gene- often a gene encoding drug resistance (*tet* in Tn10, *Kan* in Tn5)

no naturally occurring composite transposons in *E. coli* K12- come from other places- drug resistance plasmids and ISs usually not the same as find in *E. coli* xsome

Both IS elements have inverted repeats and can transpose independently if transposase made-

**Draw on OH- return to at end:  
Point out -like 4 transposons in one**



Draw left ends of 2 and 3- say Won't transpose- why? Ends not inverted

Noncomposite transposons

also often carry drugR, no IS component

-Tn3

-phage Mu- transposon that is virus (pp 390-391 3rd)

## II. Mechanism

-Most fundamental question: conservative or replicative

PP

Replicative- half-open, half-filled

conservative- donor damaged and might be lost (=”cut-and-paste”)

Remember: transposon always inserted in something else- doesn't have own replication origin

Even though may draw a "free" transposon- always understood that embedded in other DNA that is providing replication functions

First studies providing insight into mechanism- Tn3 (noncomposite tn)-showed its mechanism is replicative

PP-These studies identified three genes encoding proteins and three sites

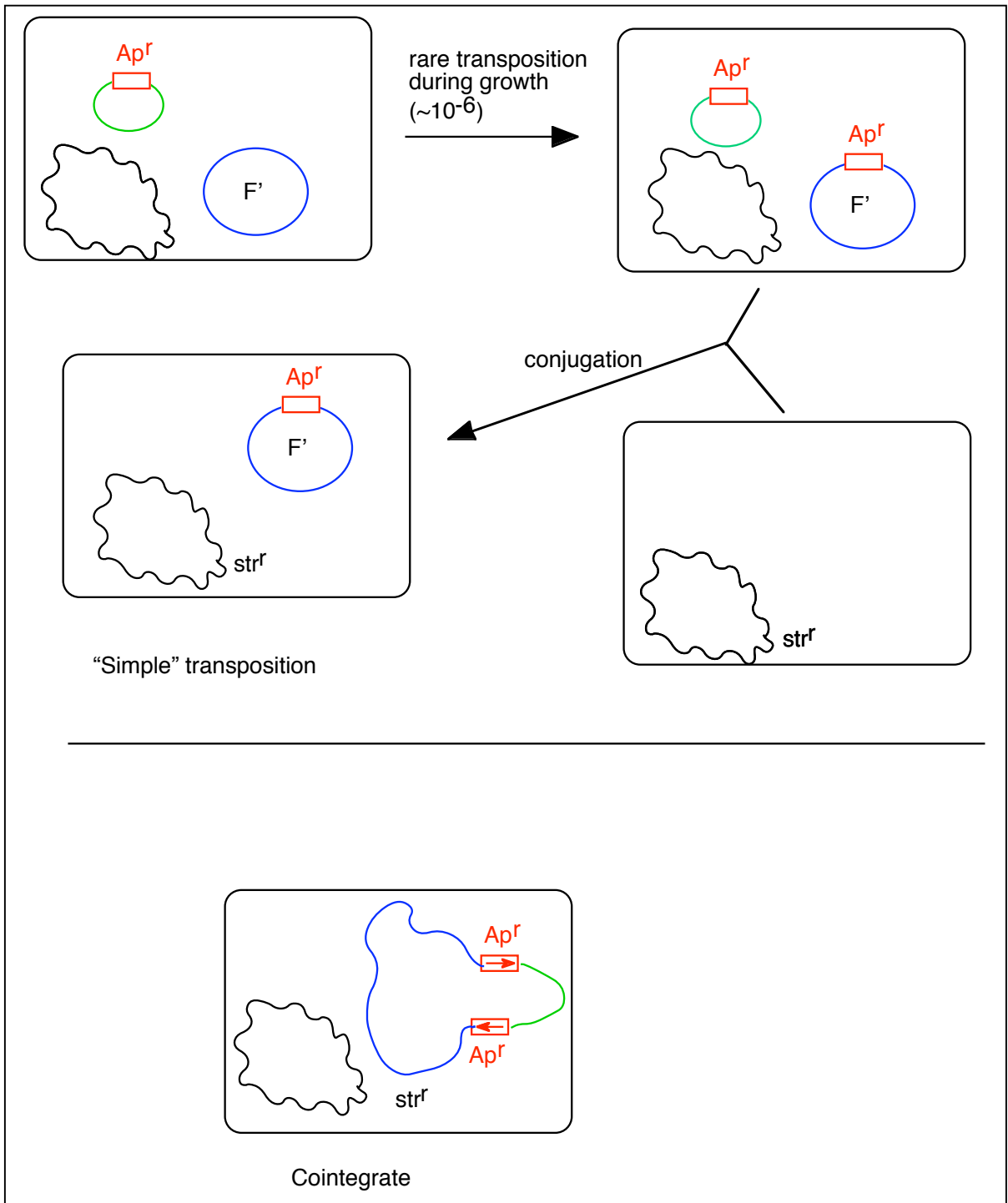
### Genetic “dissection” of Tn3

1. Generate many small deletion mutations randomly in Tn3
2. Examine phenotypes
  - a. Ampicillin resistance
  - b. Transposition

Think back-originally, just a blank piece of DNA that carried ampicillin resistance and could transpose into new sites- Make small insertions and deletions (few bps) at different sites throughout Tn3-

–first examine ampR and then ability to transpose of different deletion mutants

Mating-out assay for transposition (draw) and Tn negative mutants (Fig 9.11 and 9.12 of 3rd)



Surprise - cointegrates (draw)

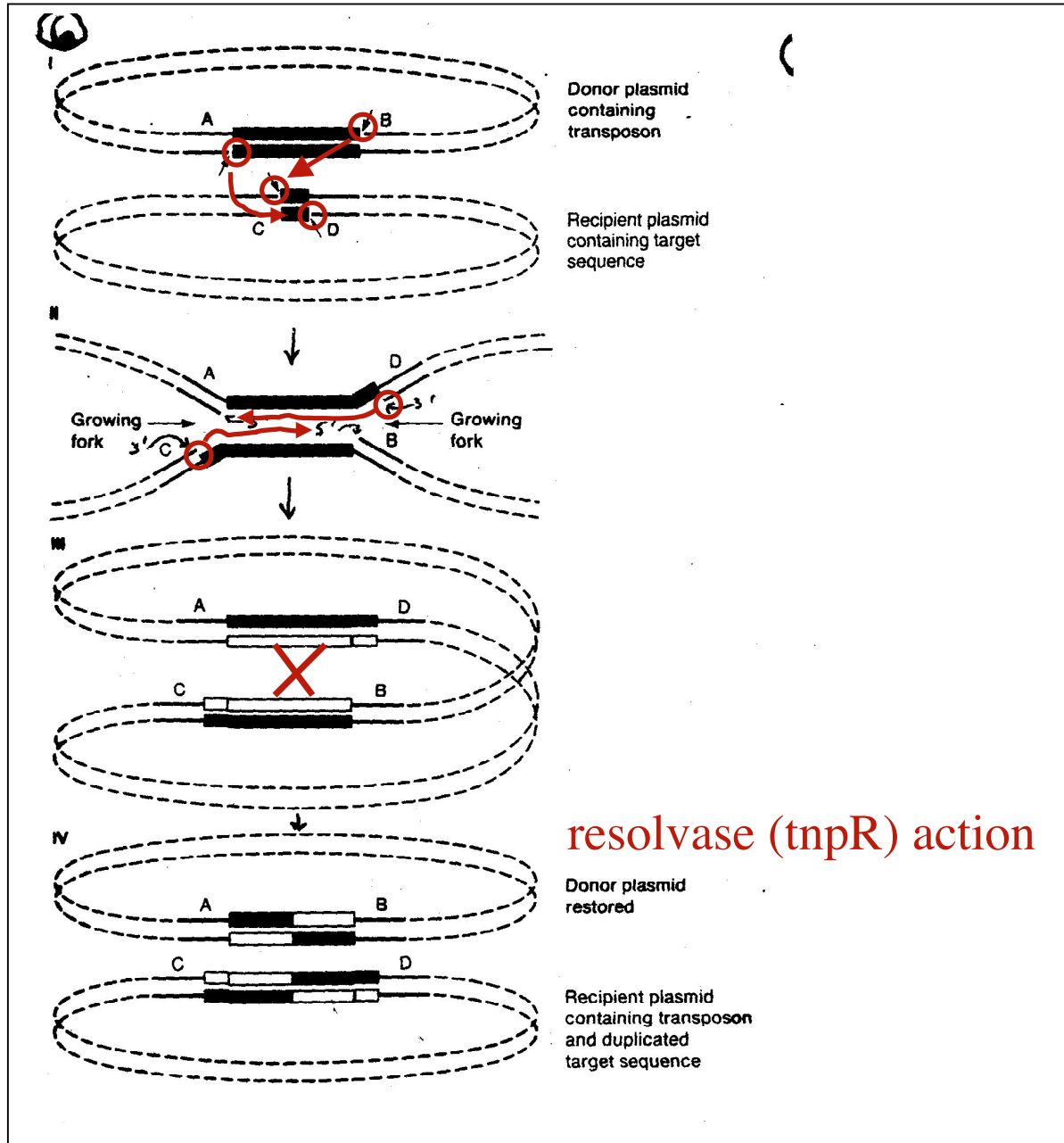
Cointegrates-consist of both plasmids joined together

Complementation- test of whether mutations affect proteins (or RNAs) or DNA sites

where proteins act- remember lac constitutives- I- are complemented; O- are not

overall model of Tn3

Shapiro model (Fig. 9.13 in text S/C3)



Tn10

Experiments with Tn10 led to a different picture

never saw cointegrates

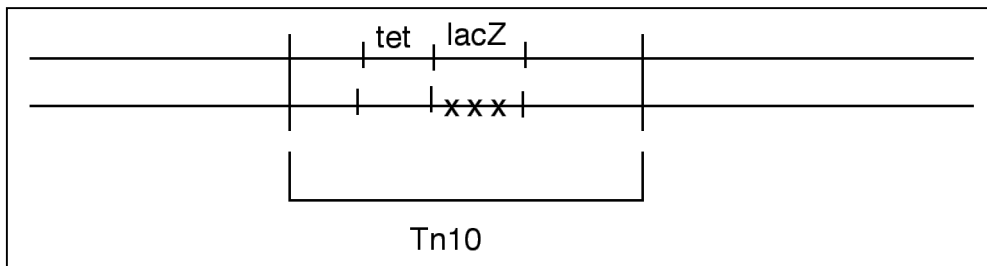
-go back to conservative vs replicative

-conservative tn??

Very elegant experiment to test (Fig 9.15, 9.16 of S/C3)

**Draw on OH:**

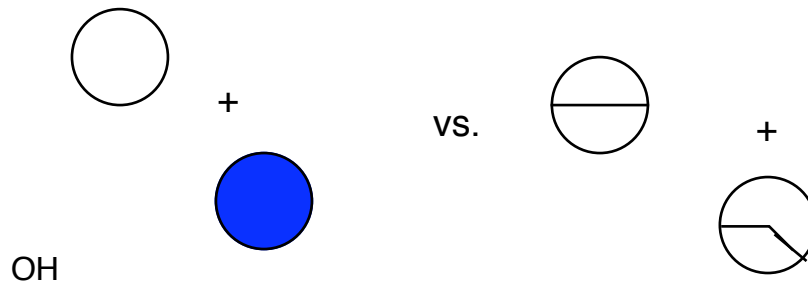
1. a phage lambda DNA, but not just any lambda- replication minus, integration-minus; Tn10, but not just any Tn10- has lacZ in Tn10
2. lacZ heteroduplex of wt and triple mutant



How destabilizing would expect the three mismatches would be- 3/50,000 bps?

Because replication-, integration- injected DNA sits there- Tn10 can transpose into chromosome and cells become tetracycline<sup>R</sup>

PP- compare replicative and conservative tn expectations



Find lots of sectored colonies (1/3)

Might have expected mismatch repair to be important, but didn't- why not? Because only an issue if DNA newly replicated. (But what if repaired double methylated DNA randomly instead of not at all?)

So conclusion: OH: Tn10 transposition is conservative

WIERD: One tn (Tn3) replicative  
Other tn (Tn10) conservative

How can there be two mechanisms of TN??

Gets weirder-

Phage Mu- combines properties of a phage and a transposon- pp 390-391

Discovered as phage able to make lysogens, but lysogeny due to random insertion into xsome

OH showing Mu lysogeny and lytic growth

So one particular Tn can be either replicative or conservative (both use A + B)

Suggests that conservative and replicative transposition may be related- using one of the same proteins after all

How can this be?

PP- first go back to earlier diagram

PP- different representation of same thing

C. Genetic consequences - Transposon insertion normally inactivates a gene it inserts into- but that's hardly all

Bizarre and unfortunate things happen to a gene unlucky enough to be in the neighborhood of an active transposon. Such genes mutate at very high frequencies- usually as part of chromosome rearrangements. Draw out gene with Tn nearby. Show on OH as well as PP (gene X<sup>+</sup> → X<sup>-</sup>)

1. Composite transposon (e.g., Tn10) -mediated rearrangements

1. Simple insertions
2. Cointegrate formation
3. Adjacent deletion
4. Adjacent inversion

Want to go through these rearrangements take place

2. Remember- composite transposon like Tn10 is four transposons in one- show orientations of ISs

3. Major Board action- see HO for transposon-mediated rearrangements- conservative and replicative

- a. two molecules
- b. one molecule



Point out that linear molecules lost due to damage

