

# Chapter 9: Integrons and Mobile Gene Cassettes

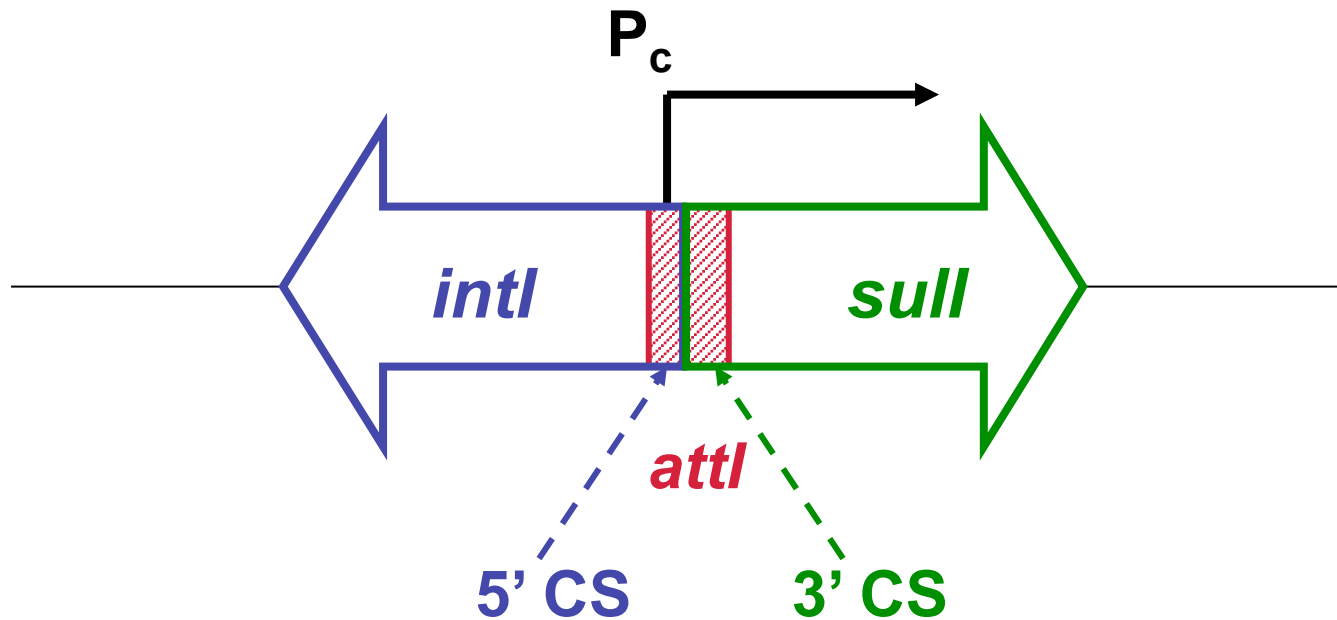
**Integron: genetic unit that includes genes of a site-specific recombination system capable of capturing and mobilizing genes contained in gene cassettes**

- ***intl* region: encodes a site-specific integrase**
- ***sull* region: confers resistance to sulphonamides**
- **variable region: gene cassette that contains one or more resistance genes**

**Gene cassette: mobile genes that can exist in free circular form**

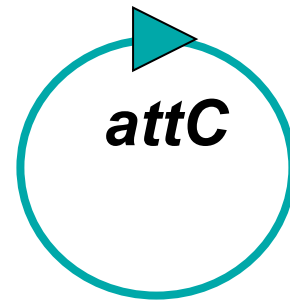
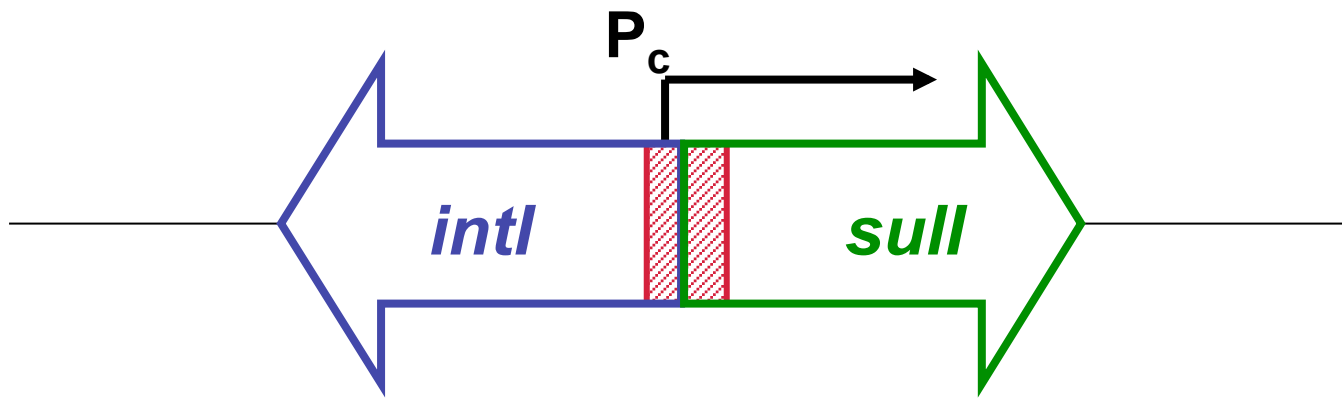
- **genes confer resistance to range of antibiotics**

# Cassette-free integron

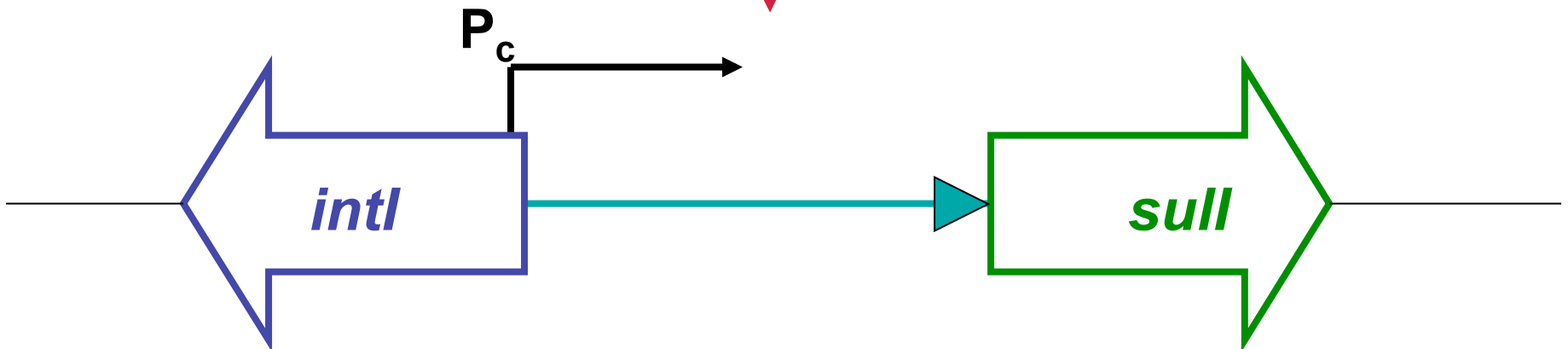


***attI*: receptor site for insertion of the gene cassette**

- Interacts with 59 base element of cassette (*attC*)
- Consists of a 5' and 3' conserved sequence
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Free mobile gene cassette



## Sequence of events for integration of cassettes

1)

2) Free mobile gene cassette associates with the integron and the integrase incorporates the cassette via site-specific recombination between the *attI* and *attC* sites

➤ Note that this a site-specific recombination system in which the integrase is supplied by the recipient DNA

3) Due to the orientation of the CS in the *attI* site and the 59 base element within the *attC* site, all genes of the mobile cassette are incorporated in the same orientation



# Assembly of integrons with multiple gene cassettes

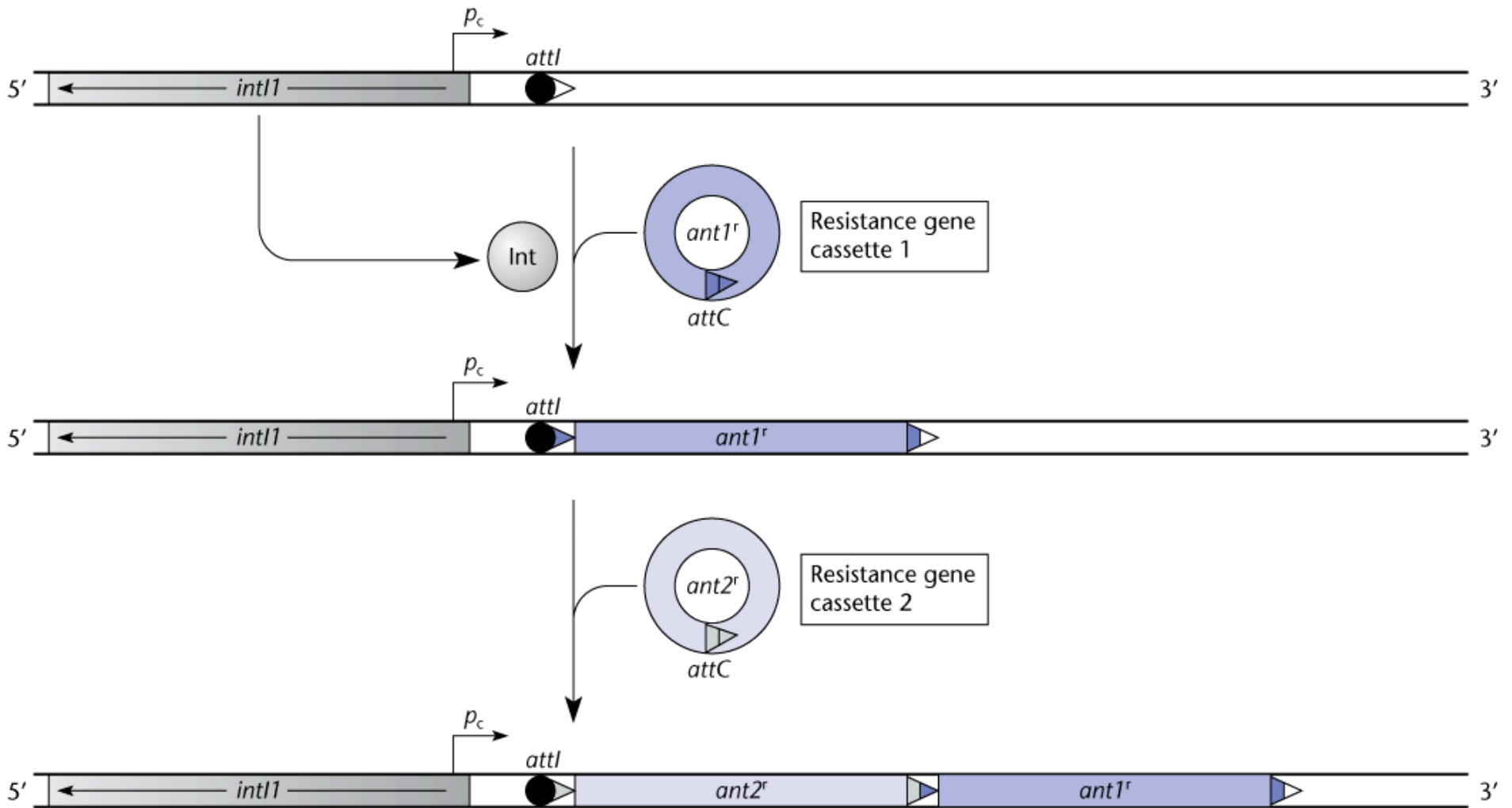


Fig. 9.29

# Superintegron of *Vibrio cholerae*: integron with > 100 cassettes

## Chromosomal superintegron (*V. cholerae*)

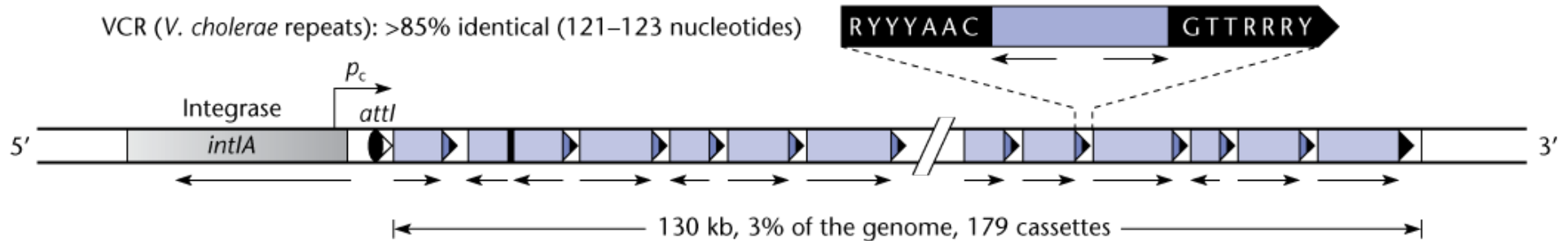
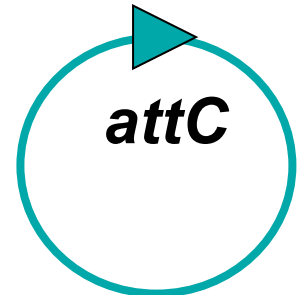
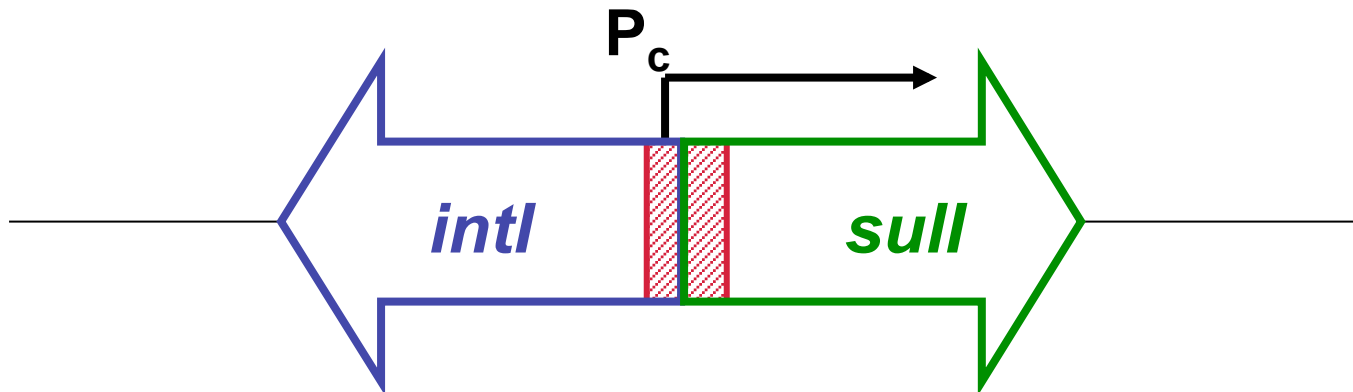


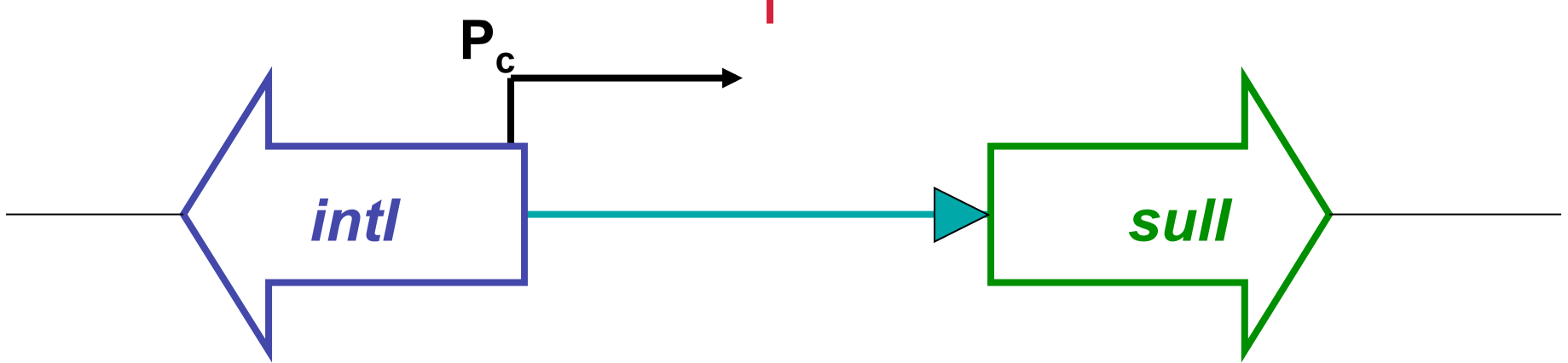
Fig. 9.30

## Loss of cassettes

- **Excision of the mobile gene cassette is dependent on the activity of the integrase**
- **Excision results in a circular mobile cassette that is free to participate in integration into another integron**
  - **Free circular cassettes are unable to replicate, so must be integrated into a replicon to be transferred to progeny cells**
  -



Free mobile gene cassette



## Possible outcomes if two integron-containing plasmids exist in the cell

- Insertion/excision events mediated by the integron encoded integrase can lead to co-integrate formation between the two plasmids
- If cointegrate resolution occurs at sites other than the *att* sites, a reciprocal acquisition and loss of gene cassettes can occur resulting in:

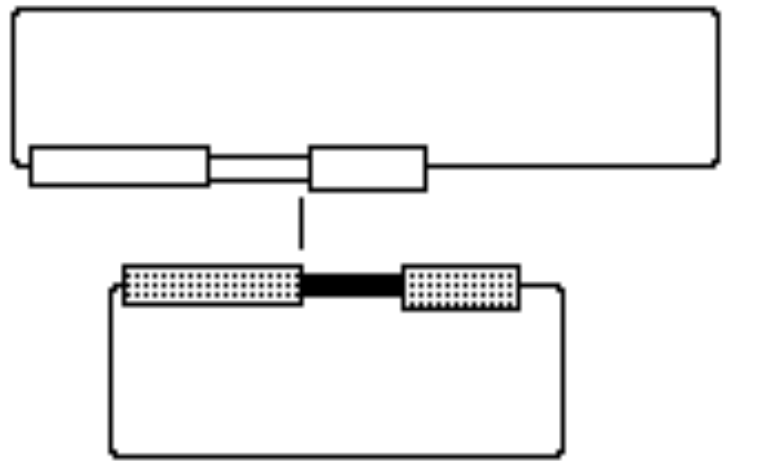


- An integron with two integrated gene cassettes

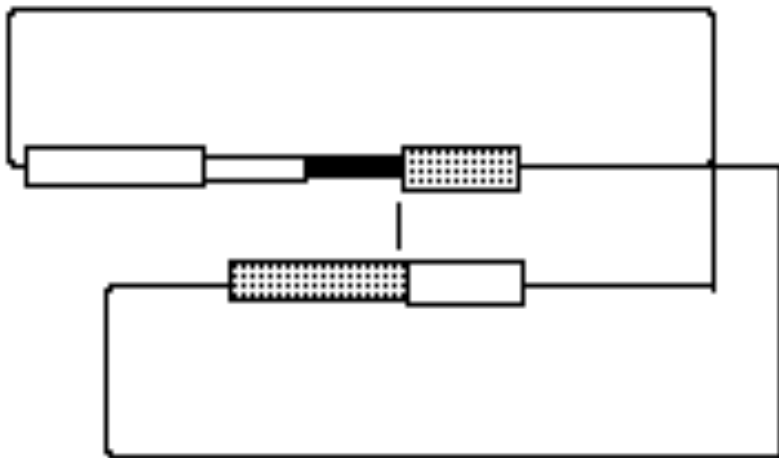


**Significant to the spread of antibiotic resistance if this molecule is a transferable plasmid with broad host range**

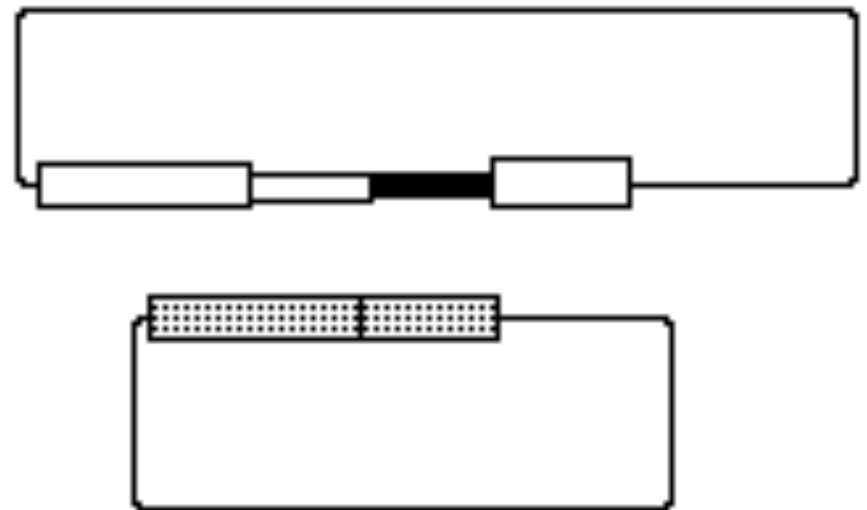
## Reciprocal acquisition and loss of gene cassette



↓  
IntI



→  
IntI



## Origin of gene cassettes?

**Possibly from reverse transcription of mRNA**

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- **The 59 base element (*attC* region) may have originated from a terminator**

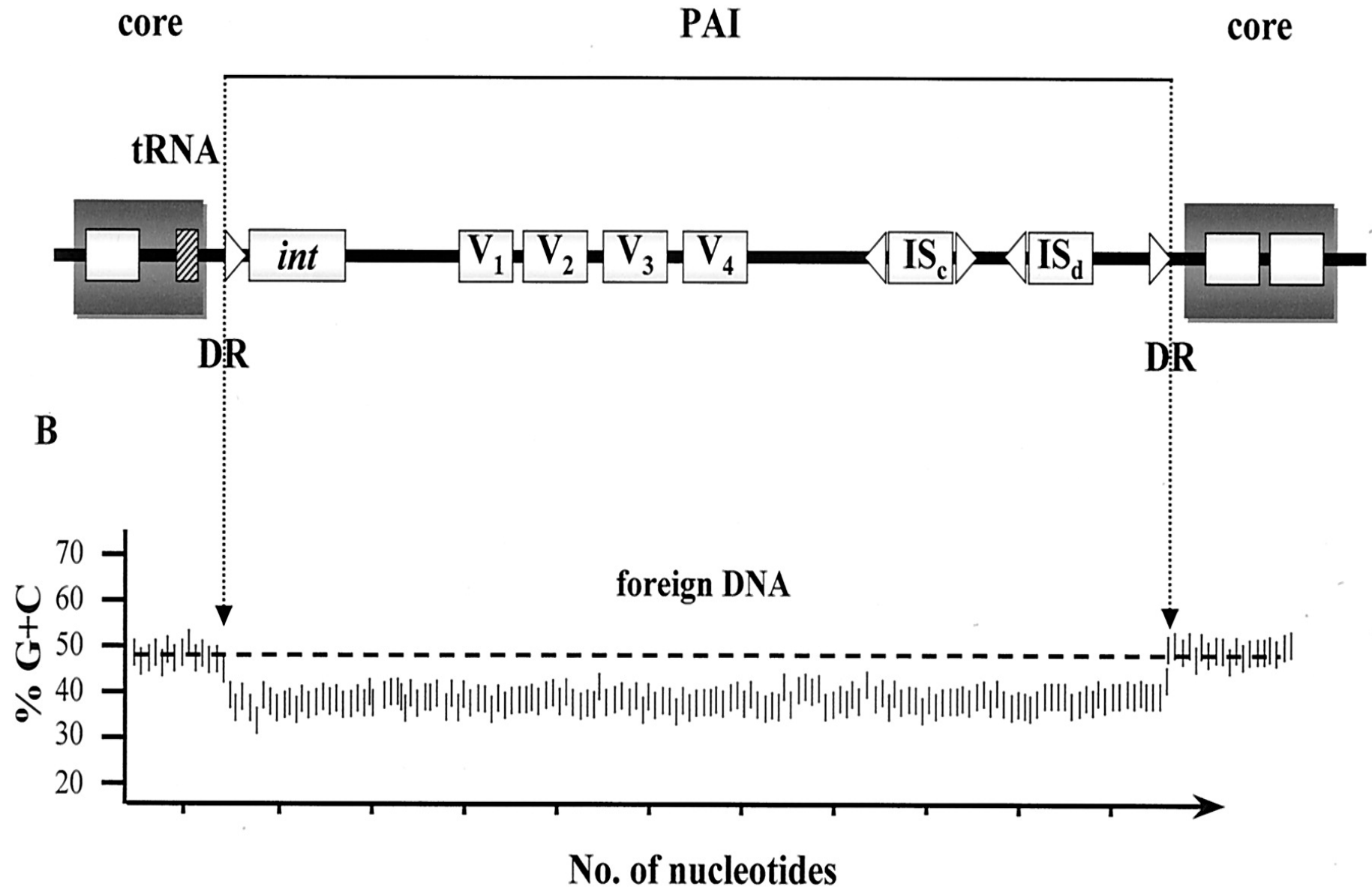
- **Recent evidence suggests that these elements can form a stem-loop and function as rho-independent terminator sequences**

# Pathogenicity Islands (PAI)

DNA elements integrated into the chromosome that carry genes whose products are required for pathogenicity

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- **Presence in pathogenic strains, and absence in less pathogenic strains**
- **Possess a different G+C ratio than the rest of the genome**
- **Occupy a large region of the genome: 10 - 200 kb**
- 
- **Often inserted into tRNAs**
- **Presence of 'mobility' (often cryptic) genes (IS elements, integrases, transposases,**
- **Unstable if mobility genes are functional**

# Schmidt and Hensel. 2004. CMR 17: 14-56



## Acquisition of pathogenicity islands

- **Region of DNA possibly originated from a plasmid or phage and was inserted into a tRNA**
  - **Part of the tRNA is usually duplicated on the PAI, so that the tRNA is functional**
- **Insertion likely facilitated by the inverted repeats on the island and a site-specific recombinase (integrase)**
  - **tRNA genes are fairly conserved between bacteria, thus regions of tRNAs can be analogous to *att* sites**
- **Most PAIs have not been demonstrated to move**
- **Contain cryptic integrase genes --> mutations acquired that render the products nonfunctional**



## Examples:

- ***Streptomyces***: plant pathogenicity
- ***Shigella***: carries resistance to multiple antibiotics
- ***E. coli***: hemolysin and fimbriae
- ***Yersinia***: scavenging and storing iron
- ***Helicobacter pylori***: secretion system for toxin release
- ***Staphylococcus aureus***: toxic shock syndrome
  - **PAI that mobilizes by behaving like a satellite phage**

## ***Staphylococcus aureus* SaPI1 and toxic shock**

- **SaPI1 is a PAI that encodes gene products that lead to toxic shock**
- **SaPI1 (encodes a functional integrase) was the first PAI whose movement was demonstrated**
- **SaPI1 moves by specifically parasitizing the *S. aureus* phage 80 $\alpha$** 
  - **When 80 $\alpha$  infects a *S. aureus* bacterium, SaPI1 is excised from the genome and replicated by the 80 $\alpha$  machinery**
  - **SaPI1 DNA is then packaged by 80 $\alpha$  head and tail proteins and the released for infection**



## ***E. coli* uropathogenic strain 536 harbors two different PAIs: PAI-1 and PAI-2**

- **PAI-1: is inserted into the 3'-end of the *selC* gene (selenocysteine tRNA)**
- **PAI-2: is inserted into the 3' end of the *leuX* gene (minor leucyl-tRNA)**
- **Both *selC* and *leuX* have been shown as insertion sites for prophage, thus the PAIs may have originated from prophage**
  - ***leuX* is also involved in the regulation of other *E. coli* virulence factors including type 1 fimbrae, enterobactin, serum resistance factors, and flagella**



## Gram positive bacteria contain different PAIs

- PAIs from *Clostridium*, *Listeria* and *Streptococcus* do not seem to have specific junction sequences like direct repeats or tRNA loci
- 
- Likely derived from a different source and evolved along a different path than their Gram-negative counterparts.

### Example: *Clostridium difficile* (pseudomembranous colitis)

- 19 kb PAI that encodes enterotoxins and cytotoxins localizes to a 115 bp region of the genome
- Non-pathogenic strains that do not have the PAI have a 20 bp repeat capable of forming a hairpin within this 115 target region