Genetic support of Extended-Spectrum ß-Lactamases

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ESBLs

- **TEM-like (*Enterobacteriaceae* + *P. aeruginosa*)**
- **SHV-like (*Enterobacteriaceae* + *P. aeruginosa* + *A. baumannii*)**
- **CTX-M-like (*Enterobacteriaceae* + *A. baumannii*)**

- **Other enzymes**

(*Enterobacteriaceae* + *A. baumannii* + *P. aeruginosa*)

  - **PER-1, PER-2**
  - **VEB-1**
  - **GES-like (IBC)**
  - **BEL-1**

Variety of genetic elements

- Transposons
  - Class II transposons
  - Composite transposons
- Integrons
  - Class 1 integrons
  - *sul1*-type integrons
- Insertion sequences
- Other features

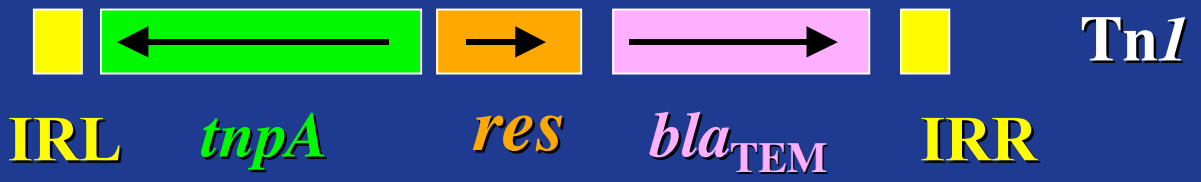
The *bla*$_{TEM}$-like genes

- Point mutant derivatives leading to ESBL enzymes but with similar genetic contexts

- Reservoir of *bla*$_{TEM}$ genes ?

Tn*3*-type transposons

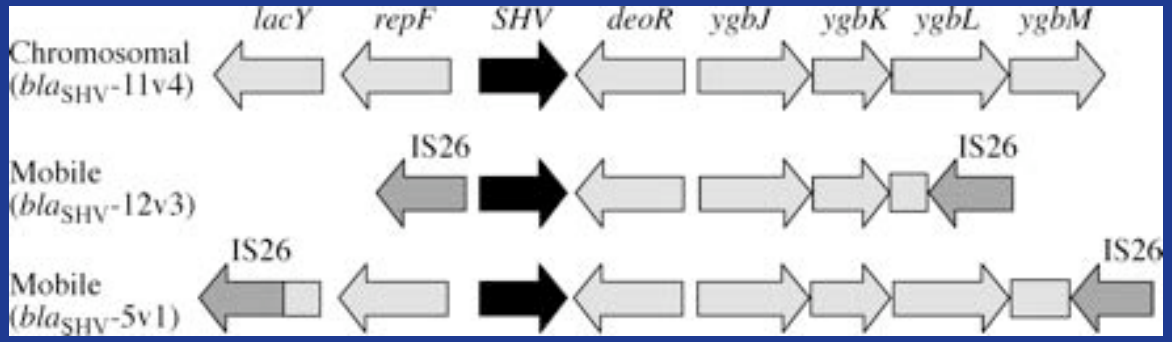
- 2 extremities corresponding to inverted repeat sequences (IR)
- 1 transposase (TnpA) => recognizes IR extremities in the transposition process and cuts target DNA
- 1 resolvase (TnpR) : negative regulator of TnpA expression and recombinase mediating the resolution of the transposon integration process



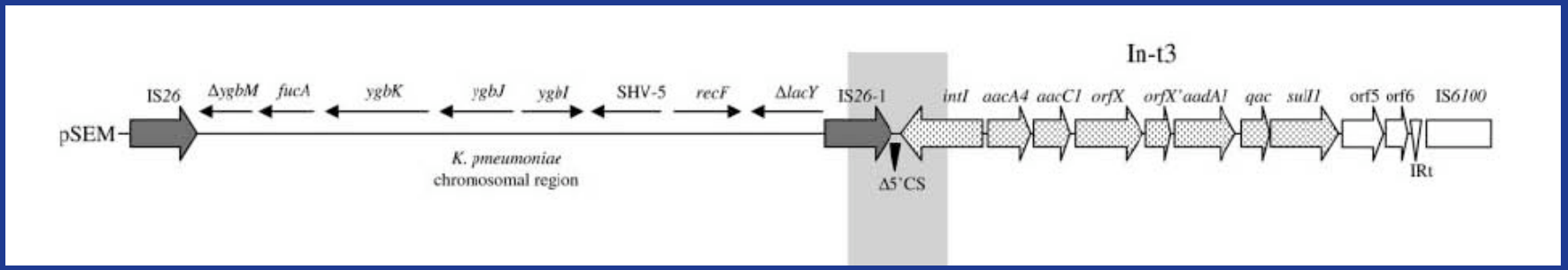
The *bla*$_{SHV}$-like genes

- Point mutant derivatives leading to ESBL enzymes
- Originating from *Klebsiella pneumoniae*

IS*26*-mediated mobilization of bla_{SHV}



Ford & Avison, JAC 2004



Miriagou, Carattoli et al., AAC 2005

The *bla*$_{\text{CTX-M}}$-like genes

- **Five main groups made of**
  - **CTX-M-1**
  - **CTX-M-2**
  - **CTX-M-8**
  - **CTX-M-9**
  - **-CTX-M-25**

- **Originate from *Kluyvera* spp.**

Main genetic structures for *bla*$_{\text{CTX-M}}$ acquisition

IS*Ecp1*

CTX-M of different groups

sul1-type integrons

(including CR1 element)

CTX-M-2 and CTX-M-9

Phage-related elements

CTX-M-10 (CTX-M-1 group)

A peculiar insertion sequence : IS*Ecp1*

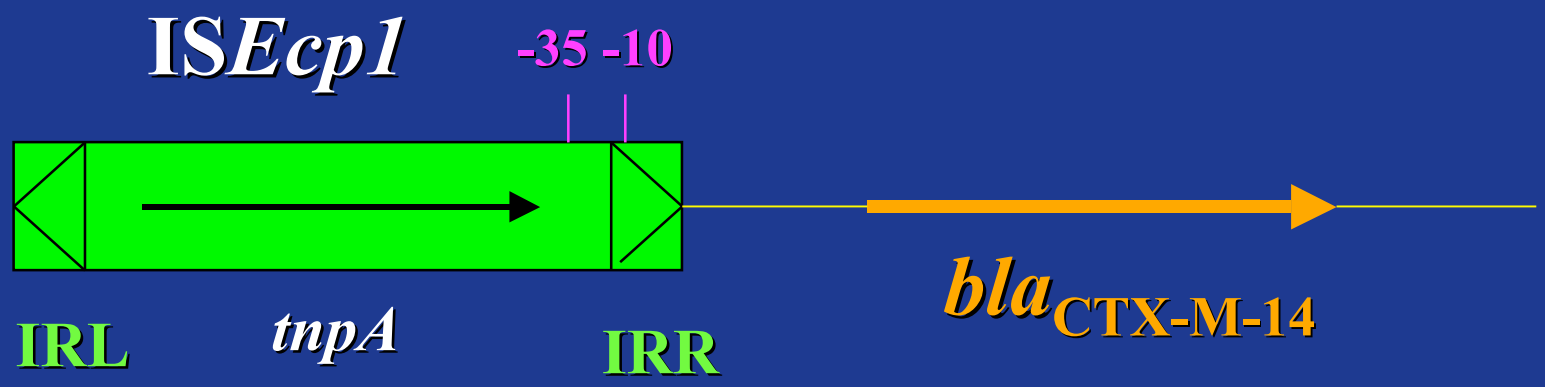
- IS*Ecp1*, insertion sequence responsible for mobilization and expression of ß-lactamase genes by transposition using imperfect right inverted repeats

- Implications in term of mobilization and dissemination of genes coding for ESBL

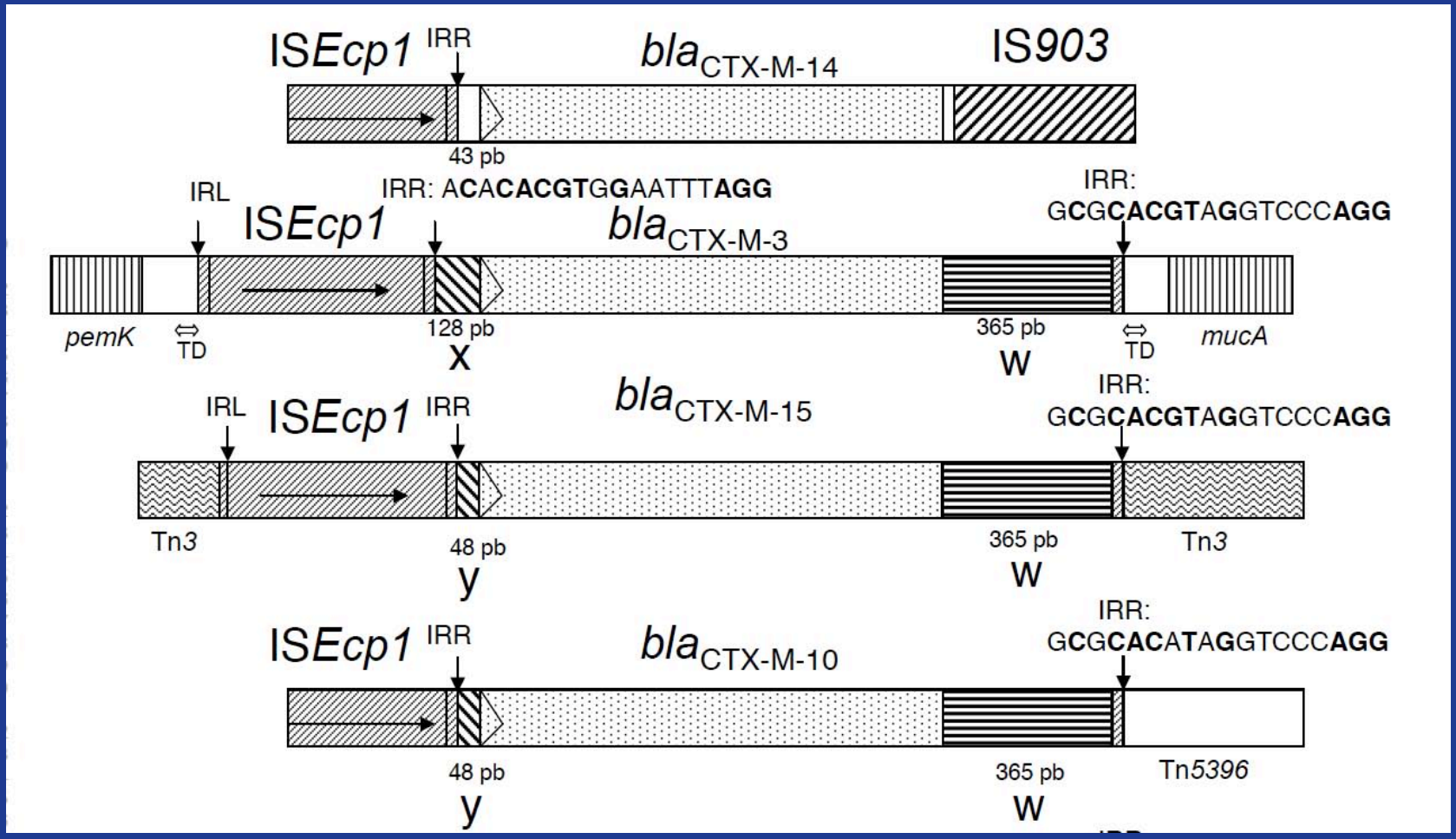
Poirel et al., AAC 2003;47:2938

Poirel et al., AAC 2005;49:447

Role of IS*Ecp1* in expression of *bla*$_{\text{CTX-M-14}}$



Distinct mobilization events

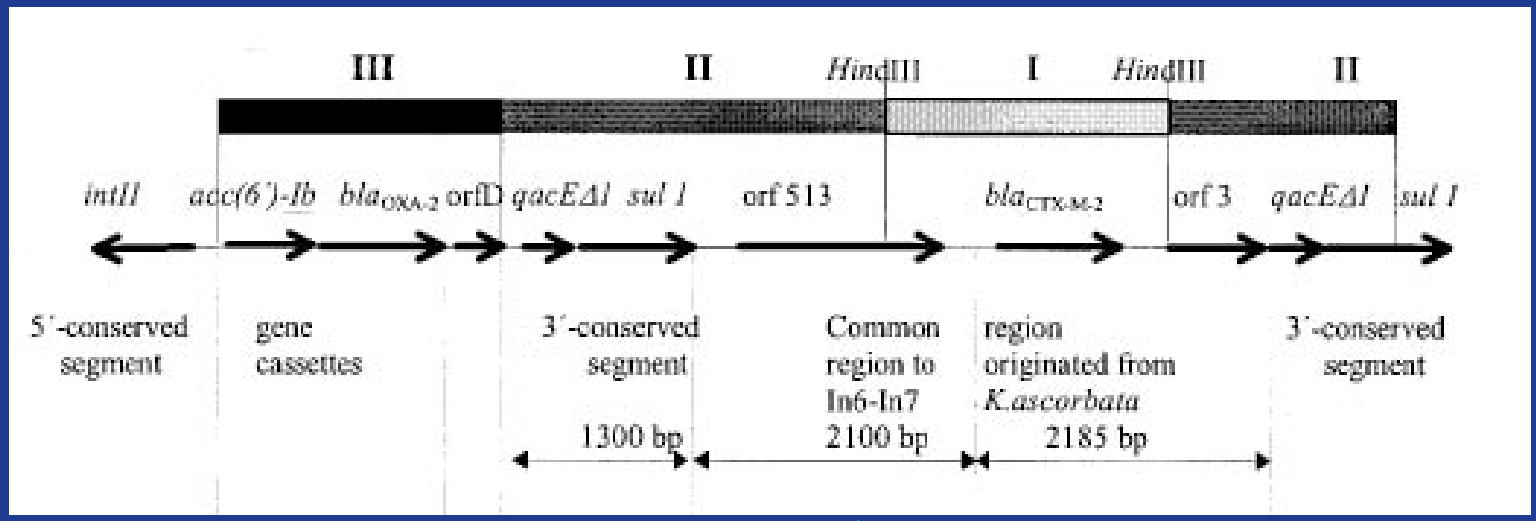


Lartigue, Poirel, Nordmann, FEMS Microb. Lett. 2004

bla$_{\text{CTX-M}}$ genes associated to *sul1*-type integrons

A « complex » *sul1*-type integron

Example; In35 in a *Proteus mirabilis* isolate

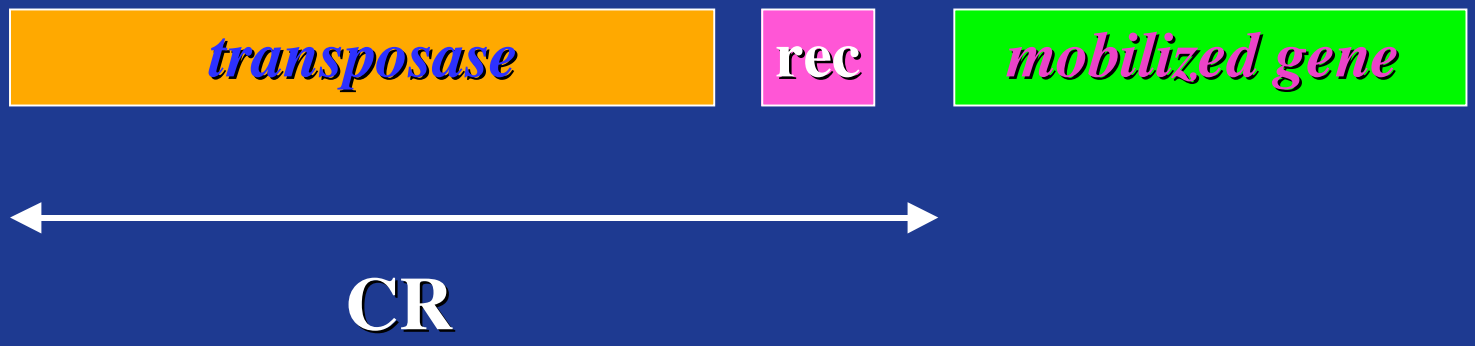


Arduino et al., AAC 2002, 2303

CR (Common region)

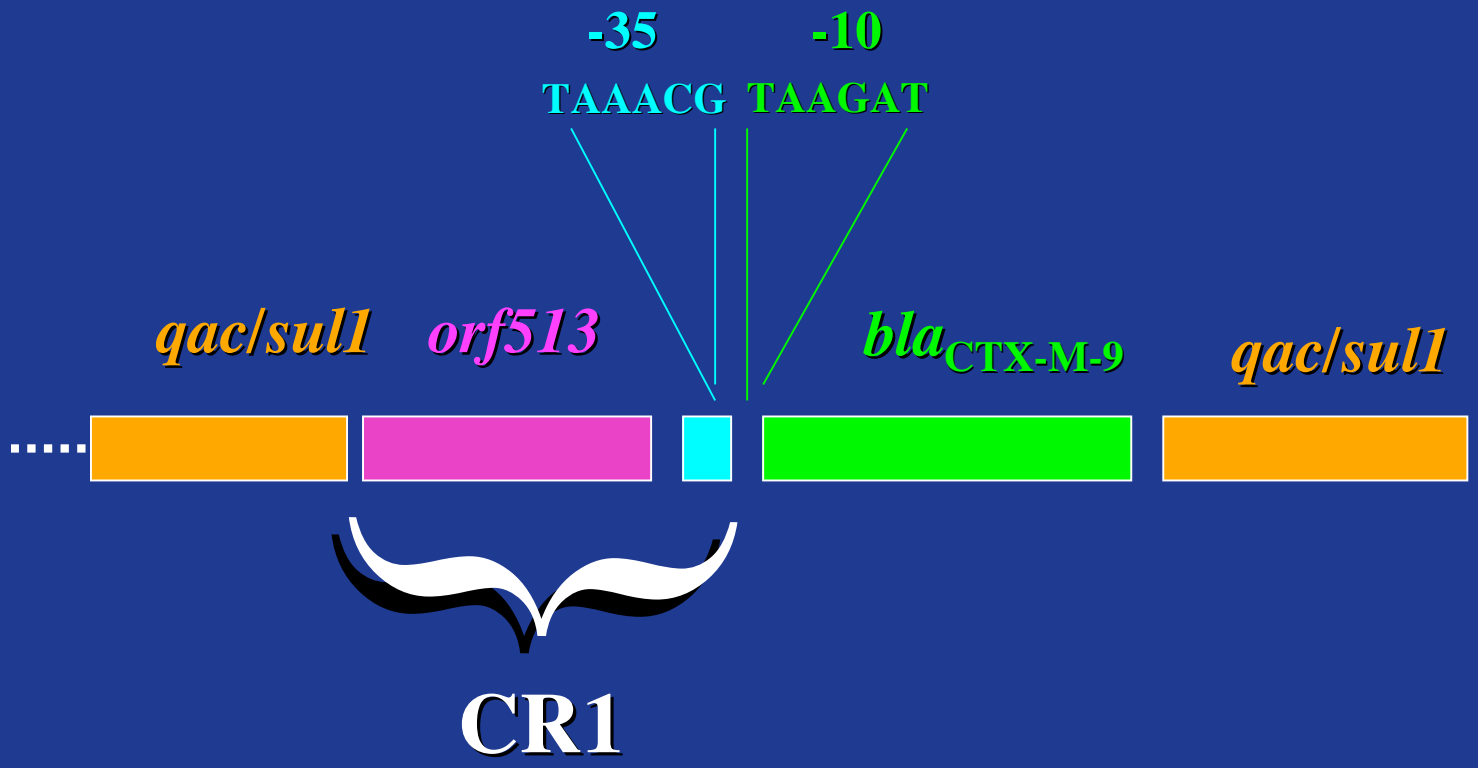
First Definition

- A transposase-like protein
- One extremity serves as a recombination site



(Partridge and Hall, AAC 2003)

CR1 enhances the expression of *bla*$_{CTX-M-2}$ and *bla*$_{CTX-M-9}$

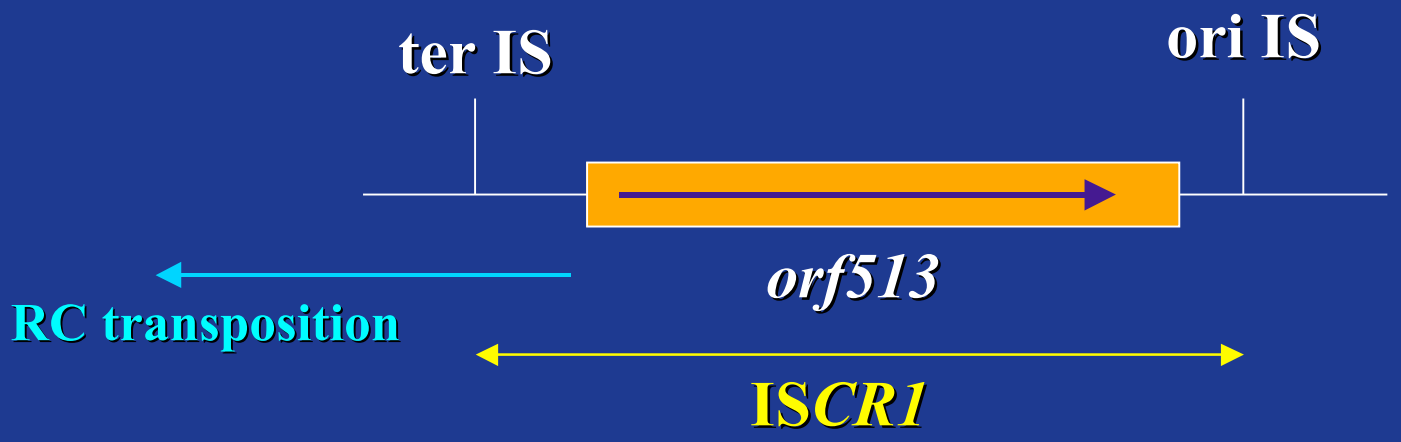


J.M. Rodriguez-Martinez, L. Poirel, R. Canton & P. Nordmann *et al.*, AAC 2006, in press

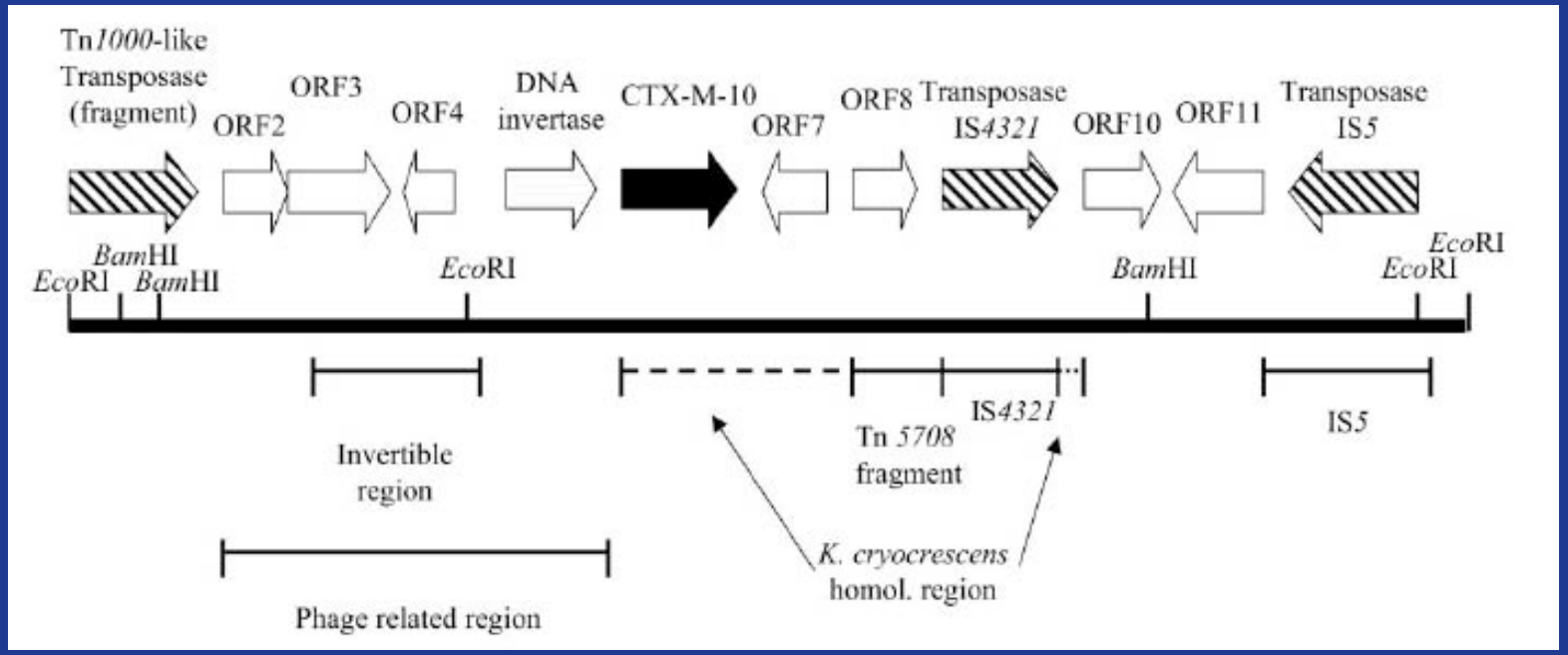
Update on the definition of CR1

T. Walsh, personal communication

- CR1 is an IS*91*-like transposable element
- IS*CR1* lack the terminal IRs typical of most IS elements
- IS*CR1* moves by rolling-circle (RC) transposition
- Orf513 is a transposase
- No target site duplication is generated by RC transposition



Phage-associated structure at the origin of *bla*$_{\text{CTX-M-10}}$ acquisition in *Klebsiella pneumoniae* in Spain



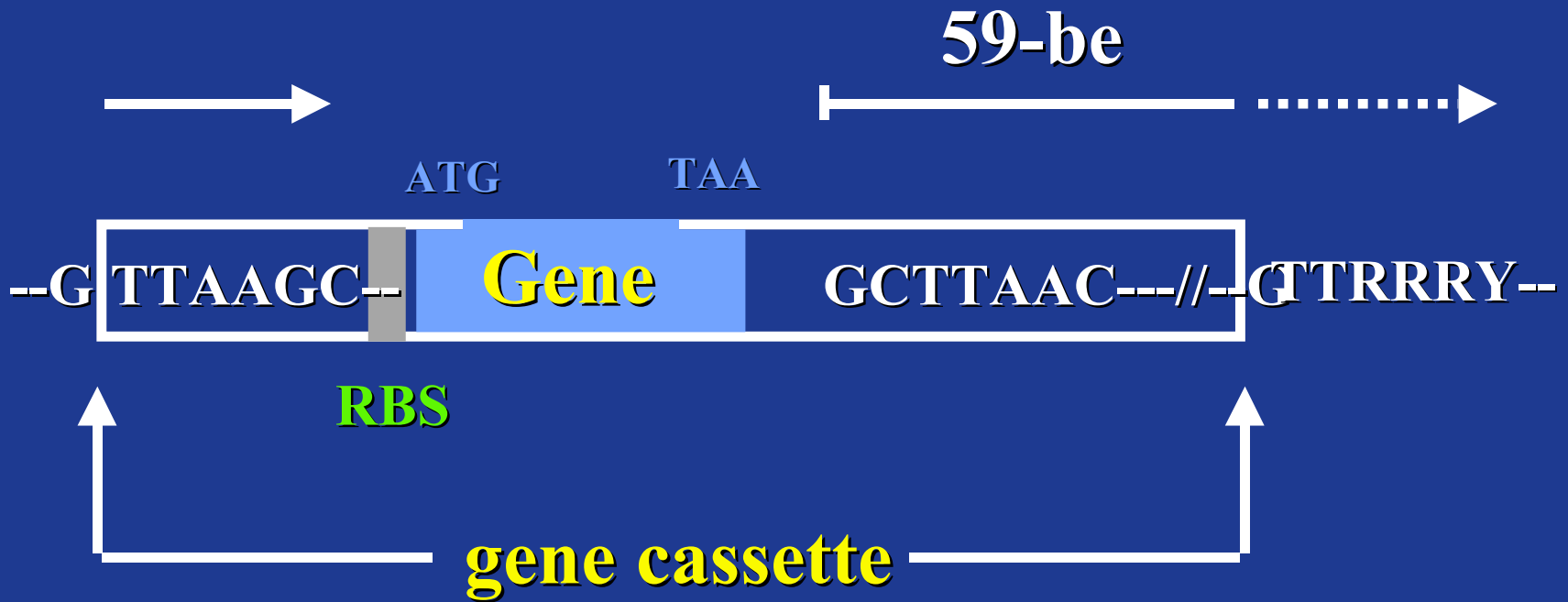
Oliver et al., AAC 2005;49:1567

Other ESBL-encoding genes

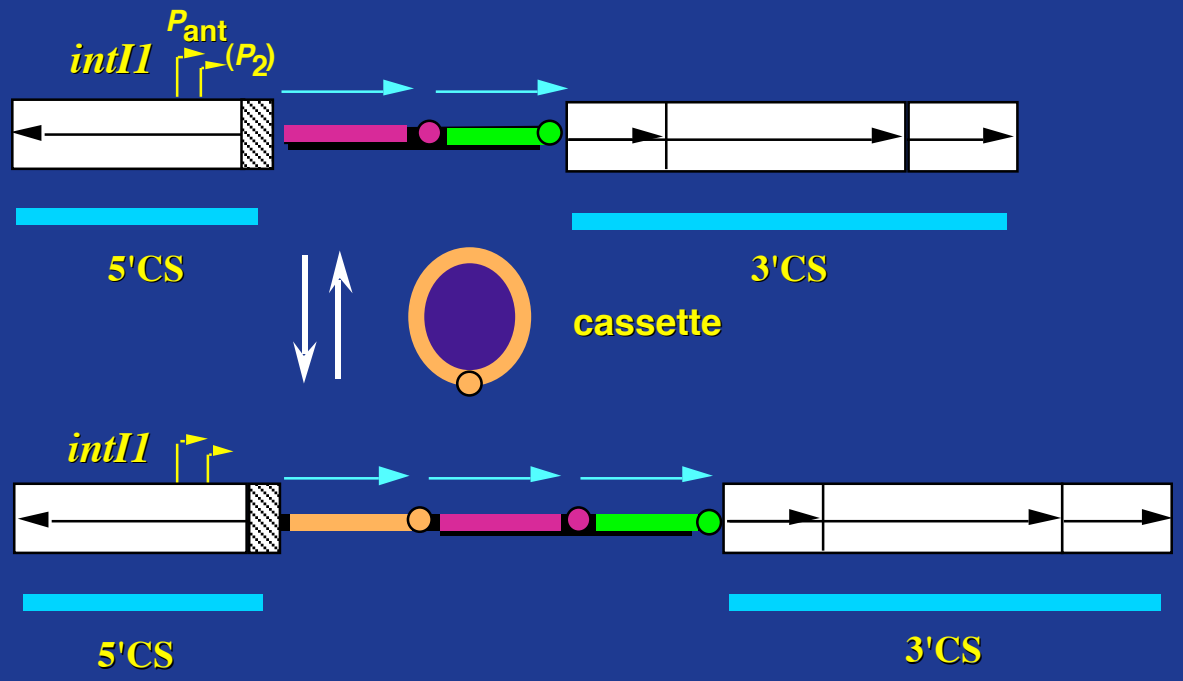
Integrons as a source of ß-lactamase gene acquisition

The ESBL gene is part of a gene cassette

Structure of a gene-cassette

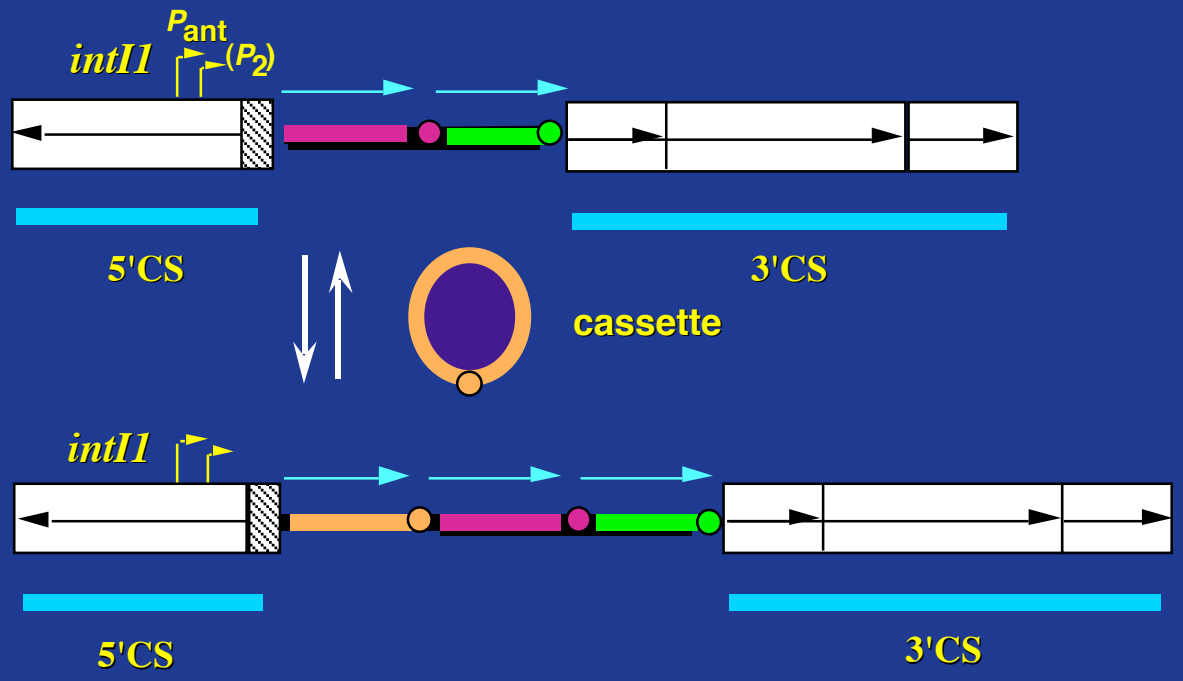


Class 1 integrons



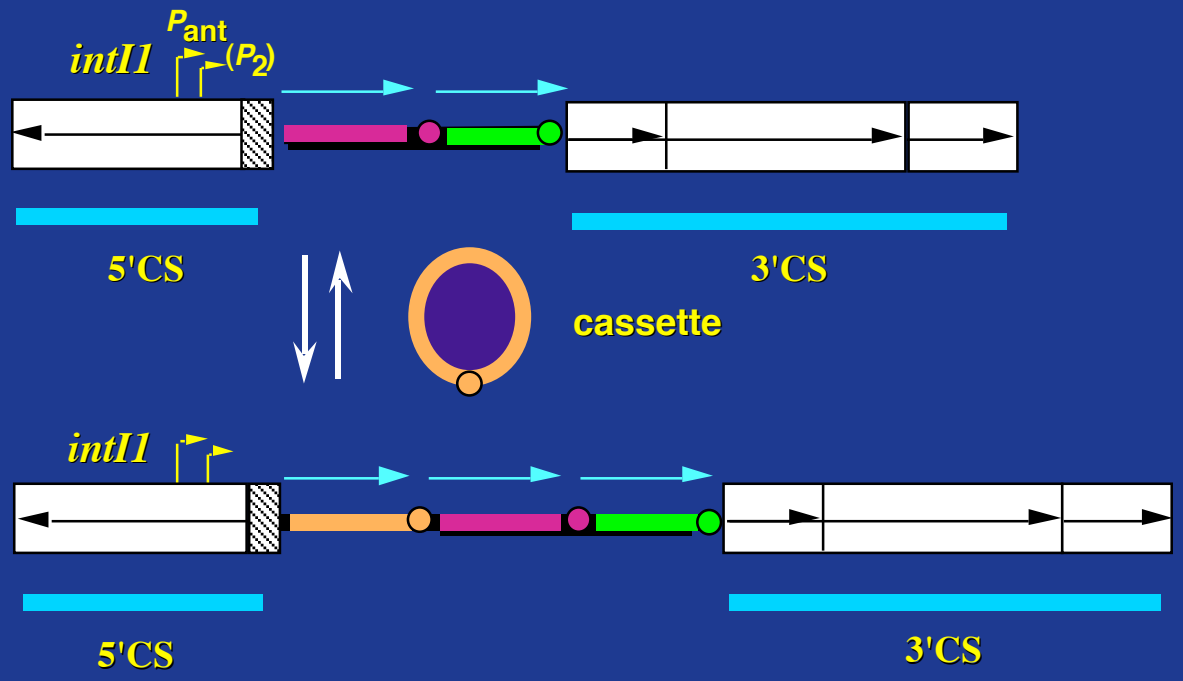
- Consequences : co-resistance

Class 1 integrons



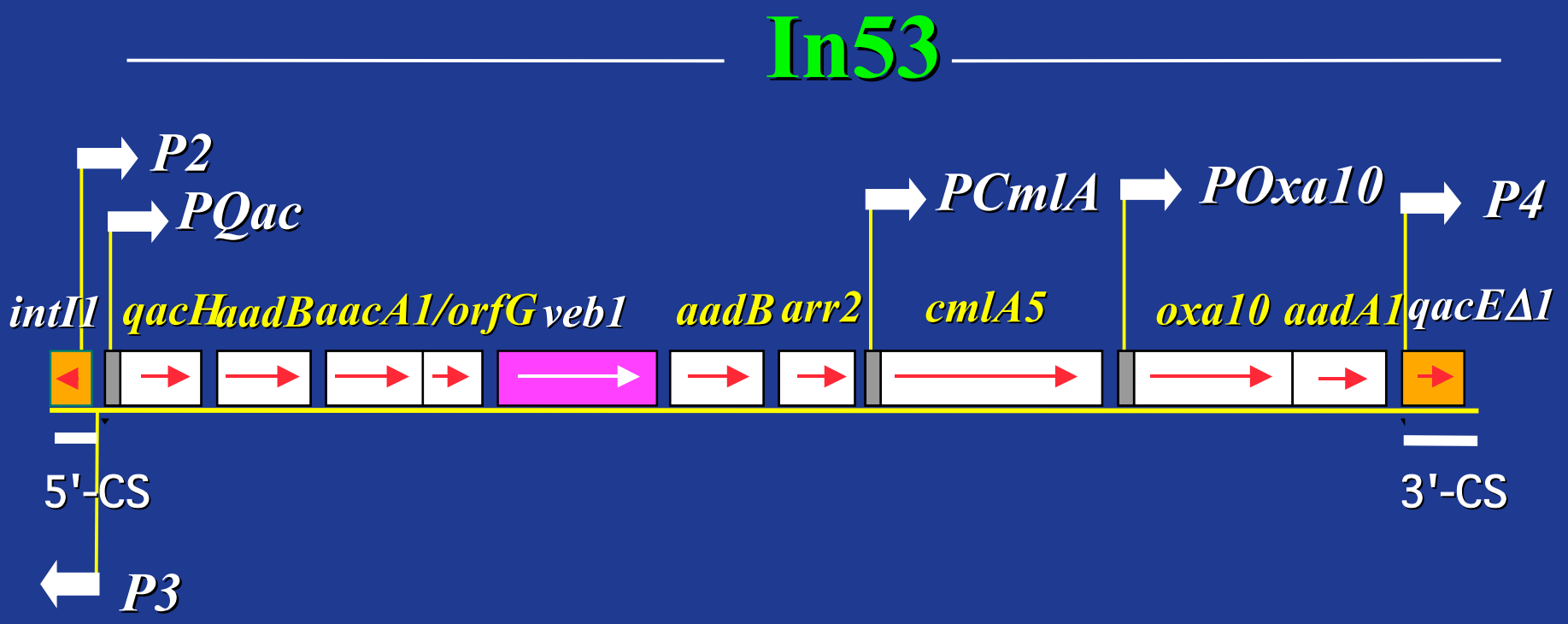
- Consequences : co-resistance ; co-expression

Class 1 integrons



- Consequences : co-resistance ; co-expression ; co-selection

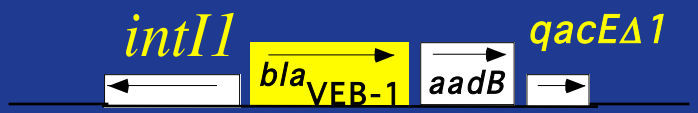
bla_{VEB-1} as a gene cassette identified in In53 in *E. coli*



Naas et al., J Bact 2001

bla$_{\text{VEB-1}}$ in *P. aeruginosa*

1



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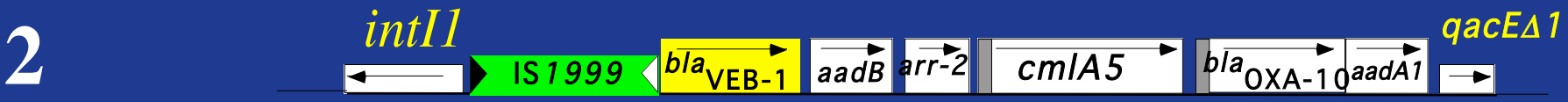

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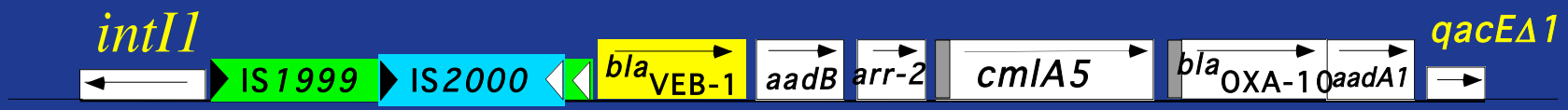
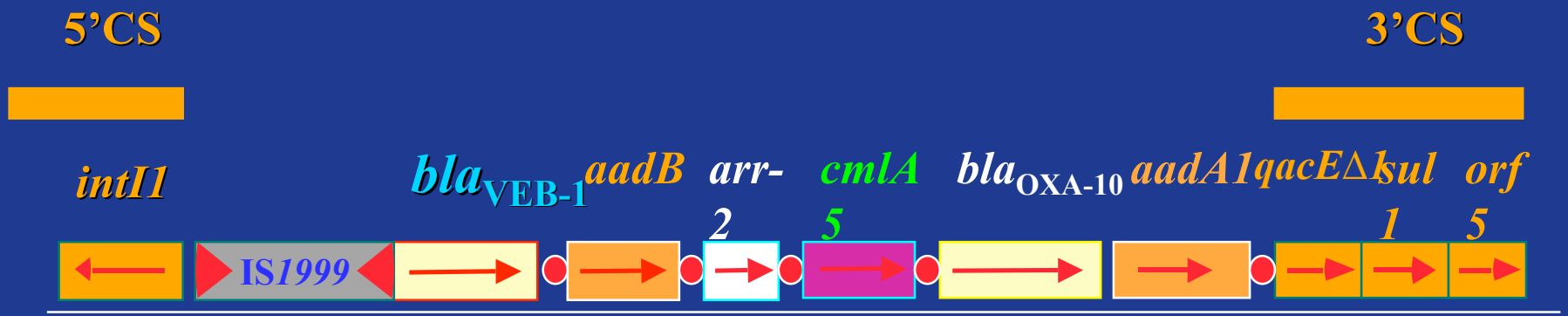


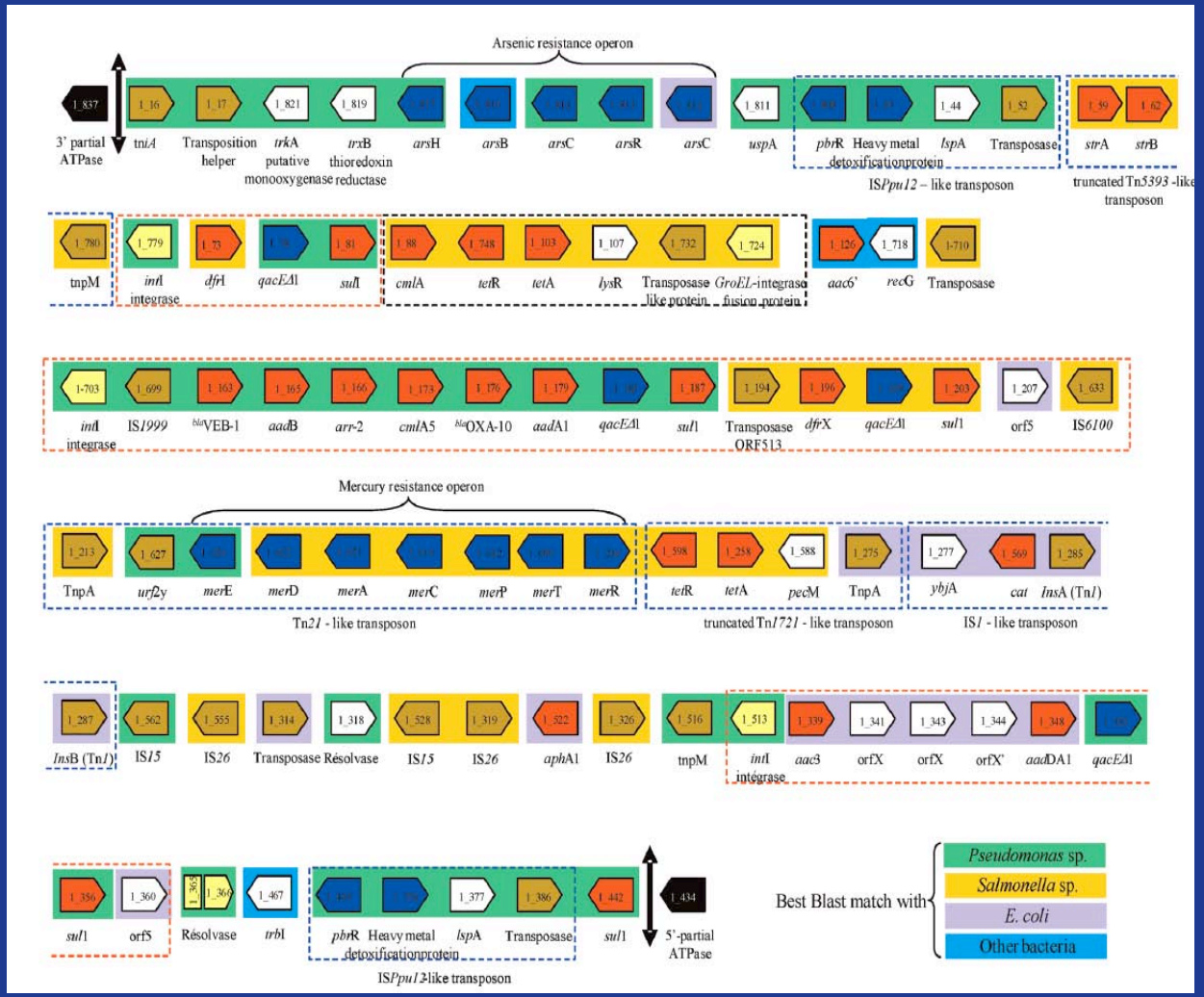
Girlich *et al.*, CID 2002

Structure of the *bla*$_{VEB-1}$-containing integron identified in the epidemic clone of *A. baumannii* in France



Poirel *et al.*, JCM 2003;41:3542

An 86-kb resistance island



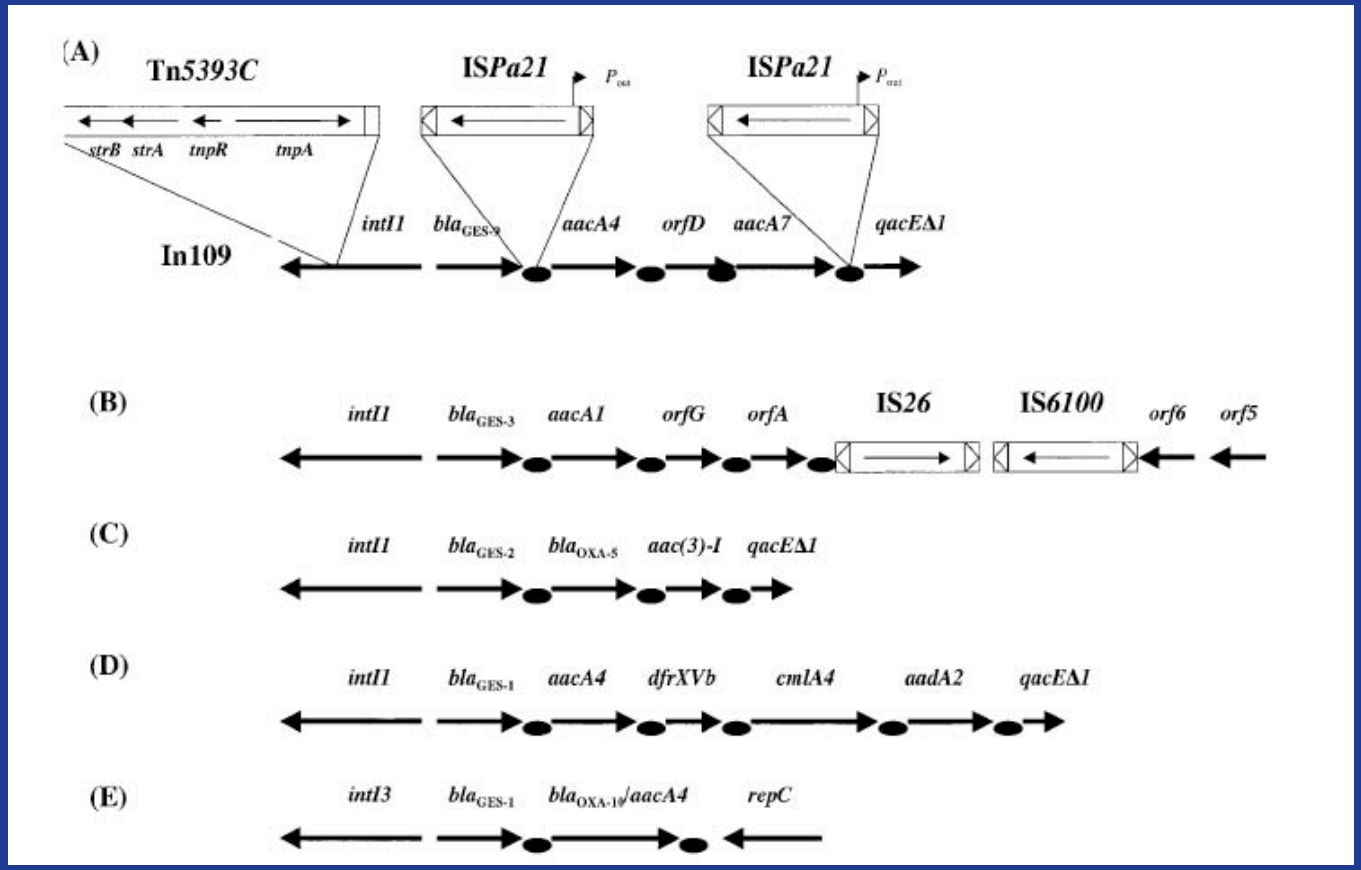
Fournier et al., PLOS Genetics 2006

Other ESBLs encoded by gene cassettes located into class 1 integrons

- GES-type enzymes (GES-1 to -9)
(France, Greece, South Africa, Asia, South America)

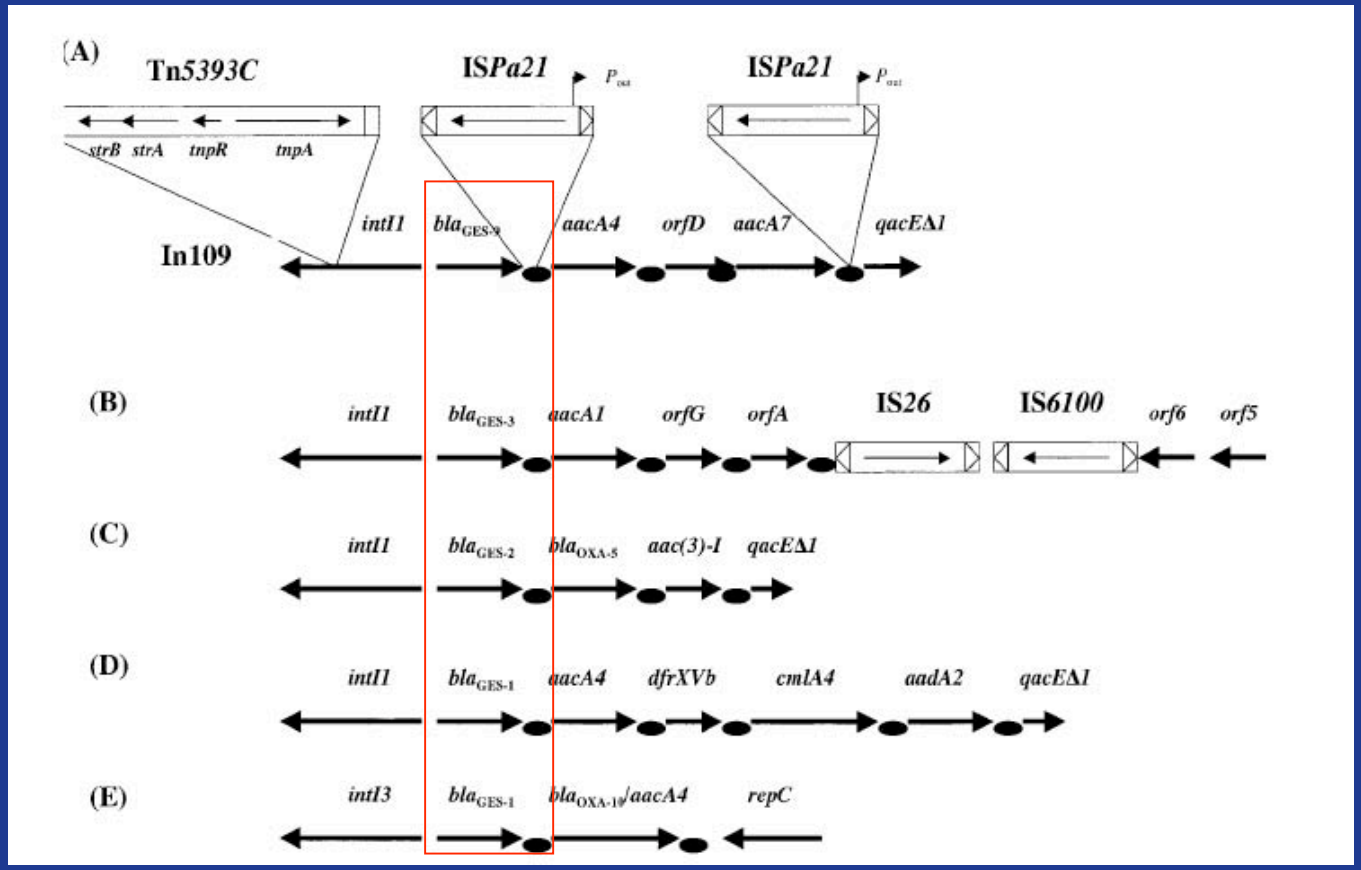
- BEL-1
(Belgium)

Structures of integrons harboring *bla*$_{\text{GES-like}}$ gene cassettes



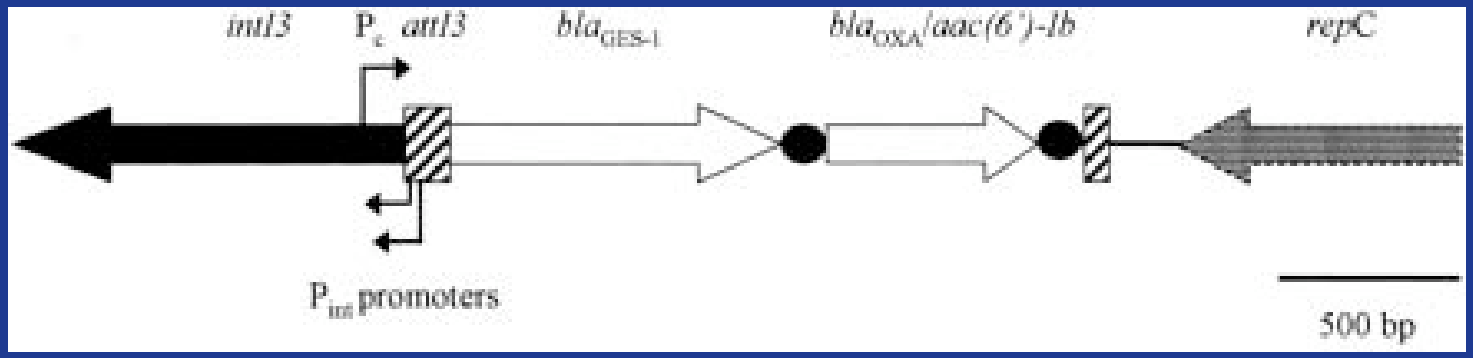
Poirel et al., AAC 2005;49:3593

Structures of integrons harboring *bla*$_{\text{GES-like}}$ gene cassettes



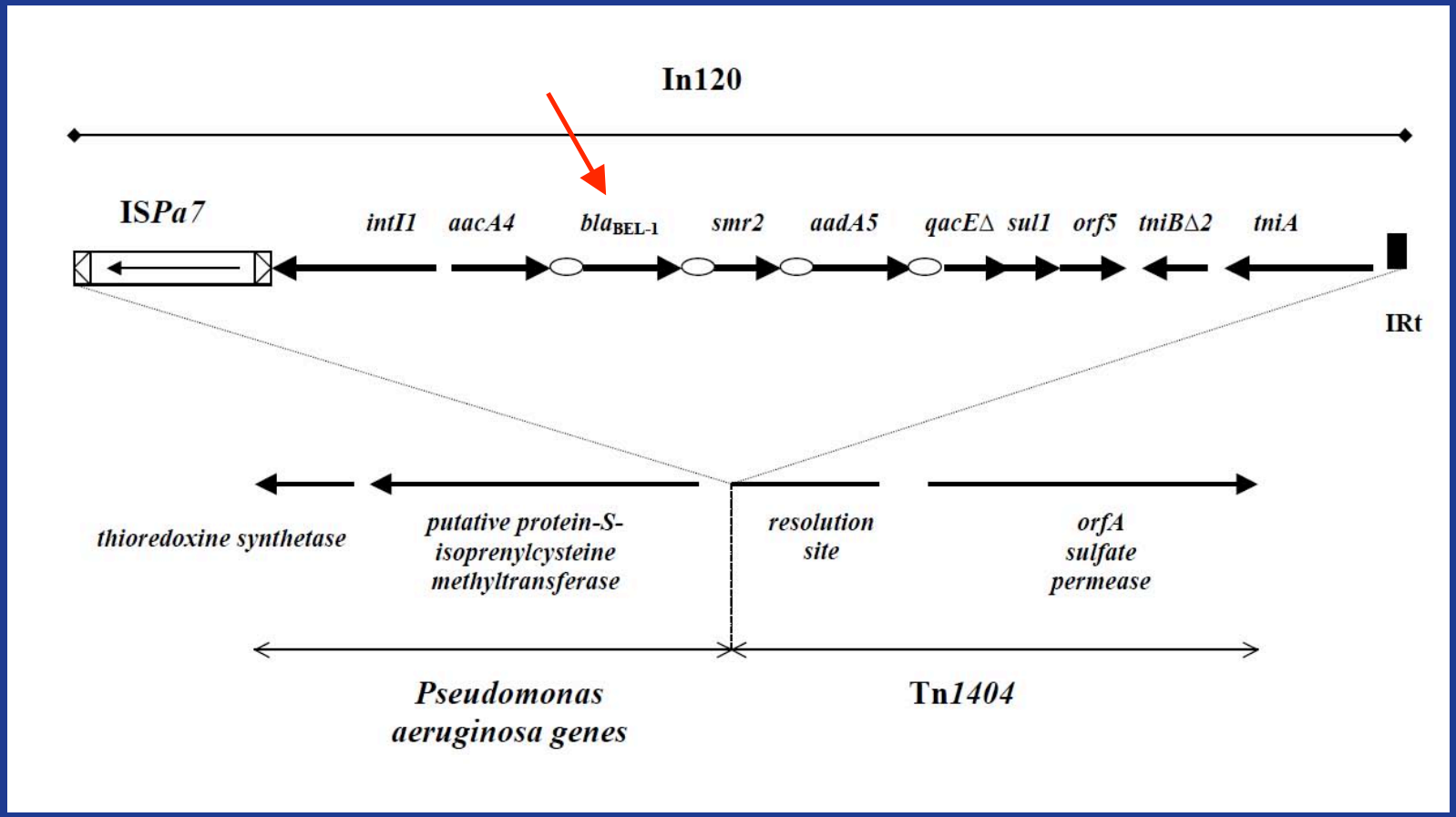
Poirel et al., AAC 2005;49:3593

A class 3 integron at the origin of bla_{GES-1} acquisition



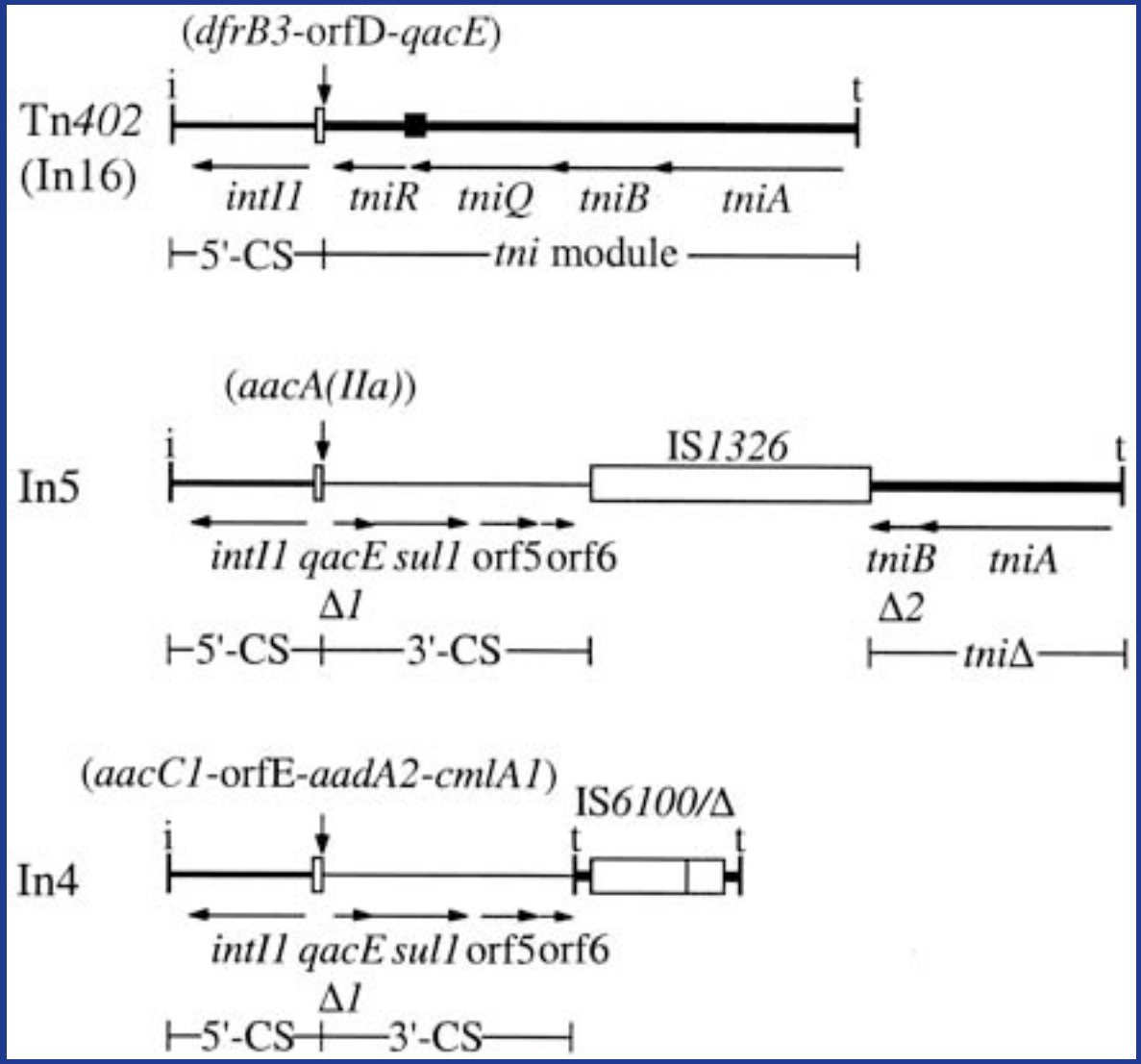
Correia *et al.*, AAC 2003

bla$_{BEL-1}$ and its integron In120



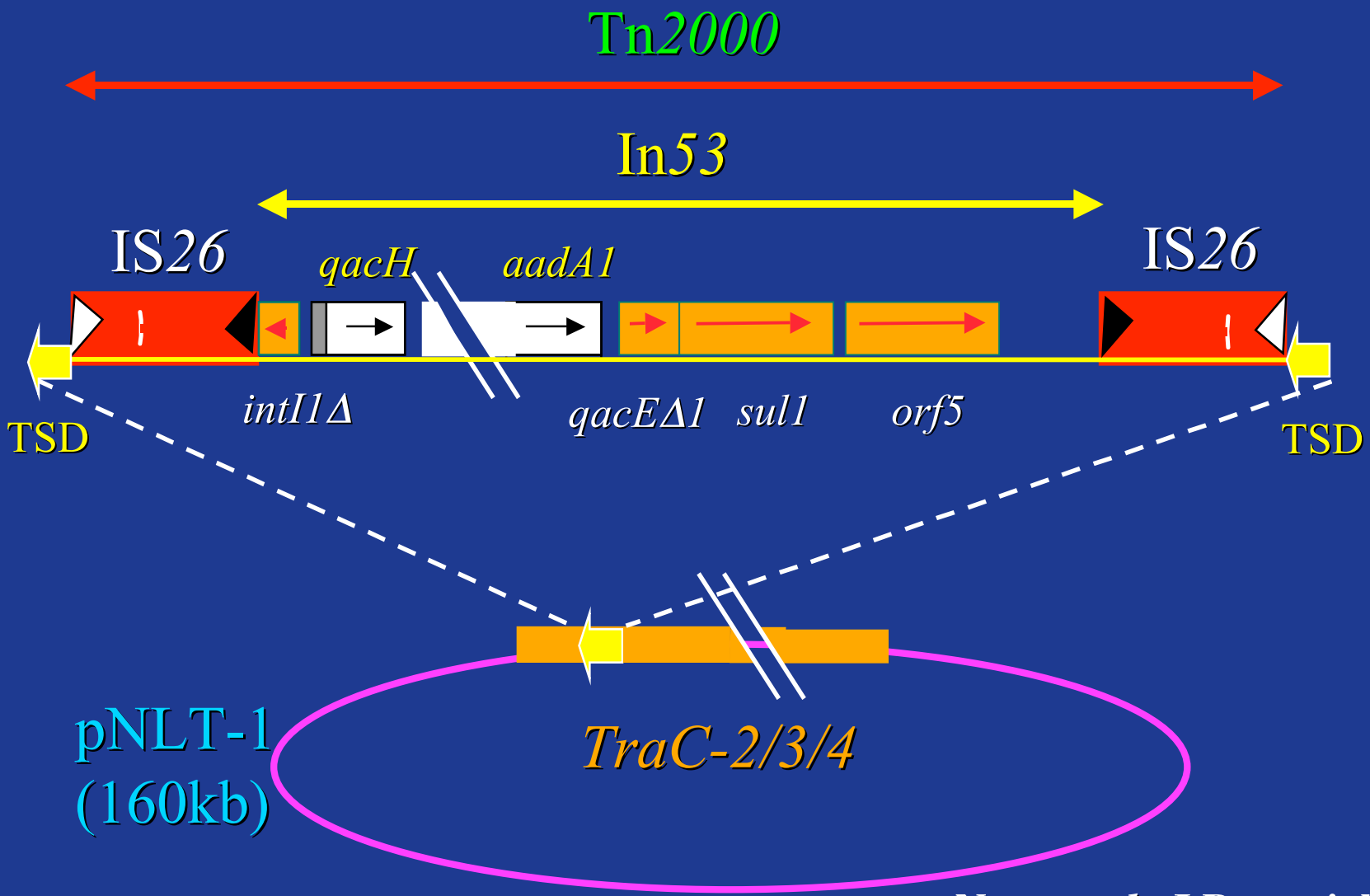
Poirel et al., AAC 2005;49:3743

The integron-borne transposons



Partridge et al., AAC 2001

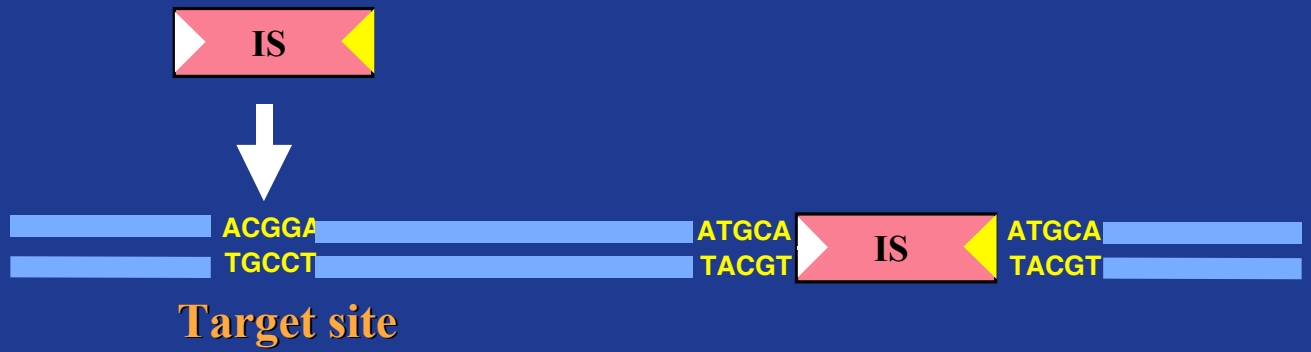
Structure of Tn*2000*



Naas *et al.*, J Bacteriol 2001

Mobilisation of resistance genes

- Composite transposon



Mobilisation of resistance genes

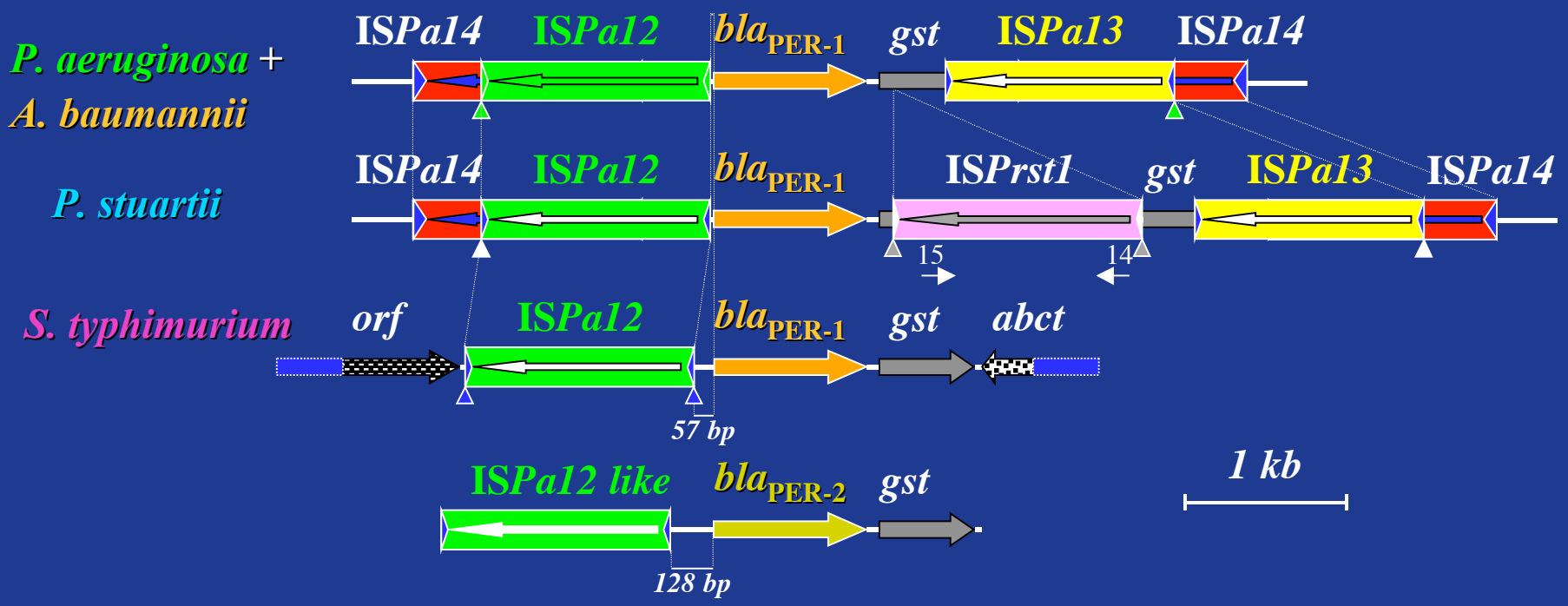
- Composite transposon = two IS bracketing the transposed fragment

Mobilisation of resistance genes

- Composite transposon = two IS bracketing the transposed fragment

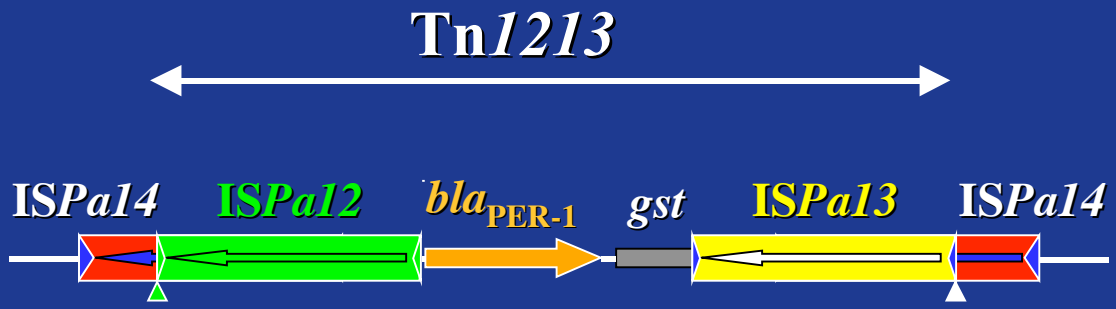
A peculiar composite transposon at the origin of *bla*$_{\text{PER-1}}$ acquisition

Genetic environment of bla_{PER-1} (1)

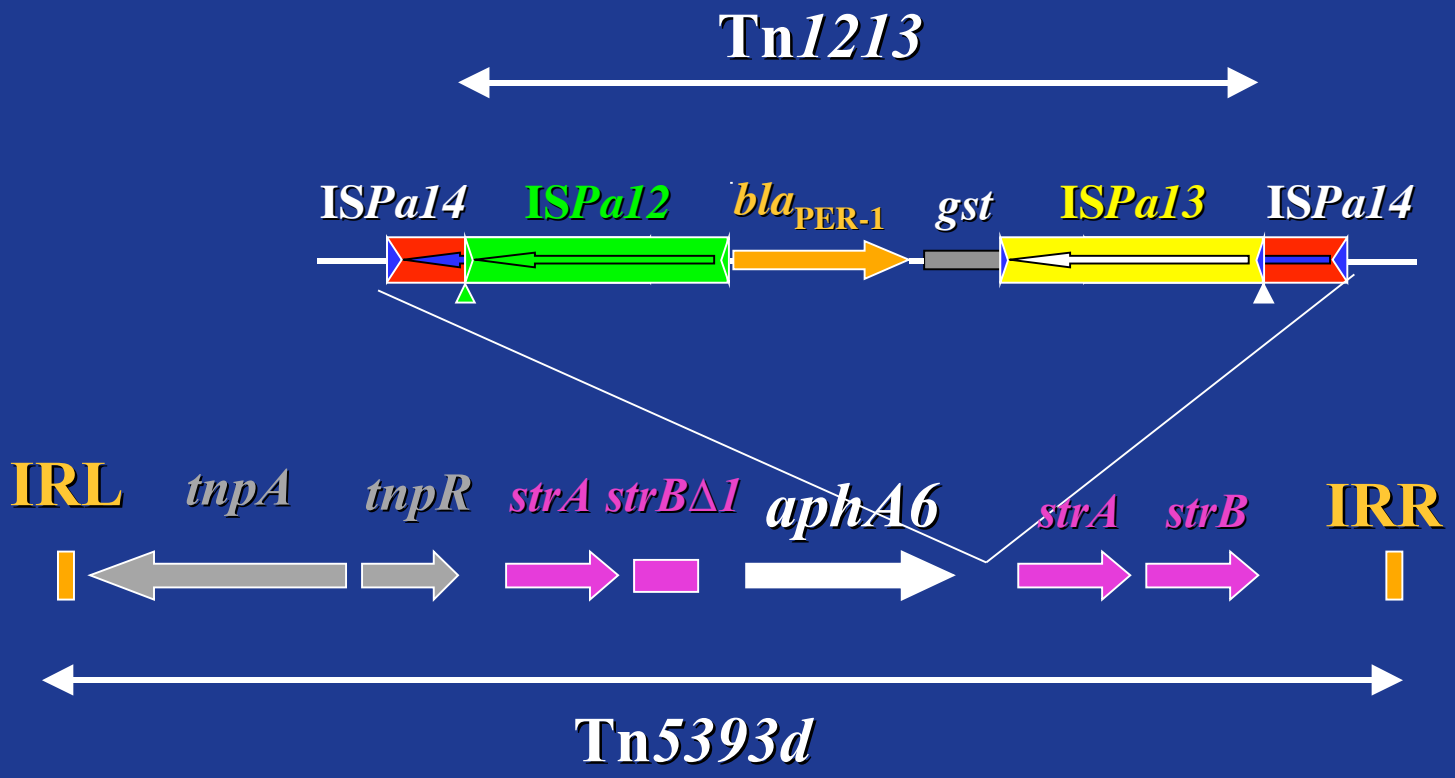


Poirel et al., AAC 2005;49:1708

Genetic environment of *bla*$_{PER-1}$ (2)



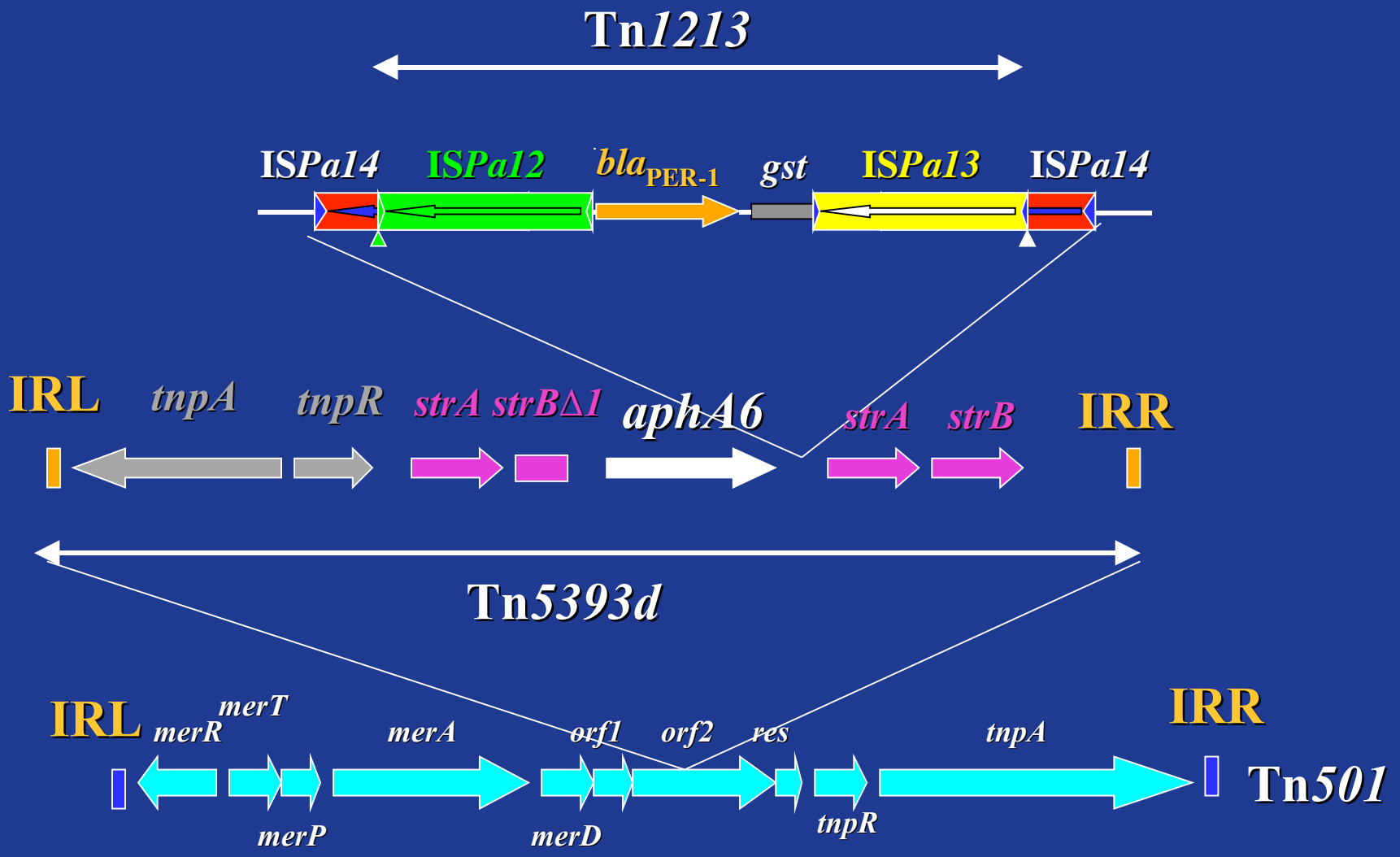
Genetic environment of bla_{PER-1} (2)



Alcaligenes faecalis, Italy

Mantengoli & Rossolini, AAC 2005

Genetic environment of bla_{PER-1} (2)

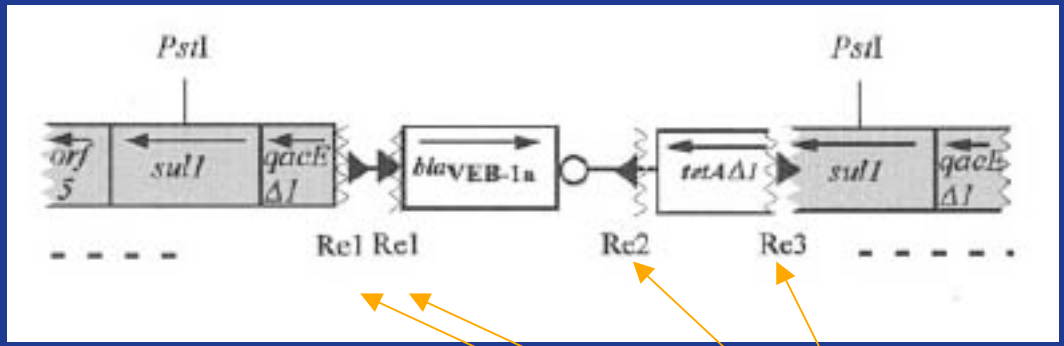


Alcaligenes faecalis, Italy

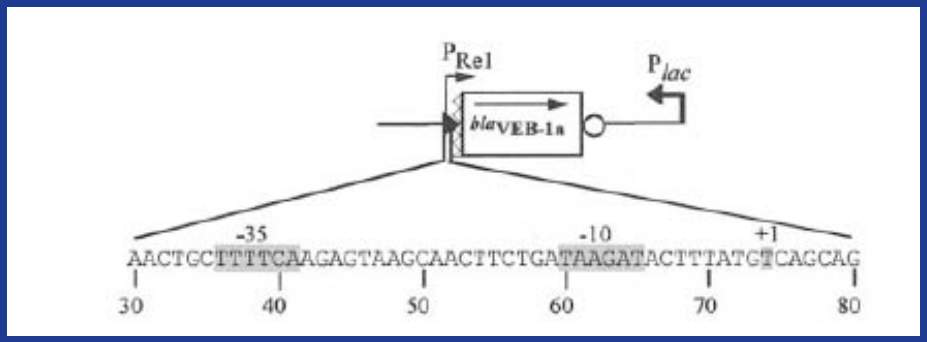
Mantengoli & Rossolini, AAC 2005

Other features

Re elements and *bla*$_{\text{VEB-1}}$ acquisition

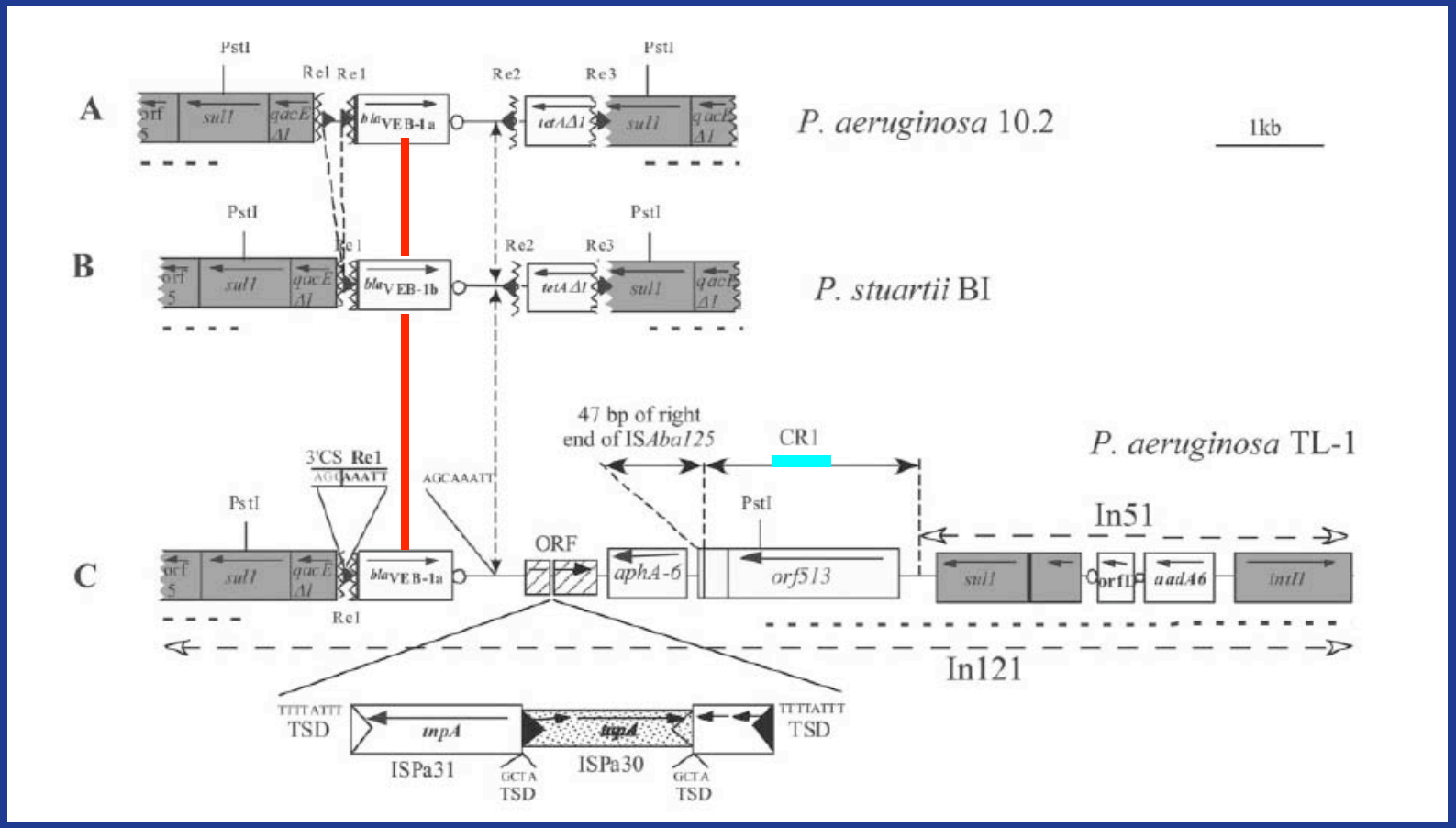


Re = 135 bp



Aubert et al., AAC 2004

bla$_{\text{VEB-1}}$ may be part of a *sul1*-type integron



Naas et al., AAC 2006

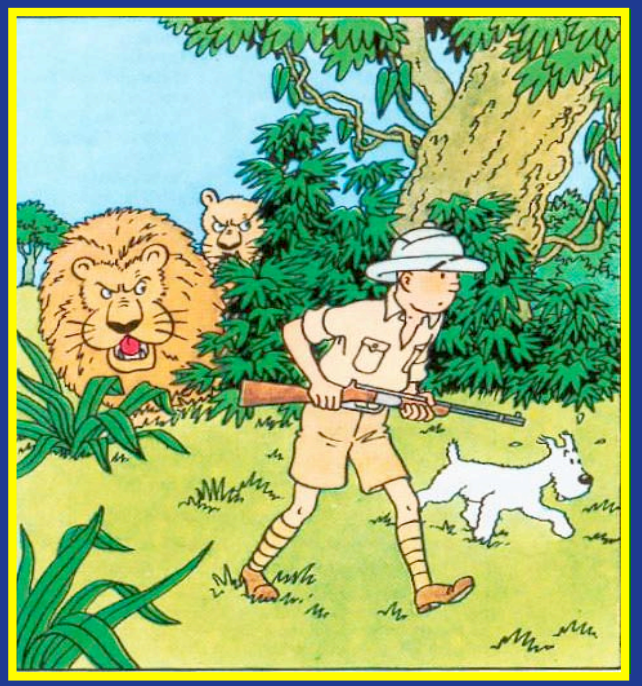
Genetic elements involved in mobilization of genes encoding class A ß-lactamases

- Transposons for *bla*$_{TEM}$
- IS*26* element for *bla*$_{SHV}$
- Class 1 integrons for *bla*$_{VEB}$, *bla*$_{GES}$
- CR1 element for some *bla*$_{CTX-M}$ genes (*sul1*-type integrons)
- IS*Ecp1* for other *bla*$_{CTX-M}$ genes
- Peculiar composite transposons for *bla*$_{PER-1}$

Conclusions

- More and more vehicles for ESBL genes acquisition and dissemination are identified
- Genetic plasticity at the origin of ESBL gene diffusion => increasing spread

- Searching for the reservoirs
  - Mobile elements
  - Resistance genes by themselves



Acknowledgments

P. Nordmann

T. Naas