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Review

New insights about excisable pathogenicity islands in *Salmonella* and their contribution to virulence

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Abstract

Pathogenicity islands (PAIs) are regions of the chromosome of pathogenic bacteria that harbor virulence genes, which were probably acquired by lateral gene transfer. Several PAIs can excise from the bacterial chromosome by site-specific recombination and in this review have been denominated "excisable PAIs". Here, the characteristic of some of the excisable PAIs from *Salmonella enterica* and the possible role and impact of the excision process on bacterial virulence is discussed. Understanding the role of PAI excision could provide important insights relative to the emergence, evolution and virulence of pathogenic enterobacteria.

Keywords: *Salmonella*; Pathogenicity island; Excision; Virulence

1. Introduction

Pathogenicity islands (PAIs) are large clusters of genes (10–100 kb) present in the genome of pathogenic bacteria and absent in the genome of non-pathogenic strains of the same or closely related species [1]. Usually, the G + C content and codon usage of a PAI differ from that of the rest of the genome [2], a fact that supports the notion of PAI acquisition through lateral gene transfer. The presence of virulence-associated genes, whose protein products facilitate the process of infection, favors the infective process of pathogenic bacteria and contributes to the selection of bacteria that harbor the newly acquired PAI in the genome. Among virulence genes found in PAIs are type III and type IV secretion systems [3,4], superoxide dismutases [5], adhesins, hemolysins [6], Shiga-like toxins [7] and genes encoding for capsule production [8].

Many PAIs are able to excise from the chromosome and to re-integrate into it, and in this review they have been named excisable PAIs. The spontaneous excision of PAIs was first described in the uropathogenic strain *Escherichia coli* 503, which possesses the excisable PAIs I and II containing the genes for hemolysin and P-related fimbriae. Deletion mutants who have lost both PAIs showed decreased virulence [9]. More recently, a large number of studies showed that the excision

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process occurs in many pathogenic bacteria, such as *Legionella pneumophila*, *Yersinia pseudotuberculosis*, *Salmonella* and others [10–13].

Excisable PAIs often possess genes or pseudogenes coding for mobility-related proteins, such as integrases, transposases, and excisionases/recombination directionality factors (RDFs), all of which show similarity to proteins found in phages or transposons [14,15]. Moreover, most PAIs that encode P4-type integrases are inserted proximal to tRNA genes [12,16]. These genes are frequent insertion points for genomic islands and several hypotheses have been proposed to explain this [16–18]. Excisable PAIs are also flanked by direct repeated sequences (DRS) generated during the insertion into the chromosome by site-specific recombination [19], after the PAI acquisition by lateral gene transfer [16,20]. When the PAI is inserted in the 3′ end of a tRNA gene, the recombination process generates a sequence duplication that corresponds to the last 15–100 bp of the tRNA, producing the two flanking DRS that are called *attL* and *attR* (left and right DRS respectively) (Fig. 1A) [12,21]. These DRS will act as integration sites for bacteriophages, other PAIs or mobile genetic elements, allowing the incorporation of new genetic material to the PAI [22,23]. Indeed, some PAIs have a mosaic-like structure, presenting two or more fragments that have been acquired sequentially over time by insertion in the same target site [23].

In general, the excision of a PAI occurs by site-specific recombination between the flanking DRS, which are recognized by integrases and excisionases/RDFs [10,24–26], whose genes can be located inside or outside the PAI itself [27]. The excision results in the formation of a circular, extrachromosomal element, which keeps one copy of the DRS (also called *attP*). The other DRS copy remains in the bacterial chromosome and it is called *attB* (Fig. 1B). If the excision is precise, the genomic sequence will return to its original state, before PAI insertion. The circular element can re-integrate into the chromosome or remain episomal, to eventually segregate and get lost (Fig. 1A and B) [8,20,27]. In addition, it was recently observed that some of these elements could be transferred by conjugation [28,29]. Due to the characteristics described above, the isolation of pathogenic bacterial strains lacking a complete PAI has been reported under laboratory conditions or from clinical samples, although the excision frequency is very low [8,12,28,30,31].

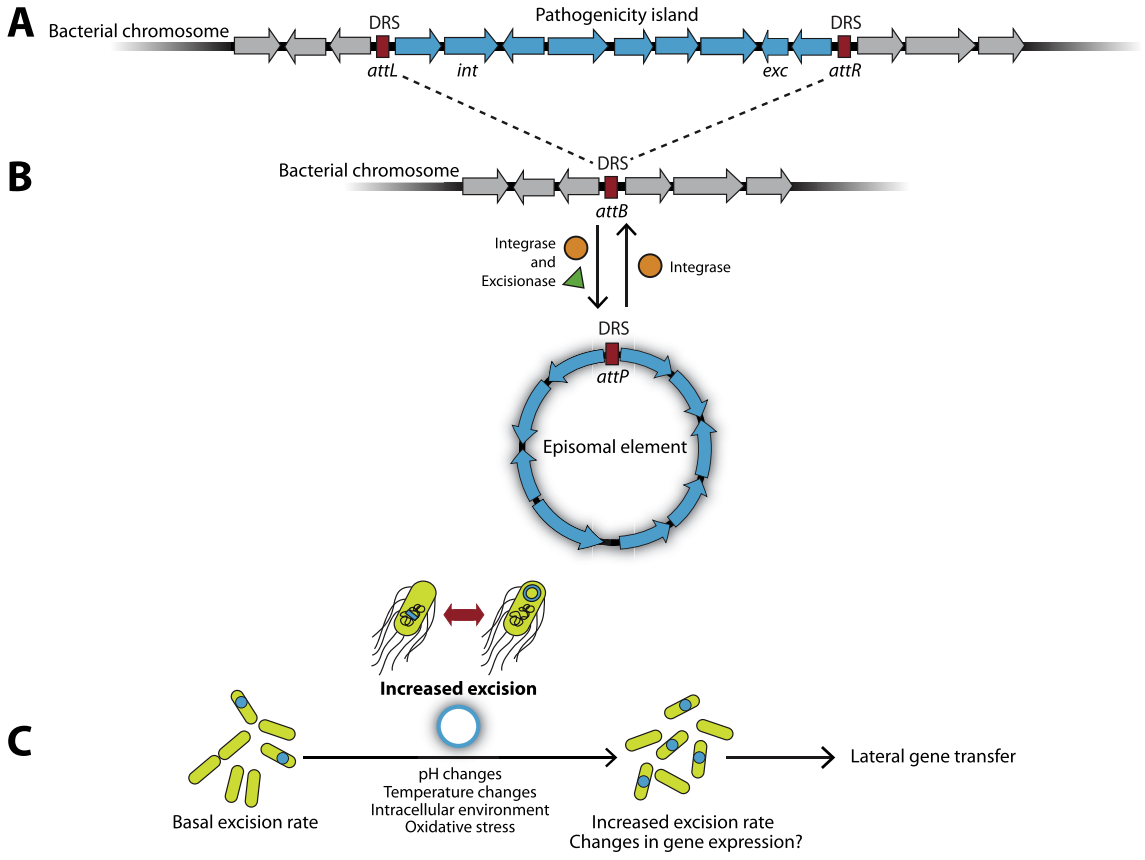

Fig. 1. **The excision process of pathogenicity islands (PAIs).** (A) PAIs are genomic fragments harboring virulence genes in pathogenic bacteria. Some PAIs have the ability to excise from the bacterial chromosome, and in this review they have been denominated "excisable PAIs". This ability is due to the presence of Direct Repeated Sequences (DRS) in their ends, also named *attL* (left DRS) and *attR* (right DRS). DRS/*att* sites are recognized by integrases and excisionases/recombination directionality factors that catalyze the excision. (B) The excision of the PAI generates an episomal element that contains one of the *att* sites (*attP*), while the other *att* site remains in the chromosome (*attB*). (C) The basal excision rate is low, but it can increase due to changes in pH, temperature, intracellular environment, and oxidative stress. An increased excision rate could lead to changes in the expression of the island genes, and also increase passage of the episomal element to other bacteria, through lateral gene transfer.