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THE MOLECULAR BASIS OF INFECTIOUS DISEASES: PATHOGENICITY ISLANDS AND OTHER MOBILE GENETIC ELEMENTS

A REVIEW*

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Bacterial genomes generally consist of stable regions termed core genome, and variable regions that form the so-called flexible gene pool. The flexible part is composed of bacteriophages, plasmids, transposons as well as unstable large regions that have been termed genomic islands. Genomic islands encoding virulence factors of pathogenic bacteria have been designated "pathogenicity islands". Pathogenicity islands were first discovered in uropathogenic *Escherichia coli* and presently more than 30 bacterial species carrying pathogenicity islands have been described. This review summarises the current knowledge on bacterial genomic islands and their general features, and discusses their putative role in the evolution of microbes in the light of genomics of pathogenic bacteria.

Keywords: flexible gene pool, genomic islands, pathogenicity islands, evolution

1. Introduction

The typical bacterial genome consists of a core part containing the genes encoding essential functions such as DNA replication, cell division, ribosomal proteins etc. and a flexible gene pool encompassing genes that are only required

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under certain environmental conditions. While the core genes have a relatively homogenous G+C content and are located in stable regions of the chromosome, the flexible gene pool is often located on mobile genetic elements [1]. Plasmids represent one class of mobile genetic elements that have the capacity of autonomous replication and of conjugative transfer. Furthermore, bacteriophages may have the capacity to be incorporated into the chromosome of bacteria where they exist as prophages. After induction, prophages can be transferred via transduction. So-called "conjugative transposons" have the capacity to be transferred from one bacterium to the other but they are not able to replicate autonomously [2]. Finally, integrons as well as IS elements and "classical" transposons belong to the flexible gene pool.

Several years ago, it was demonstrated that certain regions of bacterial genomes are unstable because they can be deleted with relatively high frequencies [3]. Later on it was shown that such regions exhibit features typical for mobile genetic elements. These elements, which have been termed "genomic islands" (GIs) contain specific structures distinguishing them from the rest of genome [4, 5]. The first GIs have been described for pathogenic *E. coli* bacteria that have the capacity to cause urinary tract infections. They have been termed "pathogenicity islands" (PAIs) because the functions encoded by these islands contribute to pathogenicity [6].

2. Pathogenicity islands of uropathogenic *Escherichia coli*

2.1. Escherichia coli as a model to study pathogenicity

Escherichia coli is a normal inhabitant of the gut of humans and animals. In contrast to commensal *E. coli*, pathogenic strains have the capacity to cause infectious diseases. Intestinal pathogenic *E. coli* (IPEC) cause infections of the gut including cholera- and shigellosis-like diseases. Furthermore, certain *E. coli* strains are able to cause infections outside the gut. The group of extraintestinal pathogenic *E. coli* (ExPEC) causes urinary tract infections (UTI) in humans and animals, as well as sepsis and meningitis.

Uropathogenic *E. coli* (UPEC) represent, from a quantitative point of view, the most important group of bacterial pathogens in industrialised countries. In Germany, more than two million cases of UTI are documented per year. UPEC produce virulence factors that allow them to infect the bladder as well as the kidney. Specific fimbrial adherence factors, e.g. P-fimbriae, S-fimbriae and others enable

the bacteria to adhere to the epithelium of the urinary tract. Capsules and specific membrane proteins play a role in serum resistance and immune evasion. Furthermore, toxins such as α-hemolysin or the cytotoxic necrotizing factor (CNF) play a role in tissue destruction [7, 8]. Some of the bacteria have also the capacity to invade eukaryotic cells. Iron-uptake systems, such as enterobactin, yersiniabactin or aerobactin are important for a successful adaptation of the bacteria to the conditions in the urinary tract [9]. As the majority of these factors are produced by UPEC but not by commensal strains, *E. coli* represents an excellent model to study the specific features of pathogenic microbes.

2.2. Pathogenicity islands of E. coli strain 536

In our laboratory we analyse UPEC strain 536, isolated from a case of pyelonephritis more than 15 years ago. This strain belongs to the serotype O6:K15 and produces a number of virulence factors such as S-fimbriae, P-fimbriae and type 1 fimbriae. Furthermore, two α-hemolysins and four iron-uptake systems as well as the K15 capsule are produced by strain 536 (see Figure 1). Using genetic methods, we were able to demonstrate that the genes encoding these virulence factors are located on large regions of the chromosome which have been termed "pathogenicity islands" (PAIs) [6, 10]. As shown in Table I, five PAIs of strain 536 have been described up to now. PAI I_{536} carries the genes responsible for

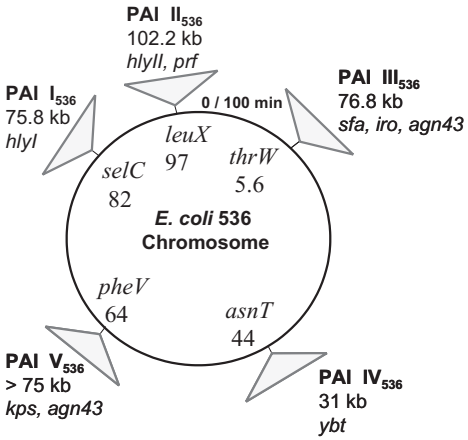

Figure 1. Pathogenicity factors of UPEC strain 536. The respective factors are indicated. In the genome the presence of five pathogenicity islands (PAIs) is given

Table I

Main features of PAIs of UPEC strain 536

Designation	Insertion site (unit)	Target tRNA	Size (kb)	Virulence encoded genes	Integrase * cryptic	Boundary
PAI I_{536}	82	*selC*	75.8	α-hemolysin	CP4-like*	DR
PAI II_{536}	97	*leuX*	102.2	α-hemolysin P-fimbriae	P4	DR
PAI III_{536}	5.6	*thrW*	76.8	S-fimbriae Ag43	Sfx	DR
PAI IV_{536}	44	*asnT*	31	Yersiniabactin	P4-like*	–
PAI V_{536}	64	*pheV*	> 75	Capsule Ag43	P4-like	(DR)

α-hemolysin. PAI II_{536} also encodes α-hemolysin in addition to P-fimbriae. S-fimbriae are encoded by PAI III_{536}. PAI IV_{536} is identical to the core of the so called "high-pathogenicity island" (HPI) which was first described in pathogenic *Yersinia* species that encodes the iron uptake system yersiniabactin [11]. PAI V_{536} is responsible for the production of the capsular antigen. All PAIs are located in specific regions of the chromosome, they are linked to tRNA genes and (with the exception of PAI IV_{536}) are flanked by direct repeats at their ends. Interestingly, some of the PAIs are unstable, that means they can be deleted from the chromosome with a relatively high frequency [12].

3. General features of pathogenicity islands

3.1. Physical characteristics

Following the detection of the first PAIs in UPEC in 1990, PAIs have also been identified in other *E. coli* pathotypes as well as in different Gram-positive and Gram-negative bacteria [reviewed in 13]. Based on this knowledge, it is possible to summarise the characteristics common to most of the PAIs of different pathogens. PAIs have been defined as (i) large genomic regions that (ii) carry virulence associated genes, (iii) have a G+C content different from the rest of the chromosome, (iv) are frequently associated with tRNA genes, (v) are often flanked by repeat structures, (vi) contain mobility genes such as integrase and transposase genes and (vii) are often unstable. It is worth mentioning that not all of the PAIs of more than 30 species will exhibit all the features indicated above. Nevertheless, the majority of PAIs show these physical features demonstrating that they have been acquired by horizontal gene transfer.

3.2. Virulence traits encoded by PAIs

The PAIs of UPEC strains have already been described in detail (see paragraph 2.2). Similarly, PAIs from other *E. coli* pathotypes and other species may carry genes specific for adherence factor genes. Besides P- and S-fimbriae, the "locus of enterocyte effacement" (LEE) of enteropathogenic and enterohemorrhagic *E. coli* (EPEC, EHEC) encodes an adherence factor called intimin [14]. Furthermore, type 4 pili of *Vibrio cholerae* are encoded on a PAI [15].

PAIs often encode secretion systems that are essential for the transport of virulence factors to the surface or directly to the host cells. In Gram-negative bacteria the genes encoding type III and type IV systems are frequently found on PAIs. This is especially true for *Salmonella enterica*-specific PAIs, the LEE islands of EHEC and EPEC isolates as well as for plant pathogens of the *Pseudomonas* group [14, 16, 17, 18]. Type IV secretion systems have been found to be essential for full virulence in several pathogens such as *Helicobacter pylori*, *Bordetella pertussis*, *Legionella pneumophila* and *Agrobacterium tumefaciens* [19]. Toxin genes are also frequently located on mobile genetic elements, such as bacteriophages or plasmids and consequently they are also associated with PAIs [20]. The α-hemolysin produced by UPEC has already been described. The activity of the cytolysin encoded by a recently described PAI of *Enterococcus facealis* is based on a similar mechanism [21]. Furthermore, toxins encoded on PAIs have been described for *Shigella flexneri* and *Stapylococcus aureus* [22, 23].

In enterobacteria, capsular antigen determinants are also located on PAIs [24]. This may also be the case for non-enterobacterial species. The acquisition of iron is required for bacteria to multiply in ecological niches of the eukaryotic host. In Gram-negative bacteria iron-uptake systems are frequently encoded by PAIs. This is true for pathogenic *E. coli* as well as for other enterobacteria. Interestingly, the *ybt*-genes encoding yersiniabactin are located on the so-called "high pathogenicity island" (HPI) which has meanwhile been detected in many enterobacterial species [25, 26]. In some *E. coli* and *S. flexneri* isolates the hydroxamate siderophore aerobactin which is associated with increased virulence in enteric bacteria is also encoded on PAIs [27, 28].

3.3. Other functions encoded by PAIs

Several PAIs encode regulatory proteins that control their respective virulence genes, but also act on loci outside the island. This is true for regulators of

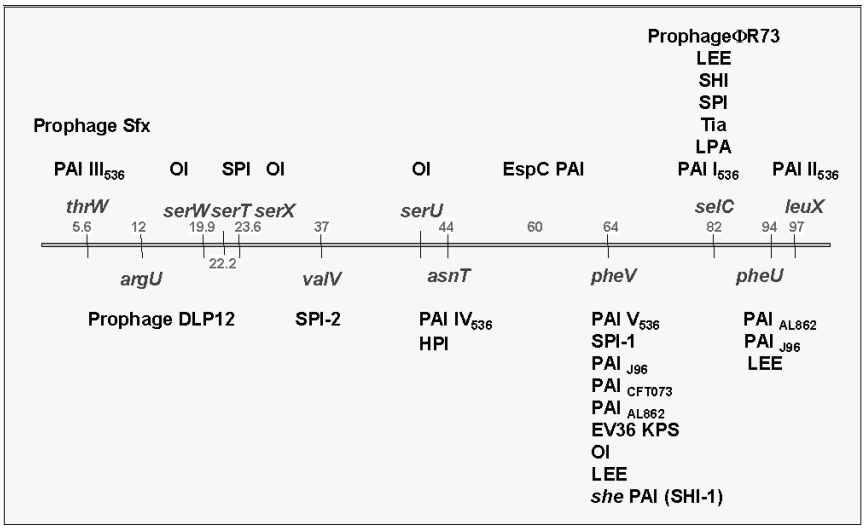

Figure 2. Locations of various PAIs in enterobacteria. The numbers indicate units corresponding to the *E. coli* K-12 genome. The letters above the units represent tRNA genes. The respective PAIs as well as the presence of inserted prophages are indicated. Abbreviations: OI, O – island; SPI – *Salmonella* pathogenicity island; for other abbreviations see text

Vibrio cholerae and for genes that regulate fimbrial adherence molecules of pathogenic *E. coli* [15, 29]. Furthermore, so-called "mobility genes" are often parts of PAIs. Most frequently, tyrosine recombinases are associated with PAIs. As such factors are very often encoded by bacteriophages, the presence of these integrase genes supports the model that parts of PAIs originate from bacteriophages [13]. Also, IS-element and transposon sequences have been identified on various PAIs. At least in Gram-negative bacteria, tRNA genes are often associated with PAIs. Figure 2 summarises insertion points of PAIs in enterobacteria. It has been wondered why tRNA genes are preferred integration sites of PAIs. It seems that the high conservation of the nucleotide sequences of tRNA genes is one reason for their usage as integration sites.

4. PAIs and microbial evolution

4.1. PAIs in non-pathogenic microbes

As already mentioned, PAIs are most frequently found in pathogenic organisms. Some of the PAIs, however, have also been detected in non-pathogenic or-

ganisms. One example is the HPI which has been first described in pathogenic *Yersinia,* but which has also been found in commensal *E. coli* strains and in non-pathogenic isolates of *Salmonella enterica* [25, 26]. These islands have been termed "genomic islands" (GI) and it turns out that GIs enable certain organisms living in a specific niche to enhance their adaptation. Thus, iron-uptake systems serve as fitness factors and promote cellular metabolism under iron-limiting conditions. In the case of pathogenic bacteria, the siderophore systems serve as additional virulence factors. In other bacteria, the genomic islands could not be regarded as PAIs as they contribute to increased colonisation of bacteria in the new niche rather than to their pathogenicity. GIs have been described for many bacterial species. From our point of view, GIs represent formerly transferred mobile genetic entities that have evolved from horizontal gene transfer and DNA recombination events.

4.2. PAIs and "evolution in quantum leaps"

Pathogenic and non-pathogenic species of the same genera often differ only with respect to certain virulence factors which may be encoded by PAIs. Furthermore, pathogenic and non-pathogenic variants of the same species can be described with respect to loss and gain of these large virulence blocks. Table II shows a few examples of pathogenic and non-pathogenic variants of the same or related species. It seems that the acquisition of defined genetic elements, e.g. plasmids, bacteriophages, integrons or PAIs is responsible for the differences in the virulence of the respective strains. The process of the development of the different pathotypes has been defined as "evolution in quantum leaps". Therefore, mobile or mobilisable genetic elements contribute to horizontal gene transfer and conse-

Table II

Examples for non-pathogenic and pathogenic variants of the same or related species

Non-pathogenic	Pathogenic
Listeria innocua	*Listeria monocytogenes*
Staphylococcus carnosus	*Stapyhlococcus aureus*
Neisseria lactamica	*Neisseria meningitidis*
Corynebacterium diphtheriae	*Corynebacterium diphtheriae*
Dtx^-	Dtx^+
Staphylococcus epidermidis	*Staphylococcus epidermidis*
Ica^-	Ica^+
E. coli K-12	Intestinal, extraintestinal *E. coli*

quently to the evolution of pathogens. The occurrence and genetic organisation of PAIs mirror the importance of gene acquisition and genome reduction events in microbial evolution as they exhibit several features of horizontal gene transfer as well as of the accessory genetic elements. Therefore, PAIs represent one example of the process of evolution of microbial genomes.

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