Ecological fitness, genomic islands and bacterial pathogenicity

A Darwinian view of the evolution of microbes

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The compositions of bacterial genomes can be changed rapidly and dramatically through a variety of processes including horizontal gene transfer. This form of change is key to bacterial evolution, as it leads to 'evolution in quantum leaps'. Horizontal gene transfer entails the incorporation of genetic elements transferred from another organism—perhaps in an earlier generation—directly into the genome, where they form 'genomic islands', i.e. blocks of DNA with signatures of mobile genetic elements. Genomic islands whose functions increase bacterial fitness, either directly or indirectly, have most likely been positively selected and can be termed 'fitness islands'. Fitness islands can be divided into several subtypes: 'ecological islands' in environmental bacteria and 'saprophytic islands', 'symbiosis islands' or 'pathogenicity islands' (PAIs) in microorganisms that interact with living hosts. Here we discuss ways in which PAIs contribute to the pathogenic potency of bacteria, and the idea that genetic entities similar to genomic islands may also be present in the genomes of eukaryotes.

Introduction

Bacteria, which have existed for more than 3 billion years, represent the most ancient forms of life on the earth. The enormous evolutionary potential of these organisms is illustrated by the fact that the innumerable species currently living differ in many properties including metabolic capacities, cell surface compositions, life styles, ecological niches and host specificities (Doolittle, 1999). From a Darwinian point of view, every living organism is a result of the driving forces of evolution, which include the plasticity of the genome and the rate of phenotype generation, as well as the selective pressures exerted by the environment (Arber, 2000). The capacity for change, as determined by these factors, forms the basis of evolutionary progress.

In eukaryotes, genetic variability is primarily the result of sexual reproduction, which involves chromosomal recombination during meiosis. In prokaryotes, where this form of shuffling is not available, other factors determine the rate of evolution. These include the frequent occurrence of point mutants, high levels of recombination and gene silencing, and the transfer of genetic material between different bacterial species—even genera. In particular the latter process, referred to as horizontal gene transfer, represents a cornerstone of bacterial evolution, and it has led to dramatic changes in the composition of microbial genomes over relatively short time periods (Ochman et al., 2000).

Prokaryotic genomes: core and flexible gene pools

Bacterial genes may be transmitted between different organisms via conjugation, transduction and natural transformation. The former two processes require specific gene ferries, such as plasmids or bacterial viruses, which transport bacterial DNA along with their own sequences from donor to recipient cells. The majority of the horizontally transferred DNA is part of the flexible bacterial gene pool. In addition to phages and plasmids, the flexible gene pool comprises conjugative transposons, 'simple' transposons, integrons, 'genomic islets' (<10 kb), and 'genomic islands' (>10 kb) (Figure 1). In contrast, the core gene

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The DNA elements comprising the core as well as the flexible gene pools are presented in the circles. Functions encoded by the pools are given in the lower part of the diagram.

Genomic islands: elements of the flexible gene pool

In recent years, ‘pathogenicity islands’ (PAIs) have attracted a great deal of attention (Kaper and Hacker, 1999). First described in the genomes of pathogenic E. coli, they were subsequently also found in other pathogens, where they form specific entities associated with bacterial pathogenicity (Blum et al., 1994). Sequencing of several entire genomes revealed that PAIs are much more widespread than previously thought, and represent a paradigm of more general genetic entities that are present in the genomes of many bacterial species and are termed genomic islands (Strauss and Falkow, 1997; Hacker and Kaper, 2000).

Genomic islands are part of the flexible bacterial gene pool and are somewhere between 10 and 100 kilobases (kb) in length (see Figure 2). They frequently harbor phage- and/or plasmid-derived sequences, including transfer genes or integrase and IS elements. These particular blocks of DNA are most often inserted into tRNA genes and may be unstable. This instability appears to be mediated by flanking direct repeats which are often homologous to phage attachment sites and promote integration into, and excision out of, the bacterial genome (Hacker et al., 1997; Buchrieser et al., 1998). In addition to mobility loci, genomic islands carry gene clusters with specific functions. As for other elements of the flexible gene pool, the majority of these islands differ from the core genome with respect to their G+C content and codon usage.

A wide range of functions

Sequence analysis revealed that genomic islands carry selfish genes, especially of the type that encode proteins with transfer, recombination and restriction/modification properties. However, the majority of the clusters located on these genetic elements encode functions that can be useful for the survival and
Transmission of the microbes (Table I). Thus, they may provide a selective advantage to the island-carrying organisms within a population. For instance, DNA elements encoding sucrose-uptake in *Salmonella senftenberg* are necessary for metabolic adaptation of these bacteria to their hosts (Hochhut et al., 1997). Other genomic islands encode iron-uptake systems which enhance the capacity of bacteria to grow and disseminate in the soil or in a host. This holds true for many enterobacteria and for bacteria of the *Pseudomonas* group, which are part of the plant rhizosphere. Other *Pseudomonas* strains carry genomic islands that encode enzymes involved in degradation of phenolic compounds (Ravatn et al., 1998). Genomic islands may also carry genes encoding factors that confer resistance to antimicrobial substances. For example, the *mecA*-region of staphylococci enhances survival of its carriers, both in soil compartments in which antibiotic-producing microbes exist, and in hospitals with strong antibiotic pressure (Ito et al., 1999). In addition, the symbiosis islands of rhizobia carry nitrogen fixation genes whose products are necessary for the interactions of the bacteria with plant cells (Sullivan and Ronson, 1998). Other genomic islands encode toxins or adherence factors involved in pathogenicity.

**Table I. Functions encoded by fitness islands**

<table>
<thead>
<tr>
<th>Subtypes of fitness islands</th>
<th>Function</th>
<th>Organism</th>
<th>Increased pathogenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAI</td>
<td>iron uptake</td>
<td><em>Yersinia</em> spp.</td>
<td>+</td>
</tr>
<tr>
<td>SAI</td>
<td>iron uptake</td>
<td>fecal <em>E. coli</em></td>
<td>–</td>
</tr>
<tr>
<td>ECI</td>
<td>iron uptake</td>
<td><em>Klebsiella</em> spp.</td>
<td>–</td>
</tr>
<tr>
<td>ECI</td>
<td>sucrose uptake</td>
<td><em>Salmonella senftenberg</em></td>
<td>–</td>
</tr>
<tr>
<td>ECI</td>
<td>degradation of phenols</td>
<td><em>Pseudomonas putida</em></td>
<td>–</td>
</tr>
<tr>
<td>PAI</td>
<td>toxin production</td>
<td><em>Vibrio cholerae</em></td>
<td>+</td>
</tr>
<tr>
<td>SAI</td>
<td>adhesins</td>
<td>fecal <em>E. coli</em></td>
<td>–</td>
</tr>
<tr>
<td>PAI</td>
<td>adhesins</td>
<td>urinary <em>E. coli</em></td>
<td>–</td>
</tr>
<tr>
<td>ECI</td>
<td>methicillin resistance</td>
<td><em>Staphylococcus aureus</em></td>
<td>–</td>
</tr>
<tr>
<td>ECI</td>
<td>multi-resistance</td>
<td><em>Shigella flexneri</em></td>
<td>–</td>
</tr>
<tr>
<td>SYI</td>
<td>nitrogen fixation</td>
<td><em>Mesorhizobium loti</em></td>
<td>–</td>
</tr>
<tr>
<td>PAI</td>
<td>type III-system</td>
<td><em>Salmonella enterica</em></td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>type III-system</td>
<td><em>Shigella flexneri</em></td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>type III-system</td>
<td><em>Yersinia</em> spp.</td>
<td>+</td>
</tr>
<tr>
<td>SYI</td>
<td>type III-system</td>
<td><em>Sinorhizobium fredii</em></td>
<td>–</td>
</tr>
<tr>
<td>PAI</td>
<td>type IV-system</td>
<td><em>Helicobacter pylori</em></td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>type IV-system</td>
<td><em>Legionella pneumophila</em></td>
<td>+</td>
</tr>
<tr>
<td>EAI</td>
<td>type IV-system</td>
<td><em>E. coli</em> F-plasmid</td>
<td>–</td>
</tr>
</tbody>
</table>

PAI, pathogenicity island; SYI, symbiosis island; SAI, saprophytic island; ECI, ecological island.

Increasing bacterial fitness and driving evolution

The progress of evolution is determined by an increase in the fitness of the organism. Fitness, in this context, is considered to be a set of properties that enhance the survival, spread, and/or transmission of an organism within a specific ecological niche (Preston et al., 1998). The Darwinian laws (‘survival of the fittest’) are valid for the development of eukaryotes as well as for prokaryotes (Arber, 2000). Therefore, carrying a genomic island may provide a selective advantage under specific environmental conditions (stress, *in vivo* conditions, exposure to antibacterial substances) because it enhances microbial transmission, survival or colonization within a niche. From a functional point of view, then, genomic islands that increase the fitness of the recipient microbes should be termed ‘fitness islands’, as already suggested by Preston et al. (1998) (Figure 3). Under these circumstances, genomic, fitness islands confer new properties which enhance the adaptational capacity of their bacterial host.

Fitness islands can be subdivided into different subsets, depending on the life-style of the microbe (its niche) (Figure 3), rather than on the intrinsic composition of the islands. Fitness islands that help microorganisms to live in the environment or to persist as saprophytes in a host may be considered ‘ecological islands’ and ‘saprophytic islands’, respectively. Other bacteria reside temporarily or permanently in a host (another microorganism, a plant or an animal), where they either provide some benefits to the host-organism (symbiont) or cause damage to it (pathogen). Accordingly, a ‘symbiosis island’ is a specific type of fitness island that helps bacteria to positively interact with their hosts, while a fitness island that participates directly or indirectly in the induction of lesions is a true pathogenicity island.

Pathogenicity islands may influence microbial evolution

PAIs represent a subset of genomic islands and share the same general composition and organization (Hacker and Kaper,
The actions of these pathogenicity factors seems to result from direct evolutionary pressures. This is true not only for enteric, but also respiratory pathogens, where the action of pathogenicity factors supports their transmission and therefore positively influences microbial evolution.

**Contributions to ecological adaptation and to pathogenesis**

As already mentioned, the division of fitness islands into different subtypes is not based on their intrinsic genetic composition, but on their effects in a specific niche and within a particular organism. In other words, the same fitness island may act as an ecological island when the bacterial recipient resides outside of a host, but become a pathogenicity island when the bacterium enters a host. For example, the genes encoding an iron-uptake system termed yersiniabactin are part of a genomic island that was first identified in highly pathogenic strains of the genus *Yersinia* (Carniel et al., 1996). This 'high pathogenicity island' (HPI), however, is not only present in pathogenic *yersiniae*, but also in harmless *E. coli* of the intestinal flora and in *Klebsiella* from the soil (Schubert et al., 1998; Bach et al., 2000). The iron-uptake system seems to have evolved to adapt certain enterobacteria specifically to iron-limiting conditions. In bacteria that reside in the environment, this island can be considered as an ecological island with a role in cellular metabolism. If, on the other hand, the island is present in a bacterium with a host, and it carries additional virulence features, it is a pathogenicity island. If it is integrated in the chromosome of a non-virulent bacterium, it may constitute a saprophytic island.

Like the iron-uptake system, adhesins may exhibit 'dual' functions in bacteria (Finlay and Falkow, 1997). For example, certain adherence factors in *E. coli* (e.g. P-, S-, and F1C-fimbriae) are encoded by genomic islands and are produced by commensal strains that are part of the normal human gut flora (Hacker, 2000). If the adhesins are involved in colonization of the gut, the genetic entity is a saprophytic island. Under special circumstances, however, P-, S- or F1C-positive *E. coli* may reach the urinary tract, where they cause infections of the bladder or the kidney (Khan et al., 2000), becoming true PAIs. In other words, the PAIs of uropathogenic *E. coli* were originally selected as 'pure' fitness islands in the gut, but then helped a particular bacterial pathotype to emerge as the microbes colonized a new niche, the kidney or the bladder.

Other PAIs carry genes whose products form secretion systems of type III or IV. Again, if these secretion systems transport proteins involved in the infectious process, they can be considered PAIs. This is true for strains of the *Salmonella*- *Galán and Collmer*, 1999), *Shigella* - (Parsot and Sansonetti, 1999), and *Yersinia*-groups (Cornelis et al., 1998) for the type III system, and for *Legionella pneumophila* (Vogel et al., 1998) and *Helicobacter pylori* (Cesini et al., 1996) for the type IV system (Table I). In other words, the secretion systems transport proteins or even DNA molecules of non-pathogenic organisms, as in the case of the type III system of *rhizobia*, or the type IV system of F plasmids, they do not form PAIs but rather symbiotic islands or ecological islands which enhance the fitness of bacteria in their natural niche (Preston et al., 1998). Therefore, the subtypes of fitness islands depend on several criteria including not only the genetic
composition of the island itself, but also the genetic background of its bacterial host, and the ecological habitat of the microorganism.

Driving bacterial evolution

PAIs have been selected during evolution because their presence conferred selective advantages to their bacterial host. However, in some instances, their acquisition might subsequently have oriented the evolution of their host bacteria. This has probably been the case for Y. pestis, the agent of plague. Y. pestis is a highly clonal species that emerged recently (1500 to 20 000 years ago) from Y. pseudotuberculosis (Achttmann et al., 1999). In contrast to its progenitor which uses the oral route to contaminate human and animal hosts, Y. pestis is transmitted by flea bites and the sepsicaemia that systematically occurs in the host at the pre-mortem stage of plague is a prerequisite for Y. pestis transmission by fleas. By promoting the systemic dissemination and thereby the efficient transmission of the bacteria in vivo, the HPI presumably served as one of the key factors in the emergence of this highly dangerous microorganism. In other words, Y. pestis would probably not have evolved from Y. pseudotuberculosis if the genome of the latter had not already harbored the HPI.

Genomic islands in eukaryotes?

Horizontal gene transfer represents an important mechanism in the evolution of eubacteria, and genomic islands belong to the group of genetic elements that are involved in evolutionary progress. Recently, it has become evident that laterally transferred DNA is also present in the genomes of archeabacteria (Doolittle, 1999), and questions regarding a role for horizontal gene transfer in the evolution of eukaryotes (de la Cruz and Davis, 2000; Kurland, 2000) have arisen. From our point of view, there are good indications that this is the case, and genetic elements with features of genomic islands have been found in eukaryotic genomes. First, mobile genetic elements such as retrottransposons are present even in the genomes of mammals, where they have the capacity to jump into 3′ ends of tRNA genes, a process that was first identified in bacterial genomes. Secondly, the well-characterized Ti plasmid is able to transfer genes, a process that was first identified in bacterial genomes. Therefore, we can envisage that some of the features of genomic islands have been selected during evolution because their composition of the island itself, but also the genetic background of its bacterial host, and the ecological habitat of the microorganism.

References


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