Genomic islands: tools of bacterial horizontal gene transfer and evolution

Mario Juhas1, Jan Roelof van der Meer2, Muriel Gaillard2, Rosalind M. Harding3, Derek W. Hood4 & Derrick W. Crook1

1Clinical Microbiology and Infectious Diseases, Nuffield Department of Clinical Laboratory Sciences, University of Oxford, Oxford, UK; 2Department of Fundamental Microbiology, University of Lausanne, Lausanne, Switzerland; 3Departments of Zoology and Statistics, University of Oxford, Oxford, UK; and 4Molecular Infectious Diseases Group, The Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, UK

Correspondence: Mario Juhas, Clinical Microbiology and Infectious Diseases, Nuffield Department of Clinical Laboratory Sciences, University of Oxford, Headington, Oxford OX3 9DU, UK. Tel.: +44 1865 222194; fax: +44 1865 222195; e-mail: mario.juhas@ndcls.ox.ac.uk

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Abstract

Bacterial genomes evolve through mutations, rearrangements or horizontal gene transfer. Besides the core genes encoding essential metabolic functions, bacterial genomes also harbour a number of accessory genes acquired by horizontal gene transfer that might be beneficial under certain environmental conditions. The horizontal gene transfer contributes to the diversification and adaptation of microorganisms, thus having an impact on the genome plasticity. A significant part of the horizontal gene transfer is or has been facilitated by genomic islands (GEIs). GEIs are discrete DNA segments, some of which are mobile and others which are not, or are no longer mobile, which differ among closely related strains. A number of GEIs are capable of integration into the chromosome of the host, excision, and transfer to a new host by transformation, conjugation or transduction. GEIs play a crucial role in the evolution of a broad spectrum of bacteria as they are involved in the dissemination of variable genes, including antibiotic resistance and virulence genes leading to generation of hospital ‘superbugs’, as well as catabolic genes leading to formation of new metabolic pathways. Depending on the composition of gene modules, the same type of GEIs can promote survival of pathogenic as well as environmental bacteria.

Introduction

Genomes of bacterial species can evolve through a variety of processes including mutations, rearrangements or horizontal gene transfer. Information gathered over the past few years from a rapidly increasing number of sequenced genomes has shown that besides the core genes encoding essential metabolic functions, bacterial genomes also harbour a variable number of accessory genes acquired by horizontal gene transfer that encode adaptive traits that might be beneficial for bacteria under certain growth or environmental conditions (Schmidt & Hensel, 2004).

Many of the accessory genes acquired by horizontal transfer form syntenic blocks recognized as genomic islands (GEIs). GEIs are typically recognized as discrete DNA segments between closely related strains, but it is currently thought that their formation contributes to the diversification and adaptation of microorganisms, thus having a significant impact on the genome plasticity and evolution, the dissemination of antibiotic resistance and virulence genes, and formation of catabolic pathways. More and more DNA elements are being detected by bacterial genome sequencing projects, and, at the same time, more information has become available on the life-style of GEIs. This review will summarize recent advances in our understanding of their distribution, evolution and mechanistic modes of behaviour. One of the emerging ideas is that GEIs comprise an overarching family of elements, including previously recognized mobile DNA elements such as integrative and conjugative elements (ICEs), conjugative transposons and some prophages. One GEI-type, a class of highly conserved ICEs occurring in Beta- and Gammaproteobacteria will be particularly highlighted.

General features of GEIs

GEIs are in essence discrete DNA segments differing between closely related bacterial strains to which usually some
past or present mobility is attributed. If we accept this
definition it will comprise an overarching family of elements
with different functional life-styles. The concept of patho-
genicity islands (PAIs) was originally coined in the late 1980s
by J. Hacker and colleagues, who investigated the genetic
basis of virulence of uropathogenic isolates of Escherichia
coli (UPEC) (Hacker et al., 1990). PAIs found in their study
were unstable chromosomal regions with variable virulence-
associated characteristics and phenotypes (Groisman &
Ochman, 1996). Nowadays, it is appreciated that GEIs
represent a much broader and more diverse group of DNA
elements than PAIs only, with a large variety of sizes and
abundance in bacterial genomes (Dobrindt et al., 2004).
Different GEI families, some probably evolutionarily very
ancient, have been recognized on the basis of predicted
sequence and functional homologies (Burrus et al., 2002;
Juhas et al., 2007b; Vernikos & Parkhill, 2008). The coding
capacity of GEIs is not limited to pathogenicity functions,
but can be very diverse, including such traits as symbiosis
(Sullivan et al., 2002), sucrose and aromatic compound
metabolism (Gaillard et al., 2006), mercury resistance and
siderophore synthesis (Larbig et al., 2002b). Bioinformatics
studies have shown that GEIs tend to carry more 'novel'
genes (i.e. those that do not have orthologues in other
species) than the rest of the genome (Hsiao et al., 2005).
This suggests that GEIs have become strongly selected for
adaptive and auxiliary functions. The fact that GEIs come in
a large spectrum of varieties in terms of genetic organization
and functionality makes it more difficult to provide an exact
definition of a GEI. Here, we propose that the term GEI
should be used for the overarching family of discrete 'DNA
elements', which are part of a cell's chromosome and can
drive or have driven strain differentiation.

Most GEIs known to date share the following features
(Fig. 1):

1. GEIs are relatively large segments of DNA, usually be-
tween 10 and 200 kb detected by comparisons among closely
related strains. Discrete DNA regions detected by comparative
genome sequencing with sizes smaller than 10 kb have been
named genomic islets (Hacker & Kaper, 2000).

2. GEIs may be recognized by nucleotide statistics (e.g. GC
content, cumulative GC skew, tetranucleotide frequencies or

Fig. 1. General features of GEIs. GEIs are relatively large segments of DNA whose nucleotide characteristics often differ from the rest of
the chromosome. GEIs are often inserted at tRNA genes and flanked by DR. GEIs typically harbour genes encoding factors involved in genetic mobility, such
as integrases, transposases and insertion sequences (IS). According to their gene content, GEIs can be described as pathogenicity, symbiosis, metabolic,
fitness or resistance islands.
GEIs are often inserted at tRNA genes, in which case they might be ICEs.

(4) GEIs are often flanked by 16–20-bp perfect or almost perfect direct repeats (DR). DRs usually arise by the site-specific integration of the GEIs into the target site and can act as recognition sequences for their enzymatic excision (Schmidt & Hensel, 2004).

(5) GEIs often harbour functional or cryptic genes encoding integrases or factors related to plasmid conjugation systems or phages involved in GEI transfer.

(6) GEIs often carry insertion elements or transposons, which may have been implicated in mobilizing genetic material onto or deleting DNA from the element (Buchrieser et al., 1998; Gal-Mor & Finlay, 2006).

(7) GEIs often carry genes offering a selective advantage for host bacteria. According to their gene content, GEIs are often described as pathogenicity, symbiosis, metabolic, fitness or resistance islands (Dobrindt et al., 2004; Schmidt & Hensel, 2004).

Evolutionary origins of GEIs

Although most of the GEIs known so far fit the above-described definition, a significant number of elements lack one or more of the hallmark indications. As many GEIs have only been put forward on the basis of data from sequencing projects, and not on experimental evidence of their mode of action, many elements may actually be in an evolutionary state of regression, as has been suggested earlier by Dobrindt et al. (2004). Sequence and phylogenetic comparisons show that GEIs tend to fall within structurally distinct genus-specific families, but looking across different genus boundaries leads to the emergence of universally distributed structural GEI components (Vernikos & Parkhill, 2008). This suggests that GEIs may have arisen multiple times independently during evolution, and can thus only be seen as a superfamily of elements on the basis of analogous core and structural features, rather than being phylogenetically related (Juhas et al., 2007b; Vernikos & Parkhill, 2008). As the original definition of GEIs was put forward 10 years ago when only 12 complete bacterial genomes were available, it is plausible that other GEIs with novel and unusual features will be discovered with the increasing number of bacterial genomes being sequenced. In the more overarching view we propose here, GEI would encompass other categories of elements, such as ICE/conjugative transposons [which were proposed to be one functionally similar group of ICEs (Burrus et al., 2002; Burrus & Waldor, 2004)], integrated plasmids, nonreplicative but excisable elements [nonreplicating Bacteroides unit (NBU) from Bacteroides (Shoemaker et al., 2000)], and perhaps even cryptic or damaged prophages (Fig. 2).

Work over the last few years has suggested that GEIs have had multiple parallel evolutionary origins, as some of them clearly contain phage and others conjugative plasmid-related genes. In addition, it has become clear that mobile elements form by a combination of functional modules, which makes it more difficult to categorize them (Toussaint & Merlin, 2002; Frost et al., 2005; Leplae et al., 2006). For example, as it is common to find multiple prophages in various stages of functionality in a bacterial chromosome, it is plausible that some have cointegrated with the chromosome and slowly acquired other genes of nonphage origin (Dobrindt et al., 2004; Klockgether et al., 2004). Indeed, for a number of GEIs, the similarities between GEI-encoded and known plasmid transfer systems are so striking that one can rightfully assume that the plasmid transfer genes formed the origin for the progenitor hybrid GEI element. This is the case, for example, for the Mesorhizobium loti R7A symbiosis island (Sullivan et al., 2002; Ramsay et al., 2006), the CTn4371 biphenyl transposon of Ralstonia oxalatica (Toussaint et al., 2003) and the SXT element of Vibrio cholerae (Beaber et al., 2002). However, this need not necessarily be the case for all GEIs. Recent experiments with a family of ICEs with deep evolutionary relationships suggested that some of them may have evolutionarily very ancient self-transfer modes for which no current plasmid relatives are known. This conclusion was drawn from observations for three members of this family, namely ICEHin1056 from Haemophilus influenzae, pKLC102 from Pseudomonas aeruginosa and ICEclc from Pseudomonas sp. strain B13. These three elements are fully functional elements, capable of integration into the chromosome of the host, excision and self-transfer by conjugation to a new host and reintegration (Dimopoulou et al., 2002; van der Meer & Sentschilo, 2003; Gaillard et al., 2006; Klockgether et al., 2007; Juhas et al., 2007a). As will be outlined further below,
their self-transfer system forms a distinct type IV secretion system (T4SS), which is only very distantly related to other known plasmid-encoded T4SSs.

**Integration, development and excision of GEIs**

As not all GEIs have the same components, it is difficult to speak of one unifying mode of GEI functioning or life-style (i.e. those functions necessary for maintenance, excision, transfer or integration). Interestingly, a large number of GEIs for which self-mobility has been demonstrated, can excise from their chromosomal location, encode the full capacity for horizontal self-transfer to another cell, and reintegrate into the target site in the new host’s chromosome. GEIs that exhibit simultaneously all these features and self-transfer by conjugation are part of an increasingly well-defined group of elements that have been named ICEs (Burrus & Waldor, 2004). ICEs also include conjugative transposons, a terminology that had been used mostly for elements that originated in gram-positive bacteria, and, according to some authors, should be reserved for elements, which can target multiple different integration sites (Burrus et al., 2002). As some GEIs do not self-transfer by conjugation but by phage packaging, release and infection, they cannot be called ICEs.

The schematic life-style of mobile GEI would thus consist of the following steps (Fig. 3):

1. Acquisition of the GEI by a host through horizontal gene transfer.
2. Integration of the GEI into the host chromosome by site-specific recombination.
3. Development of the GEI by genetic rearrangements, gene loss or acquisition of other mobile genetic elements.
4. Excision of the GEI from the chromosome.
5. Transfer of the GEI to another recipient.

As stated above, many GEIs may be in a state of evolutionary regression, with the result that one or more functionalities are missing. A number of active GEIs have been studied concerning various levels of life-style, coding capacity or regulation of transfer, and the results have contributed to knowledge about the general mechanistic modes of GEIs. For reasons only partially understood, GEIs are often inserted in the 3′-end of tRNA genes (Ravatn et al., 1998a; Larbig et al., 2002b; Williams, 2002). Insertion is catalyzed by site-specific phage-like recombinases called integrases, which are usually encoded by the GEI itself. Integrases are not strictly conserved among all GEIs, and different tRNA genes can be targeted (Williams, 2002). Several GEI-encoded integrases relate to the lambda, P4 or XerD families (van der Meer et al., 2001; Burrus et al., 2002; Mohd-Zain et al., 2004). The integrases are also implicated in the

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**Fig. 3. Integration, development and excision of GEIs.** The schematic life-style of mobile GEI consists of the following steps: (1) acquisition by horizontal gene transfer; (2) integration into the host’s chromosome by site-specific recombination; (3) development of the GEI by genetic rearrangements, gene loss (a) or gene acquisition (b); (4) excision from the chromosome; (5) transfer to another recipient.
excision of GEIs, a process that may be assisted by an excisionase (Burrus & Waldor, 2003; Lesic et al., 2004; Ramsay et al., 2006). The int gene encoding integrase is often situated at one extremity of the island and adjacent to the tRNA gene in the integrated GEI form. Following excision, both GEI ends close up to form a single copy of the recombination site (attP) (Ravatn et al., 1998b; Doublet et al., 2005; Qiu et al., 2006; Ramsay et al., 2006). Excision is mostly nonreplicative and a single copy of the recombination site is again formed on the chromosome (attB) (Burrus & Waldor, 2003; Ubeda et al., 2007). GEI can reintegrate from the excised form back into the attB site of the same host or, after transfer to a new cell, in a suitable attB site in a new cell. Integration is the result of a site-specific recombination between a 15- and 20-bp motif within the 3′ extremity of the tRNA gene (Ravatn et al., 1998a; Williams, 2002). Except in a few cases (Klockgether et al., 2004; Ubeda et al., 2007), excised GEIs do not seem to replicate independently from the host’s chromosome but rely instead on reintegration or horizontal transfer to a new host and reintegration to proliferate. Intermediate forms may also exist, as was demonstrated in a recent study on staphylococcal pathogenicity islands (SaPIs), which are phage-type GEIs. SaPIs, which have intimate relationships with certain temperate phages involving phage-induced excision, replication and packaging, were also proven to be capable of an existence in a self-replicating plasmid-like state (Ubeda et al., 2007, 2008). A large body of knowledge exists on integration and excision processes of conjugative transposons in, for example, Bacteroides, the details of which, however, lie outside the scope of this review.

**Transfer of GEIs between bacteria**

As suggested above, a wide variety of GEIs are intimately connected to phages and conjugative plasmids through their evolutionary origins. As a consequence, besides transformation, their transfer often proceeds via conjugation and transduction (Jain et al., 2002; Chen et al., 2005). GEIs do not necessarily encode the whole process of self-transfer, and several cases are known in which GEIs can be packaged by another coexisting lysogenic phage or mobilized by a plasmid or ICE conjugative system (Shoemaker et al., 2000).

Transformation has been observed in a number of gram-positive or gram-negative, pathogenic or environmental bacteria. Naturally transformable bacteria such as Acinetobacter sp. ADP1, Bacillus subtilis, Streptococcus pneumoniae, Neisseria gonorrhoeae, Pseudomonas stutzeri,Ralstonia solanacearum and H. influenzae take up free DNA from their surrounding environment (Smith et al., 1995; Hamoen et al., 2001; Barbe et al., 2004; Meier & Wackernagel, 2005; Fall et al., 2007). Part of the foreign DNA acquired by natural transformation is degraded, but part could be incorporated into the host’s genome, thus contributing to the evolution of bacterial species. Naturally transformable bacteria need to acquire a physiological state called ‘competence’ through expression of a number of genes encoding DNA uptake and processing systems before transformation. Uptake systems of naturally transformable bacteria are often composed of components resembling subunits of the type IV pili and type II secretion systems (Chen et al., 2005). Several bacteria, including N. gonorrhoeae and H. influenzae, have a strong bias to take up species-specific DNA for transformation. Efficient species-specific DNA uptake in N. gonorrhoeae and H. influenzae requires the presence of an c. 10-nucleotide-long DNA uptake sequence, which is found at a high frequency (c. 1400 copies) in the respective genomes (Goodman & Scocca, 1988; Smith et al., 1995). Recently, a novel T4SS has been identified in N. gonorrhoeae that secretes chromosomal DNA in the surrounding environment in a noncontact-dependent manner (Hamilton et al., 2005). This T4SS is localized in the large, horizontally acquired gonococcal genetic island (GGI) present in the chromosome of N. gonorrhoeae; thus by facilitating chromosomal DNA secretion, this GEI also encodes the mechanism of its own dissemination. Interestingly, the SOS response to antibiotic stress-induced DNA damage has been shown to induce genetic transformability of bacteria and hence to promote horizontal dissemination of antibiotic resistance genes (Beaber et al., 2004; Prudhomme et al., 2006).

Conjugation is the process of DNA transfer from donor to recipient through a specialized apparatus that consists of a cell-envelope spanning translocation channel joined to a tube-like structure known as a pilus in gram-negative bacteria or to the surface-associated adhesins in gram-positive bacteria (Chen et al., 2005). Conjugation systems constitute part of a large and versatile family of T4SS-dependent transport systems (Chen et al., 2005; Christie et al., 2005). T4SSs are usually encoded by multiple genes organized into a single operon. Based on the organization of genetic determinants, shared homologies and evolutionary relationships, T4SSs have been classified into several types (Juhas et al., 2008). Type F and P T4SSs, often referred to as type IVA systems, resemble the archetypal VirB/VirD4 system of Agrobacterium tumefaciens and are considered to be the paradigm of type IV secretion. Type I T4SSs, also referred to as type IVB systems, resembling the archetypal Dot/Icm system are found in two intracellular bacterial pathogens, Legionella pneumophila and Coxiella burnetii (Segal et al., 2005). The most recently described T4SSs that are evolutionarily distant from all previously described GI T4SSs, play a key role in the horizontal transfer of a wide variety of GEIs derived from a broad spectrum of bacteria, including Haemophilus spp., Pseudomonas spp., Erwinia carotovora, Salmonella enterica serovar Typhi, L. pneumophila and others (Juhas et al., 2007a, b, 2008). This lineage of
T4SSs has been named GI-type to emphasize the fact that it was found to be associated only with certain GEIs (Juhas et al., 2007a).

Transduction is the process of DNA transfer from one bacterium to another via bacterial viruses, bacteriophages. Many bacteriophages are able to transfer bacterial genes, including GEIs, as passengers in their genomes. One good example is the SaPI family of Staphylococcus aureus islands (Maiques et al., 2007). Members of this family were shown to be induced to excise and replicate by certain resident temperate phages that also play a role in their packaging into a small phage-like particles (Ubeda et al., 2003, 2007; Maiques et al., 2007) that are transferred from donor to recipient cells at frequencies commensurate with the plaque-forming titer of the phage (Ruzin et al., 2001). Interestingly, SaPlbov2, a member of the SaPI family of GEIs, has been shown to be induced to replicate by different staphylococcal phages, encapsidated and transferred to a variety of recipient bacteria, including different Staphylococcus strains (Maiques et al., 2007). Certain genomic regions of staphylococci resemble slightly deteriorated prophages that could be mobilized by other phages. GEI transfer may mechanistically resemble certain aspects indicative of an existence of phage- and plasmid-like ancestors: the transfer of such islands may include self-replicating plasmid-like states (Ubeda et al., 2007). Several other examples of GEIs from a wide variety of bacterial species have been reported recently to be transferred by bacteriophages, including Yersinia high-pathogenicity island (HPI) of Yersinia pseudotuberculosis (Lesic et al., 2004) or GEIs of the marine cyanobacterium Prochlorococcus (Coleman et al., 2006).

Active GEI transfer has been described in a number of cases, although for the great majority of potential GEIs detected by genome sequencing projects, no information is as yet available on their transfer capacities. For example, conjugative transfer of ICEHin1056, calculated as a number of transconjugants divided by a number of recipients, proceeds at a frequency of around $10^{-1}$ - $10^{-2}$ between two H. influenzae strains (Juhas et al., 2007b). ICEclc of Pseudomonas sp. strain B13, a distant member of the same ICEHin1056 subfamily, is self-transferable at similar frequencies to P. putida, Cupriavidus necator or P. aeruginosa (Gaillard et al., 2006). Recently, another member of this ICEHin1056 subfamily of GEIs, P. aeruginosa pathogenicity island-1 (PAPI-1) was shown to transfer from donor strain into P. aeruginosa recipient strains that do not harbour this island naturally. Furthermore, PAPI-1 has been demonstrated to exist in an extrachromosomal circular form and to reintegrate into the host genome following excision from the chromosome (Qiu et al., 2006). Finally, also the GEI pKLC102 of P. aeruginosa was shown to be highly mobile and capable of transferring at the frequencies similar to ICEclc or ICEHin1056 (Klockgether et al., 2007).

Interestingly, however, no excision or transfer has been demonstrated for other members of this subfamily of GEIs such as PAGI-2 and PAGI-3 (Klockgether et al., 2007).

Recent research indicates that the host background has a strong influence on the transferability of GEIs. The T4SS transfer module was found to be one of the most conserved parts of the ICEHin1056 subfamily of GEIs (Juhas et al., 2007b). However, the analysis of the conjugation transfer frequencies showed that these GEIs were transferred between closely related haemophili strains with significantly different frequencies. To test whether these differences were due to the respective host strain background or to the gene content of the island, the GEIs of haemophili were transferred from the same donor into the same recipient strain. In this setting, the conjugal transfer frequencies were almost constant, which is indicative of the different host strains introducing variations in the conjugal efficiency in the initial experiment (Juhas et al., 2007b). Interestingly, also transfer frequencies of ICEclc were largely dependent on the type of donor cell, even within closely related strains of P. aeruginosa (Gaillard et al., 2008). These studies have clearly shown that host background has a tremendous impact on the transferability of GEIs.

**Regulation of GEIs and adaptive behaviour**

There is still little information available on the regulation of or environmental conditions influencing GEI transfer. In fact, for most GEIs it was initially assumed that transfer would be ‘spontaneous’ or ‘constitutive’. However, it has been suggested that in a number of cases, tightly regulated events underlay the onset of GEI self-mobilization (Jain et al., 2003). This is not so surprising, especially given the high level of control on the life-style of temperate bacteriophages. A better understanding of the conditions for self-transfer, the factors augmenting or decreasing transfer rates, and the (self-)regulation of the onset of the transfer process is of great importance, both for our appreciation of the impact of horizontal gene transfer on the evolution of microorganisms and for the practical purpose of judging the potential distribution of transgenes or antibiotic resistance genes in natural microbial populations (Nielsen & Townsend, 2004). Evidence from the few GEI models studied so far suggests indeed quite the opposite of ‘spontaneous’ behaviour: a variety of regulatory modes and signals, which determine GEI self-transfer.

One of the most striking regulatory modes for GEI behaviour was revealed from studies on the tetracycline determinants in Bacteroides. Conjugative transfer of the ICEs CTnDOT and CTnERL from Bacteroides is stimulated up to 10 000-fold when cells are grown in the presence of tetracycline. This effect was found to be the result of an
induction of two regulatory genes, rteA and rteB, both of which stimulate transcription of a third factor rteC, which influences excision of the element (Cheng et al., 2001; Whittle et al., 2002). From the work on ICEc1c of Pseudomonas sp. strain B13, we know that ICEc1c transfer is strongly enhanced in the stationary phase in a bistable fashion (e.g. only c. 5% of all cells engage in transfer). Transfer of ICEc1c correlates to an increase of expression from the intB13 integrase gene, which is stimulated by the product of the gene inrR (Senthilo et al., 2003a). Upon excision and formation of a circular intermediate, a strong promoter – otherwise located at the other end of ICEc1c facing outwards – is placed in front of the intB13 gene favouring the reintegration process (Senthilo et al., 2003a). Transfer of the 108-kb GEI called PAPI-1, which was discovered in P. aeruginosa strain PA14 and is similar to the element pKLC102, proceeds via excision, formation of an intermediate circular form and reintegration into either of the two trNA^G3^ genes in P. aeruginosa (PA4541 and PA0976) (Qiu et al., 2006). The authors of this work could demonstrate that a soj gene encoded by PAPI-1 itself was required for the maintenance of the element, both in integrated and in circular form (Qiu et al., 2006). Their hypothesis was that Soj protects the circular form of PAPI-1 either directly from degradation or indirectly by promoting the integration of the circular form back into the chromosome. The major evidence for this was the finding that soj is expressed by the circular form at early stationary phase. The Soj protein is related to the ParA family of proteins, which are responsible for correct segregation of low-copy plasmids during cell division.

Transfer regulation proceeds differently in the SXT element of V. cholerae. Excision of SXT is favoured by an excisionase Xis (Burrus & Waldor, 2003), which, however, also inhibits its integration. In SXT, xis and int are convergent genes that do not appear to be co-regulated. SXT transfer is strongly enhanced under stress conditions and is dependent on the SOS response (Beaber et al., 2004). Interestingly, the V. cholerae SOS response is eluded in particular by two antibiotics, ciprofloxacin and trimethoprim, for which the SXT element encodes resistance determinants. The mechanism is thought to proceed as follows: in the presence of an SOS stimulus, the SXT-encoded repressor SetR is cleaved, resulting in the expression of two SXT-encoded genes setC and setD, which are activators for the int and tra genes of the element (Burrus & Waldor, 2003). Also excision of ICEBs1, a mobile element found in the genome of B. subtilis, is stimulated by global DNA damage in addition to an intercellular peptide signaling. This behaviour was found to be dependent on the factor ImmR, which regulates expression of a number of ICEBs1 genes and is responsible for immunity to superinfection (Auchtung et al., 2007). Excision of the ICEMISymR7A symbiosis island of M. loti strain R7A is also stimulated by a novel recombination directionality factor (RDF) called RdxS, which is encoded by the gene msi109 (Ramsay et al., 2006). Transfer of the ICEMISymR7A also requires a putative relaxase, RlxS. The genes rdxS and rlxS are part of the same cluster of which two other genes are homologues to the conjugative protein TraF (Ramsay et al., 2006). Similar to the clec element, also the excised form of ICEMISymR7A was more abundant in stationary than exponential phase of M. loti, and experimental evidence suggested that this excision was under quorum-sensing control (Ramsay et al., 2006).

Another example for excisionase requirement is the integrase of the HPI, which cannot alone promote efficiently the excision of HPI. In this case, it was demonstrated that a factor called Hef RDF, which is encoded by HPI, is required for excision (Lesic et al., 2004). Although the level of hef expression severely affected the rate of HPI excision, it had little or no effect on int transcription, and the authors concluded that Hef could not act as transcriptional regulator. Another RDF called Rox (for regulator of excision) was shown to stimulate excision of the Shigella resistance locus PAI in Shigella flexneri (Luck et al., 2004). Similarly to Hef, this Rox protein is not an activator of int transcription, although it showed 66% sequence similarity to AlpA from the phage CP4-57. AlpA is a transcription factor for the int gene of the phage CP4-57 in E. coli K-12, regulating the excision of the prophage from the bacterial chromosome (Trempey et al., 1994). The examples described above suggest that GEI transfer can be a highly regulated process with a variety of developed regulatory modes.

As discussed below, GEIs play an important role in bacterial genome evolution in general and in adaptation to changing conditions, in clinical, industrial or natural environments. The outcome of these adaptations is obvious from the development of antibiotic resistance, pathogenicity or catabolic functions. Thus, it would be extremely interesting to find specific features, which make GEIs so successful in self-transfer, host-entry or establishment in a host. Are GEIs considered parasites by a new host? Do GEIs have the means to trick a host and avoid its defence systems? Are all GEIs alike in this respect or do exceptions exist? Relatively little information is available concerning these questions and the nature of GEI–host interactions in general.

Most of the information on the host–DNA invader interactions comes from conjugative plasmids and phages. In general, the frequency of successful DNA exchange between bacteria belonging to different genera will depend on many factors: the degree of homology between the transferred DNA and the bacterial host, the metabolic compatibility, adaptations to their abiotic environment, gene expression systems, gene-transfer mechanisms, the mismatch repair and restriction endonuclease systems. For
example, the transfer efficiency of the broad-host range IncP-1 plasmid RK2, as measured by the number of transfer events per donor present, was dramatically influenced by the nature of the donor–recipient combination: between *E. coli* strains or from *E. coli* to *P. putida*, RK2 transfer was much less frequent than between *P. putida* strains. This was attributed to species-specific differences in RK2 gene expression (Bingle & Thomas, 2001). The recipient cell can also limit the entry or establishment of the incoming DNA by surface exclusion, a process by which a barrier seems to be created by cells that already carry the genes for a closely related transfer apparatus (Frost et al., 1994). Horizontally acquired DNA that confers a selective advantage to the host obviously has the potential to spread further among suitable recipients within a bacterial population under the appropriate selective conditions (Thomas & Nielsen, 2005).

Upon successful transfer, the newly incoming DNA must still be maintained to ensure its long-term survival in the new host. If there is a clear selective advantage conferred by the acquired DNA for the host, and no major fitness cost under nonselective conditions, it is less likely that the horizontally acquired DNA will be lost. Incoming DNA on a plasmid must be able to replicate independently but synchronously the host’s chromosomal replication and cell division. This process is usually guaranteed by a plasmid-specific system, such as killing daughter cells without partitioned plasmids. In the absence of appropriate replication, plasmids may still ‘survive’ by recombining into the host’s chromosome, which is promoted by the presence of suitable sequences for homologous recombination. On the other hand, GEIs depend on site-specific reintegration into an appropriate chromosomal target site, in the absence of which the element is unlikely to be maintained. However, once integrated, a GEI will be automatically maintained by chromosomal replication. Loss can occur when the GEI excises, as there seems to be a strong advantage for growth of cells without GEIs. Such a scenario was demonstrated recently in a study on the UPEC *E. coli* isolate 536, which contains five PAIs, some of which can become deleted during chronic infection. The authors could show that the integrase of one of the PAIs could actually mediate the excision of another PAI. This suggests the existence of unidirectional cross-talk between integrases of different PAIs (Hochhut et al., 2006). Similar cross-talk phenomena had been detailed previously by work on the *Bacteroides* element NBU1, which can be coexised and mobilized by the conjugative transposon CTnERL (Shoemaker et al., 2000).

Although it is generally stated that GEIs give a selective advantage to the host cell (Dobrindt et al., 2004), this has not been extensively experimentally tested yet. In fact, results from conjugative plasmids would suggest the opposite, namely that cells which acquire a conjugative plasmid, go through a period of fitness loss (Dahlberg & Chao, 2003). This fitness loss can be caused by particular functions carried on the plasmid which are detrimental upon expression in the new host (e.g. regulatory proteins interfering with the global gene expression network), or by proteins expressed from plasmid which have a direct phenotypic effect (e.g. antibiotic resistance) (Nguyen et al., 1989). Bacterial populations having received a newly incoming plasmid were shown to adapt to their previous fitness by spontaneous mutations which reduce or repress the plasmid specific effects (Dahlberg & Chao, 2003). Plasmids with both very low to unmeasurable cost, and very large cost to the host were detected and their ability to persist in a bacterial population was shown to be influenced by the host strain background (De Gelder et al., 2007; Schluter et al., 2007). Very recently, it was demonstrated that ICE elucidated had only a very minor effect on the fitness of host *P. aeruginosa* strains (Gaillard et al., 2008). Although so far only a single case of this has been found, it might point to this type of ICE/GEI having specific mechanisms to reduce fitness cost in the host (Gaillard et al., 2008).

There is increasing evidence that GEI expression is globally influenced by the host in which it resides. Thus, GEIs usually constitute part of global regulatory networks and genes on the particular GEI can be regulated by regulators present on the same GEI, by regulators harboured by other GEIs or by regulators encoded by the host bacterium. Similarly, GEI-borne regulators often play a role in the regulation of genes on the bacterial chromosome.

Regulators frequently contributing to regulation of GEIs comprise the two-component response regulator family, AraC family, alternative sigma factors and histone-like proteins. Paradigmatic regulatory networks involving intensive cross-talk between GEI-borne regulators and gene components of the host genome include *Salmonella* pathogenicity islands (SPI-1 and SPI-2) of *S. enterica*, *Vibrio* pathogenicity island (VPI) of *V. cholerae* and the locus of enteroctye effacement (LEE) of *E. coli* and have been reviewed elsewhere (Hacker & Kaper, 2000; Schmidt & Hensel, 2004).

Recent studies into the regulatory role of the histone-like nucleoid-structuring protein (H-NS) have shed a new light into the regulation of some bacterial GEIs. H-NS is a pleiotropic regulator that modulates gene expression of gram-negative bacteria in response to environmental stimuli, such as temperature and osmolarity (Hommais et al., 2001). H-NS represents the bacterial functional equivalent of histones, plays an important role in a local supercoiling of DNA, and has higher affinity for curved DNA (Navarre et al., 2006). Work from several laboratories exploiting recent DNA microarray technology has shown that H-NS plays a key role in the selective silencing of horizontally acquired genes (Lucchini et al., 2006; Navarre et al., 2006). Expression of > 400 genes was shown to be upregulated in the *Salmonella hns* mutant, out of which more than 90%...
were acquired by the horizontal gene transfer (Navarre et al., 2006). The GC content of most of the H-NS repressed genes was lower than the average GC content of the host *Salmonella* genome, thus leading to the conclusion that H-NS can selectively silence horizontally acquired genes by targeting sequences with proportionally high AT content (Navarre et al., 2006). Recently, conserved sequence motifs have been identified that represent the high-affinity DNA-binding sites for H-NS (Lang et al., 2007). In accordance with previously published studies, these motifs occur in AT-rich regions of DNA both within operons and in genes harboured by the pathogenicity-associated GEIs (Lang et al., 2007). As described above, the differences in the nucleotide statistics are among the characteristic features of GEIs, thus targeting sequences with different GC content by H-NS represents an elegant and efficient mechanism of regulation of newly acquired GEIs.

**Contribution of GEIs to horizontal gene transfer and bacterial evolution**

It is widely recognized that horizontal gene transfer facilitated by GEIs has played a crucial role in the evolution of bacterial species. This is attributed not only to simple acquisition and loss of GEI-borne genes, but also to the possibility of GEIs transferring parts of a host’s chromosomal DNA. Upon excision from the host genome, GEIs can play a role in the transfer of parts of the host chromosome into the recipient bacteria (Hochhut et al., 2000). Furthermore, the presence of a wide variety of secretion systems on many GEIs suggests that these can be used not only for the transfer of GEIs and the GEI-encoded products, but also for the transfer of the host’s chromosomal DNA. One good example is the secretion of chromosomal DNA in the gonococcus via the GGI-encoded T4SS (Hamilton et al., 2005). Chromosomal DNA secreted via the GGI-encoded type T4SS may be subsequently taken up by natural transformation, thus facilitating recombination that contributes to antigenic variation and the spread of antibiotic resistance (Hamilton et al., 2005). GEIs could undergo a recombination with the host’s chromosome, with a significant impact on the evolution of the host bacterium, as GEI-borne genes often encode important clinical or fitness traits. Whether encoding genes are involved in pathogenicity or biodegradation, GEIs can facilitate evolution by ‘quantum leaps’ as their acquisition or loss can rapidly and dramatically alter the life-style of a bacterium (Groisman & Ochman, 1996; Ochman et al., 2000; van der Meer & Sentschilo, 2003; Vernikos et al., 2007). It should be noted that many of the genes found in GEIs are novel and of unknown function, with no detectable homologues in other species, but nevertheless they might have a role and confer selective advantage to the host organism (Hsiao et al., 2005).

**Contribution of GEIs to evolution of pathogenic bacteria**

Antibiotic resistance represents one of the most frequent and well-studied traits associated with GEIs. The emergence and dissemination of antibiotic resistance is a serious threat to public health as it puts the successful treatment of infectious diseases in increasing doubt. This change in bacterial populations from near universal susceptibility to increasing antibiotic resistance worldwide in a few decades is illustrative of the remarkable capacity for bacterial adaptation, albeit a man-made threat. Many aspects of the accumulation of antibiotic resistance are poorly understood. One prominent aspect is how such resistance has disseminated globally so rapidly.

Investigations into the emergence of antibiotic resistance in *H. influenzae* suggest that it is an illustrative model for how resistance genes have become associated with a GEI. Sequence analysis of an *H. influenzae* antibiotic-resistant GEI, ICE*Hin*1056, revealed that this island belonged to much larger family (Mohd-Zain et al., 2004; Dimopoulou et al., 2007). Diverged GEIs of this family coevolve independently with a wide range of *Proteobacteria*, including *Haemophilus* spp., *Pseudomonas* spp., *Yersinia enterocolitica*, *S. enterica* serovar Typhi and *Ralstonia metallidurans* (Mohd-Zain et al., 2004). A recent study investigating GEIs of haemophilic has shown that the ICE*Hin*1056 subfamily of ICE/GEIs is diverse and has not recently emerged in haemophilic (Juhas et al., 2007b). However, the low-sequence diversity and distinctive GC content of the Tn3s found in the GEIs of haemophilic suggest more recent acquisition of the transposon-borne antibiotic resistance genes. Distribution between core and accessory genes (transposons) of the GEIs of haemophilic is remarkably similar to the host *H. influenzae* ‘supragenome’ and conforms to the distributed genome hypothesis (Juhas et al., 2007b). This hypothesis proposes that the full complement of genes available to a pathogenic bacterial species exists in a supragenome pool, which is not contained by any particular strain (Hogg et al., 2007). The modular structure of the core genes of the GEIs of haemophilic suggests that they are functionally constrained and, acting in concert, they play a major role in the successful propagation and survival of these GEIs within their hosts’ ‘supragenomes’. Their importance is overtly manifested by the rapid dissemination of antibiotic resistance worldwide among *H. influenzae* and *Haemophilus parainfluenzae* strains (Juhas et al., 2007b). The recent acquisition of antibiotic resistance genes and their rapid global spread over the past 30–40 years is an example of how GEIs contribute to bacterial diversification and adaptation.

*Staphylococcus aureus* is a potentially pathogenic bacterium that plays a role in a broad spectrum of diseases and is a major cause of hospital-acquired infections worldwide.
Methicillin-resistant S. aureus (MRSA) often referred to as the hospital ‘superbug’ in the press, represents one of the most serious threats to public health due to its resistance to a wide variety of antibiotics. This antibiotic resistance is facilitated mostly by genes located on a GEI-designated staphylococcal cassette chromosome mec (SCCmec) (Katayama et al., 2000). The five SCCmec subtypes identified so far range in size from 20 to 70 kb and confer resistance to methicillin, kanamycin, tobramycin, bleomycin, penicillins, heavy metals, tetracycline, macrolide, lincomamide and streptogramin (Ito et al., 2001; Deurenberg et al., 2007). GEI SCCmec is interesting in a sense that it does not contain phage-related and tra genes or transposases and is transferred between bacteria with the help of two site-specific recombinases that catalyze its chromosomal excision and reintegration (Ito et al., 2004; Noto & Archer, 2006). The origin of GEI SCCmec remains to be elucidated; however, it is hypothesized that it could originate from other staphylococci, namely Staphylococcus sciuri or Staphylococcus epidermidis. This is suggested by the high-amino acid sequence similarities of the methicillin resistance gene mecA products of S. sciuri and S. aureus as well as by an experiment where the S. sciuri mecA gene induced methicillin resistance in a formerly methicillin-sensitive S. aureus strain (Wu et al., 2001). Furthermore, S. aureus mecA was identical to that identified in an S. epidermidis isolate from the same individual, thus suggesting that MRSA strain has arisen in vivo by horizontal transfer of mecA between two staphylococcal species (Wielders et al., 2001; Deurenberg et al., 2007).

While one theory hypothesizes that all MRSA clones have a common ancestor (Kreiswirth et al., 1993), another theory suggests that SCCmec was introduced several times into different S. aureus lineages (Musser & Kapur, 1992; Fitzgerald et al., 2001; Enright et al., 2002; Qi et al., 2005).

Enterococcus faecalis is also one of the leading agents of nosocomial infections of surgical sites, the urinary tract and bloodstream (Richards et al., 2000). Most of the virulent strains of E. faecalis harbour a 150-kb GEI consisting of 129 ORFs, including those encoding a wide variety of toxins, cytolysin and the surface proteins Esp and aggregation substance (Shankar et al., 2002). Recently, the horizontal transfer of the E. faecalis GEI has been demonstrated (Coburn et al., 2007). This has many implications for the evolution and diversity of E. faecalis, as it suggests a mechanism for the conversion of commensal E. faecalis strains to virulent ones by acquisition of the GEI-encoded virulence traits (Coburn et al., 2007).

Results from recent studies have shown that GEIs also played a key role in the evolution of pathogenic human and mammalian Mycobacterium spp. (Gutierrez et al., 2005; Becq et al., 2007). Speciation events in ancestral Mycobacterium spp., that were initially environmental bacteria occurred only relatively recently, about a million years ago, and are linked to the invasion of the genome by foreign DNA (Gutierrez et al., 2005; Rosas-Magallanes et al., 2006; Becq et al., 2007). Several Mycobacterium tuberculosis GEIs harbour genes that have been identified previously as virulence genes in other bacteria (Pethe et al., 2004; Stewart et al., 2005; Becq et al., 2007). A good example illustrating the role played by the horizontally acquired GEIs in the evolution of Mycobacterium spp. pathogenic to mammals, is GEI Rv0986-8, as the Rv0986-8-borne genes are required for the binding of M. tuberculosis to eukaryotic cells and in vitro trafficking (Pethe et al., 2004; Becq et al., 2007; Rosas-Magallanes et al., 2007).

Siderophore-mediated iron uptake is important for pathogenic as well as environmental bacteria. HPI is a 36–43-kb GEI that encodes the siderophore yersinibactin-mediated iron uptake system (Lesic et al., 2004). This GEI was first found in Yersinia spp. but has been subsequently identified in a broad spectrum of Enterobacteriaceae. The presence of highly homologous HPI-borne ORFs among different species suggests recent acquisition of the HPI by these bacteria; however, the exact mechanism mediating the horizontal transfer of these islands awaits further investigation (Lesic & Carniel, 2005).

Many bacterial species exploit specialized secretion systems to transfer macromolecules across bacterial membranes. GEIs of a wide variety of bacterial pathogens encode type III secretion systems (T3SS) and T4SS, which by transfer of proteins or nucleoprotein complexes directly mediate pathogenicity and horizontal gene transfer. Salmonella enterica serovar Typhi is a gram-negative facultative intracellular pathogen that is a causative agent of gastroenteritis and typhoid fever (Shea et al., 1996). The divergence of Salmonella and E. coli from their common ancestor occurred c. 100–140 million years ago and these bacterial species have each acquired and lost more than 3 Mb of novel DNA since their divergence (Vernikos et al., 2007). The first stage of Salmonella infection, characterized by the colonization and invasion of intestinal epithelial cells, is usually followed by the replication within host’s macrophages. Two T3SSs harboured by two different GEIs, SPI-1 and SPI-2, play a crucial role in the pathogenesis of S. enterica. T3SS of SPI-1 is important for the penetration of intestinal epithelium, and T3SS of SPI-2 has been hypothesized to be required solely after bacterium has gained access to the host’s macrophages. Recent findings with a mouse model of typhoid indicate that this process is even more complicated, as SPI-2 was shown to be expressed also during early stages of pathogenesis before penetrating the intestine. This suggests that in addition to other functions, SPI-2 may be also involved in preparing Salmonella to successfully resist the harsh antimicrobial environment within macrophages (Brown et al., 2005). T3SSs harboured by Salmonella islands SPI-1 and SPI-2 are only two of many examples of GEI-
borne T3SSs enhancing the pathogenicity of their host bacteria. Besides T3SSs, GEIs can also encode T4SSs. Various T4SSs associated with GEIs include for instance the GI-like family of T4SSs described above. The T4SSs are unique among other bacterial secretion systems due to their ability to transfer both proteins and nucleoprotein complexes. They can deliver bacterial effector proteins to host cells, thus contributing directly to pathogenicity. Furthermore, they can mediate horizontal gene transfer, thus facilitating the evolution of pathogens through dissemination of virulence genes (Juhas et al., 2008).

Adhesion, either interbacterial or to specific receptors of host cells, represents another important pathogenicity trait that is often associated with GEIs. VPI encodes the toxin-coregulated pilus (TCP) that represents the major intestinal adherence factor of V. cholerae, the causative agent of cholera (Karaolis et al., 1999). TCP is a type IV pilus that mediates interbacterial adherence, resulting in formation of microcolonies, as well as secretion of a soluble colonization factor, TcpF, crucial for successful colonization of host (Kirn et al., 2003). Importance of the VPI-encoded TCP is overtly manifested by the inability of the TCP-deficient mutants of V. cholerae to colonize and cause disease in both humans and mice (Tripathi & Taylor, 2007).

Other GEI-encoded adherence factors include P fimbriae and S fimbriae of uropathogenic E. coli (UPEC) and intimin of enteropathogenic and enterohaemorrhagic E. coli (EPEC and EHEC, respectively) (reviewed elsewhere, see Hacker & Kaper, 2000). Both EPEC and EHEC infections are the leading cause of infantile diarrhoea. Multiple separate acquisitions of the LEE GEI and of the EPEC adherence factor plasmid (EAF) seem to be responsible for the evolution of the EPEC pathotype within E. coli (Lacher et al., 2007). Furthermore, microarray and whole genome PCR scanning analyses have shown that LEE GEI constitutes one of the most conserved parts of different EHEC strains. This study suggests that independent infections of similar but distinct bacteriophages carrying virulence determinants, including LEE GEI, are deeply involved in the evolution of EHEC strains belonging to different E. coli lineages (Ogura et al., 2007). Horizontal transfer of GEIs was also shown to play a major role in the evolution of a number of other bacterial pathogens, including LIPI (listeria pathogenicity island) of Listeria monocytogenes (Vazquez-Boland et al., 2001), SHI-0, SHI-1, SHI-2 of S. flexneri (Nie et al., 2006), BPAI of Bacteroides fragilis (Franco, 2004) and cag of Helicobacter pylori (Gressmann et al., 2005).

Contribution of GEIs to evolution of environmental bacteria and making the link to pathogenicity

Interestingly, although GEIs have been increasingly associated with the rise and distribution of virulence functions or antibiotic resistance genes, at the same time they appear to be implicated in a multitude of other adaptive traits, which collectively could be considered of 'environmental relevance'. For example, a number of ICE/GEIs have been identified in Beta- and Gammaproteobacteria, which carry gene clusters for the degradation of chlorinated and nitroaromatic compounds (Gaillard et al., 2006) or biphenyls (Toussaint et al., 2003). Even dehalogenases in Dehalococcoides ethenogenes have been associated with integrated DNA elements, but whether these are mobile or not remains to be determined (Seshadri et al., 2005).

One of these ICE/GEIs, the highly mobile 103-kb ICElec of Pseudomonas sp. strain B13, often used as a model for the behaviour of catabolic GEIs (Sentchilo et al., 2003a, b), is a good example of how GEIs can contribute to the adaptation of environmental strains to use polluting compounds as new carbon sources. Various classical experiments have used Pseudomonas sp. strain B13 to develop new metabolic pathways in a single step, such as for chlorobiphenyl or chlorobenzene degradation (Reineke, 1998). The recently revealed molecular basis for this metabolic ‘complementation’ of recipient bacteria also showed that ICElec is in a continuing state of further adaptation. First of all, ICElec contains an element almost 100% identical to ICEcl, except for additional genes allowing degradation of o-halobenzoates (Chain et al., 2006; Gaillard et al., 2006). Finally, in the groundwater isolateRalstonia sp. strain JS705 an element similar to ICElec was detected with a 10-kb insertion of another catabolic pathway gene cassette, necessary for chlorobenzene degradation (Muller et al., 2003). This development model for ICElec of acquisition (and loss) of gene modules became even more striking when it was discovered that a region of about 40% of the catabolic ICElec elements is highly similar to a number of other ICE/GEIs (Larbig et al., 2002b; Mohd-Zain et al., 2004; Gaillard et al., 2006) (Fig. 4). This region was therefore suspected to contain the information for self-transfer, which was more recently confirmed by the analysis of the T4SS genes of the ICEHin1056 from H. influenzae that showed significant homology to counterpart ICEelec genes (Juhas et al., 2007a). The core region of this group of elements was found in a variety of other bacteria (Fig. 4), thus showing that this evolutionarily ancient element has been very successful in transferring into a large host-range (Mohd-Zain et al., 2004; Juhas et al., 2007a). More recently, GEI fragments similar to ICElec were also detected in Xylella fastidiosa, Xanthomonas campestris, Rubrivivax gelatinosus, Azoarcus, Cupivriadius and the arsenic-oxidizing bacterium Herminiimonas arsenicoxydans (Gaillard et al., 2006; Muller et al., 2007).
Particularly interesting was the close similarity between ICEclc and a large widely distributed group of GEIs of *P. aeruginosa*, including PAGI-2 and PAGI-3 (Larbig et al., 2002a, b; Klockgether et al., 2007). PAGI-2 and PAGI-3 were initially characterized from one clinical and one environmental *P. aeruginosa* strain (Larbig et al., 2002b), but were found to be very common in a large screening of clinical and environmental isolates of *P. aeruginosa*, in addition to the even more promiscuous element pKLC102/PAPI-1 (Wolfgang et al., 2003; He et al., 2004; Qiu et al., 2006; Klockgether et al., 2007). The genetic load, in addition to the core region, was very different between ICEclc and PAGI-3, demonstrating that these GEIs can easily accommodate and distribute regions of 60–70 kb of highly dissimilar DNA. The fact that this genetic load is found mostly between the integrase gene (near one end of the element) and the core region suggests that insertions here are selectively neutral and do not compromise the functionality of the GEI. A majority of accessory genes were also found to be located in this position in the closely related *Haemophilus* spp. ICEHin1056 subfamily of GEIs (Juhás et al., 2007b). This GEI-type is therefore an extreme example of the near continuum between characteristics judged as pathogenicity (ICEHin1056 of *H. influenzae*, or PAGI-2 and PAGI-3 of *P. aeruginosa*) and environmentally (ICEclc of *Pseudomonas* sp. B13) bacteria. ATB resist., antibiotics resistance; T4SS, type IV secretion system; Integr., integration.

Fig. 4. Various functions encoded by GEIs of the same family. Modified Artemis Comparison Tool view of four GEIs of the same family: ICEHin1056, clc, ICEHpaT3T1 and PAPI. Homologous sequences (minimum cut-off = 50) are indicated by grey lines joining regions of the four GEIs. Gene modules homologous in all shown GEIs are represented by white boxes, and modules specific for individual GEIs by black boxes. The figure shows that depending on the composition of gene modules, members of the same family of GEIs can promote survival of pathogenic (ICEHin1056, ICEHpaT3T1, PAPI of *Haemophilus influenzae*, *Haemophilus parainfluenzae* and *Pseudomonas aeruginosa*, respectively) as well as environmental (clc of *Pseudomonas* sp. B13) bacteria. ATB resist., antibiotics resistance; T4SS, type IV secretion system; Integr., integration.
which provides its host bacterium with the ability to synthesize N-acyl homoserine lactones and fatty acids and also encodes further amino acid transporters and metabolic genes (Baldwin et al., 2004). Furthermore, Corynebacterium efficiens carries four GEIs, of 70, 55, 40 and 32 kb, one of which (CEGI4) has the most characteristics of a fully functional ICE. Apart from an integrase, all other ORFs encode unknown proteins (Zhang & Zhang, 2005). Recent comparison of several P. aeruginosa isolates has revealed the presence of multiple novel GEIs. One of those, the GEI RGP29 in P. aeruginosa PA2192, is a 224-kb element containing within its sequence another discernible GEI, called the dit island. The dit island encodes a full metabolic pathway for abietane diterpenoids (Mathee et al., 2008). In addition, the RGP5 element of P. aeruginosa PA14 encodes various transporters and genes known to be involved in iron metabolism (Mathee et al., 2008). Magnetospirillum gryphiswaldense contains a 130-kb unstable region of probable ancient GEI origin, but now filled with insertion elements (42 copies) that cause frequent recombinations and deletions of parts of this genomic region. Interestingly, this region encodes many genes involved in magnetosome biomineralization (Ullrich et al., 2005). Suspected ancient GEIs with deteriorated integrases for which no transfer or excision could be demonstrated were detected in the anaerobic bacterium Geobacter sulfurreducens (Butler et al., 2007). One such region has a size of 300 kb and contained many genes implicated in anaerobic metabolism of benzoate, phenol, p-cresol and 4-hydroxybenzoate. Free-living water and soil-borne bacteria with no specific pathogenicity characteristics, but which are thought to provide reservoirs for more pathogenic bacteria, have now also been found to harbour GEIs. One of those is Arcobacter butzleri, an Epsilonproteobacterium related to H. pylori (Miller et al., 2007). Three potential GEIs have been found in this bacterium, the largest of which (ABG11) had a size of 26.9 kb and was integrated in tRNA\textsuperscript{Glu} with an integrase gene nearby. The island encodes 29 genes, none of which, however, have known orthologues in other bacterial genomes. More recent genome comparisons of multiple X. fastidiosa and X. campestris strains have again reinforced the importance of GEIs in strain differentiation and provision of potential virulence functions (da Silva et al., 2007; He et al., 2007). For example, Xylella strains from citrus fruits carried one or more copies of the GI1 island, which encodes fimbriillin synthesis, haemolysin production and lipopolysaccharide synthesis (da Silva et al., 2007). Various rearrangements were observed in the largest (100 kb) island of X. campestris named XVRI3 integrated in tRNA\textsuperscript{Glu} (He et al., 2007).

**Concluding remarks**

Work over a number of years has shown the importance that GEIs have and have had in promoting horizontal gene transfer and distributing a wide range of adaptive functions for the host bacterium. GEIs seem to do the same job as many self-transferable plasmids. Conceptually, GEIs may have a number of advantages over a plasmid, one of the most notable being that GEIs are integrated in the host’s chromosome. Thus, unlike replicating plasmid molecules, GEIs do not need to continuously ensure coordinated replication, partitioning or specific maintenance, and because there is often only a single copy of the GEI present per genome, its replication ‘cost’ may not be as heavy a burden to the host cell (Gaillard et al., 2008). However, under certain conditions, those GEIs that are functionally mobile can undergo excision, self-transfer and reintegration into a new host. More interestingly, several findings, including tetracycline-induced transfer of the conjugative transposon CTnDot of Bacteroides (Cheng et al., 2000), DNA damage and SOS stress response-induced transfer of the SXT element of V. cholerae (Beaber et al., 2004), and 3-chlorobenzoate-augmented transfer of the clc element of Pseudomonas sp. strain B13 (Senticho et al., 2003b), indicate a much more regulated train of events for the transfer of GEIs that is fine-tuned in response to environmental signals. Thus, the question arises whether unwittingly, by changing the conditions for bacteria in hospitals (antibiotic stress) and environment (pollution), we are generating selective conditions which promote the success of self-transferable and responsive GEIs. As shown on multiple examples in this review, GEIs play a crucial role in the evolution of a broad spectrum of pathogenic or environmental bacteria. Furthermore, several lines of evidence suggest the existence of evolutionary ancient GEIs spread over versatile groups of otherwise unrelated bacterial species. Besides a conserved set of core genes required for maintenance, these evolutionary ancient GEIs also harbour a variable number of other gene modules whose composition is strongly dependent on the life-style of the particular host bacterial species, which can be either pathogenic or environmental (Fig. 4). The important contribution of many research groups around the world, has led to GEI-facilitated horizontal gene transfer being one of the most rapidly evolving fields of microbiology research.

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