CMLS, Cell. Mol. Life Sci. 56 (1999) 719–728
1420-682X/99/100719-10 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Evolution of bacterial pathogenesis

W. Ziebuhr[a], K. Ohlsen[a], H. Karch[b], T. Korhonen[c] and J. Hacker[a,*]

[a]Institut für Molekulare Infektionsbiologie, Röntgenring 11, D-97070 Würzburg (Germany),
Fax + 49 931 31 2578, e-mail: j.hacker@mail.uni-wuerzburg.de
[b]Institut für Hygiene und Mikrobiologie, Josef-Schneider-Str. 2, D-97080 Würzburg (Germany)
[c]Division of General Microbiolgy, University of Helsinki, PO Box 56, Viikinkaari 9, SF-00014 Helsinki (Finland)

Abstract. The evolution of bacteria is associated with continuous generation of novel genetic variants. The major driving forces in this process are point mutations, genetic rearrangements, and horizontal gene transfer. A large number of human and animal bacterial pathogens have evolved the capacity to produce virulence factors that are directly involved in infection and disease. Additionally, many bacteria express resistance traits against antibiotics. Both virulence factors and resistance determinants are subject to intrastrain genetic and phenotypic variation. They are often encoded on unstable DNA regions. Thus, they can be readily transferred to bacteria of the same species or even to non-related prokaryotes. This review article focuses on the main mechanisms of bacterial microevolution responsible for the rapid emergence of variants with novel virulence and resistance properties. In addition, processes of macroevolution are described with special emphasis on gene transfer and fixation of adaptive mutations in the genome of pathogens.

Key words. Evolution; pathogenesis; point mutation; pathogenicity islands; recombination; insertion sequences; gene transfer.

Introduction

Bacteria must conserve their genetic information from one generation to the next. The maintenance of the correct genomic sequence is ensured by complex enzymatic mechanisms managing the faithful replication and repair of DNA [1, 2]. However, bacteria have to live and survive under continuously changing environmental conditions and are therefore compelled to adapt to them. In addition to regulatory adaptive responses that act at the level of gene expression, bacteria have also evolved strategies allowing the generation of genetic diversity [3, 4]. Point mutations, recombination between homologous DNA sites, and the action of transposable genetic elements are major mechanisms by which genome flexibility is achieved. The capture and spread of genes by horizontal gene transfer mechanisms involving plasmids, phages and other mobile elements also contribute to this process. Finally, the clustering of genes on large genomic islands and their mobilization enables bacteria to gain or lose huge amounts of DNA involved in the adaption to distinct ecological niches [5, 6]. This mediates the very rapid development of new bacterial variants (within days or weeks), a process for which the term 'microevolution' was coined. However, once successfully adapted, microorganisms tend to stabilize the newly generated genotype by adaptive mutations and they can maintain it for millions of years [7]. Evolutionary processes that proceed over longer time periods are termed 'macroevolution'. They lead to the development of completely new variants of organisms, to the generation of new species and even to the emergence of new genera. Both micro- and macroevolution have contributed to the impressive diversity of the microbial world on earth.

The general processes involved in the evolution of bacteria also form the basis for the evolution of pathogens. Pathogenic bacteria often produce virulence or pathogenicity factors such as adhesins, capsules or toxins which enable them to cause infections in particular host

* Corresponding author.

organisms. In addition, pathogenic bacteria are able to express resistance factors to overcome the action of antibiotics used in human and veterinary medicine. Some of the pathogenic microorganisms are perfectly adapted to one particular host species. For example, the host spectrum of meningococci or gonococci is restricted to human beings. Other bacteria only cause disease when they are transferred to a new host organism. Transmission can be mediated by vector organisms (e.g. fleas in the case of *Yersinia pestis*), food (e.g. enterohaemorrhagic *Escherichia coli*-EHEC), water (e.g. *Vibrio cholerae*) or by technical systems (e.g. *Legionella pneumophila*) [8–11]. Finally, bacteria belonging to the normal body flora or to the environment can cause disease when the host is immunocompromised or the microorganisms are displaced to unusual body sites (e.g. uropathogenic *E. coli*, coagulase-negative staphylococci) [12].

This review describes genetic mechanisms of microevolution underlying the extraordinary capacity of pathogenic bacteria that gives rise to the continuous emergence of variants with novel virulence and resistance traits. In addition, the article provides insight into processes of macroevolution with special emphasis on gene transfer and fixation of adaptive mutations in pathogen genomes.

Point mutations, deletions and pathoadaptive mutations are involved in micro- and macroevolution of pathogens

Point mutations are considered as driving forces in slow evolutionary processes. However, in viruses, mutations or small deletions can also contribute to the rapid development of structural genes [e.g. human immunodeficiency virus (HIV) protease gene mutations, leading to protease-inhibitor-resistant HIV variants] [13]. In addition, particular genes of bacterial pathogens exhibit stretches of repeated DNA sequences in promoter regions and/or in the 5′ end of virulence genes. During replication, point mutations can be generated by slipped-strand mispairing, resulting in expression or non-expression of particular genes. In gonococci, meningococci, *Mycoplasma* sp., and *E. coli*, phase and antigenic variation of surface-associated structures is mediated by these mechanisms [14–17] (see table 1).

Regulatory genes which control coordinated gene expression under changing environmental conditions have also been found to be subject to point mutations and small deletions. In many bacteria, the stress response is regulated by alternative sigma factors [18]. The mode of action of these factors has been extensively studied in *Bacillus subtilis*, *E. coli* and *Staphylococcus aureus* [19–23]. They are involved in sporulation (*B. subtilis*), stationary and heat shock response (*E. coli*) and also in the expression of virulence genes (*S. aureus*, *E. coli*). Mutations and deletions in such genes were demonstrated in *Salmonella* sp. and *S. aureus* and exhibited a pleiotropic effect on gene expression which also influenced the virulence traits of these organisms [24–26].

Mutations in structural genes of putative virulence factors can also modify or knock out the encoded proteins and influence their function during pathogenesis. Many of these mutations represent so-called pathoadaptive mutations which enable single bacterial clones to become more pathogenic without the acquisition of additional genes. This mechanism is based on random mutagenesis which offers the bacterium a strong advantage under a selective pressure [7]. Pathoadaptive mutations are mainly observed in bacterial species that are opportunistic or non-primary pathogens (table 2). Thus, the type 1 fimbriae of enterobacteria (particular of *E. coli*) were altered by pathoadaptive mutations leading

Table 1. Genetic mechanisms involved in phase and antigenic variation contributing to microevolution in pathogenic bacteria.

Genetic mechanism	Bacterial species	Functional effect
Point mutations	*Neisseria meningitidis* and *N. gonorrhoeae*	phase variation of pili, capsules and lipopolysaccharide
Deletions	*Haemophilus influenzae*	capsule variation
	Escherichia coli	loss of P-fimbriae and haemolyisn
	Staphylococcus epidermidis	loss of biofilm production
DNA modification	*E. coli*	phase variation of P, S, K99-fimbriae
Homologous recombination	*N. gonorrhoeae*	antigenic variation of pili and surface proteins
	Mycoplasma pneumoniae	antigenic variation of surface proteins
Site-specific recombination	*E. coli*	phase variation of type-1 fimbriae
	Salmonella spp.	antigenic variation of flagellae
Insertion sequence integration/ excision	*N. meningititis*	phase variation of capsule production
	S. epidermidis	phase variation of biofilm formation

Table 2. Examples of pathoadaptive mutations that confer altered bacterial pathogenicity according to Sokurenko et al. [7].

Bacterial species	Pre-existing gene	Gene function	Functional effect of mutation	Adaptive advantage in virulence
E. coli	*fim*H	adhesion	variation of amino acid sequence	increased tropism to uroepithelial and neural basement membranes
S. aureus	*hem*B	synthesis of electron transport chain components	knockout	intracellular persistence, and increased antibiotic resistance
S. aureus	*sig*B	alternative transcription factor, regulation	knockout	increased activity of genes (e.g. toxins)
Group A *Streptococcus*	*spe*B	extracellular and cell surface protease	variation of amino acid sequence	expansion of tissue tropism and inhibition of platelet aggregation
Shigella, enteroinvasive *E. coli*	*omp*T	surface protease	knockout	surface expression of actin-polymerization factor
Shigella, enteroinvasie *E. coli*	*cad*A	lysine decarboxylase	knockout	activation of endotoxin
Haemophilus influenzae	*bex*A	polysaccharide export	knockout of one of two copies	increased capsule production
Pseudomonas aeruginosa	*muc*A	downregulation of alginate production	knockout	evasion of phagocytosis
Chlamydia trachomatis	*omp*1	adhesion, immune escape	variation of amino acid sequence	expansion of tissue tropism

to variants with increased binding capacity to extracellular matrix proteins, and enhanced tissue penetration [27, 28]. These mutations occur in a limited number of *E. coli* strains that cause urinary tract infections and meningitis and they are suggested to be part of a macroevolutionary process leading to *E. coli* variants with greater tissue invasion potential.

In addition to the modification of structural and regulatory genes, point mutations also contribute to the development of bacterial resistance to antibiotics. For example, in *E. coli*, mutations in the gyrase and topoisomerase genes cause resistance to fluorquinolone antibiotics [29]. The resistance to rifampicin and streptomycin in mycobacteria and other microorganisms is mediated by point mutations in the RNA polymerase and ribosomal genes, respectively [30]. During the treatment of an infection with these antibiotics, resistant bacterial subclones emerge very rapidly. Therefore, this mechanism can also be considered as a microevolutionary process.

The generation of large deletions of the bacterial chromosome represents another major principle of genome plasticity. Excisions are frequently observed in *Streptomyces* spp., where large deletions comprising up to 800 kb of DNA occur [31]. The deleted parts of the chromosomes are often flanked by either perfect or imperfect repeats, which may play a role in illegitimate recombination. The permanent generation of new genetic variants due to deletions may enable *Streptomyces* to adapt to its environments. In other species, such as *Bacillus subtilis* or cyanobacteria, excisions of DNA fragments which interrupt coding regions have been described [32]. The excision of 42 kb of the *B. subtilis* genome is a very good example of 'developmental' deletions because the interrupted sporulation (*spo*) genes are fused following this deletion, leading to a functional active sporulation determinant [33]. The integration and excision of plasmids into and from chromosomes as well as the site-specific excision or sequential deletion of other genetic segments (e.g. pathogenicity islands) may also play a role in bacterial adaptation during pathogenesis [6]. Thus, pathogenicity islands of pathogenic *Yersinia*, uropathogenic *E. coli* and *Helicobacter* as well as capsule genes of *Haemophilus influenzae* may undergo such deletion mechanisms leading to variants with completely new virulence traits [7] (see table 1). Recently, we were able to show that the *ica* gene cluster of *S. epidermidis* encoding the genes for the synthesis of a sugar polymer which mediates biofilm formation can undergo deletions of a DNA fragment of more than 100 kb in size (unpublished observations). Furthermore, the intergration and excision of the virulence plasmid of enteroinvasive *E. coli* (EIEC) strongly influences the expression of virulence-associated genes, presumably by alternating DNA supercoiling.

Generation of genetic diversity by homologous and site-specific recombination

Homologous and site-specific recombination events contribute considerably to both antigenic variability and to the development and spread of antibiotic resistance in important human pathogens. Homologous recombination is mediated by regions of nucleotide sequence homology in two functional DNA molecules that participate in the process. It involves DNA cleavage, homologous base pairing and the formation of a typical cross-over structure which is subsequently resolved [34, 35]. The process depends on the action of the RecA protein and can involve intragenic rearrangements on the same replicon and the uptake and integration of extracellular DNA. In *Neisseria gonorrhoeae*, for example, the nearly inexhaustible capacity for pili diversity is based on the presence of one or two copies of a pilin gene in an expression locus and, additionally, multiple silent pilin gene variants on the chromosome [15, 16, 36, 37]. Pilin variation occurs by RecA-dependent intragenic recombination between a silent locus and the expression locus on the same chromosome. A second mechanism is based on the natural competence of *Neisseria* and involves the horizontal uptake and subsequent homology-dependent integration of DNA that is released from neighbouring bacteria.

In *Streptococcus pneumoniae*, the capacity of capsule variation contributes to the evasion of the host immune system and hampers the development of an effective pneumococcal vaccine. Serotype switchs are based on recombinational exchange at the *cps* locus which carries clustered genes that are common to all pneumococci and other, non-homologous genes that mediate the structural differences between the capsular serotypes [38]. Genes adjacent to the *cps* locus as well as homologous stretches within the gene cluster have been identified as possible cross-over points during capsule gene recombination. The emergence of penicillin resistance is another serious problem in these human pathogens [39]. It is due to penicillin-binding proteins (PBPs), with diminished binding affinity to beta-lactam antibiotics, which had arisen from recombinational exchange between the PBP-encoding genes of *S. pneumoniae* and the corresponding DNA from related, intrinsically resistant streptococcal species [40, 41]. The capacity of pneumococci for uptake and integration of extracellular DNA by natural competence and transformation is regarded as the driving force in these processes.

In contrast to the recombination events described above, some genetic systems promote recombination only at specific sites. Site-specific recombination is characterized by the presence of unique nucleotide sequences in both DNA molecules that participate in recombination [42]. These sequences are recognized by recombinases, which then catalyze the joining reactions. Several bacterial genomes use site-specific recombination to control phase variation of surface antigens (e.g. type-1 fimbriae expression in *E. coli*, flagella phase transition in *Salmonella*) [43–46] (table 1). The integration of some bacteriophages (e.g. λ phage in *E. coli*) takes place by site-specific recombination [47]. Another important phenomenon is the capture, spread and expression of antibiotic resistance determinants by integrons [48]. Integrons are genetic units comprising a site-specific recombinase gene, a specific *attI* nucleotide sequence for the integration of mobile gene cassettes and a promoter for the expression of the integrated cassette genes. The gene cassette itself represents independent mobile elements that can also exist outside from integrons in free circularized forms. Most cassettes encode antibiotic resistance genes and many of the resistance traits observed in Enterobacteriaceae and pseudomonads are part of integrons. However, in *V. cholerae*, integrons can also harbour genes that are involved in pathogenesis, and it is presumed that the acquisition and spread of DNA by integron-like structures are an evolutionary process that predated the antibiotic era [49]. It is therefore likely that integrons play a more general role in the evolution of the bacterial genome.

Bacterial insertion sequence elements mediate mutations and DNA rearrangements

Bacterial insertion sequence (IS) elements are small mobile DNA units that encode only features necessary for their own mobilization [50]. In general, they consist of the genetic information for a transposase protein and inverted repeat sequences that exactly define the borders of the element. IS elements can be regarded as repetitive DNA sequences that are randomly distributed on the bacterial chromosome. They also occur on plasmids, phages and in composite transposons where the IS often forms the ends of the element. IS elements have the capacity to cause irreversible inactivation of genes by random and in some cases also by site-specific transposition [51, 52]. However, in *N. meningitidis* and *S. epidermidis*, distinct elements (i.e. IS*1301* and IS*256*, respectively) contribute to the reversible inactivation of virulence genes (table 2, fig. 1) [53, 54]. As indicated in figure 1, the *ica* genes of *S. epidermidis*, encoding the production of the polysaccharide adhesin PIA, represent the target site for the integration and precise excision of IS*256*, leading to repression and reactivation of the *ica*-specific genes. In *N. meningitidis*, IS*1301* also mediates phase variation of capsule synthesis by a similar mechanism. In addition to simple transposition, IS elements also give rise to complex DNA rearrangements

including deletions, inversions, gene amplifications and the fusion of two DNA molecules by cointegrate formation [4]. Most of these reactions are mediated by specific actions of the IS transposase. However, IS elements can also be passively involved in recombination, since their nucleotide sequences represent homologous DNA stretches which can serve as recombinational cross-over points. These IS-mediated mechanisms apparently play a major role in genome flexibility. They are involved in the creation of new antigenic variants, and since many IS elements are associated with antibiotic resistance genes, they also contribute considerably to the spread of these genes among bacterial populations [55, 56].

Plasmids, phages and pathogenicity islands

Virulence genes of pathogenic bacteria, which encode toxins, adhesins, invasins or other virulence factors may be located on transmissible genetic elements such as transposons, plasmids or bacteriophages. In addition, such genes may be part of particular regions on the bacterial chromosome, termed 'pathogenicity islands' (Pais) [6]. Pais allow bacteria to gain or lose huge amounts of DNA and they are regarded as elements that mediate bacterial macroevolution in 'quantum leaps' [5]. Pais are found in both Gram-negative and Gram-positive bacteria (table 3). They are present in the genome of pathogenic strains of a given species but absent or only rarely present in those of non-pathogenic variants of the same or related species. They comprise large DNA regions (up to 200 kb DNA) and often carry more than one virulence gene (fig. 2). The G + C content often differs from that of the remaining bacterial genome and, in most cases, Pais are flanked by specific DNA sequences, such as direct repeats or IS elements. In some bacteria (e.g. uropathogenic *E. coli*, *Yersinia* spp., *Helicobacter pylori*), Pais represent unstable genetic elements and tend to delete at high frequencies. In addition, they may undergo duplications and amplifications. Pais are often associated with tRNA loci, which may represent target sites for their chromosomal integration. The presence of bacteriophage attachment sites and cryptic genes on Pais, which are homologous to phage integrase genes, replication origins of plasmids or IS elements, indicate that these particular genetic ele-

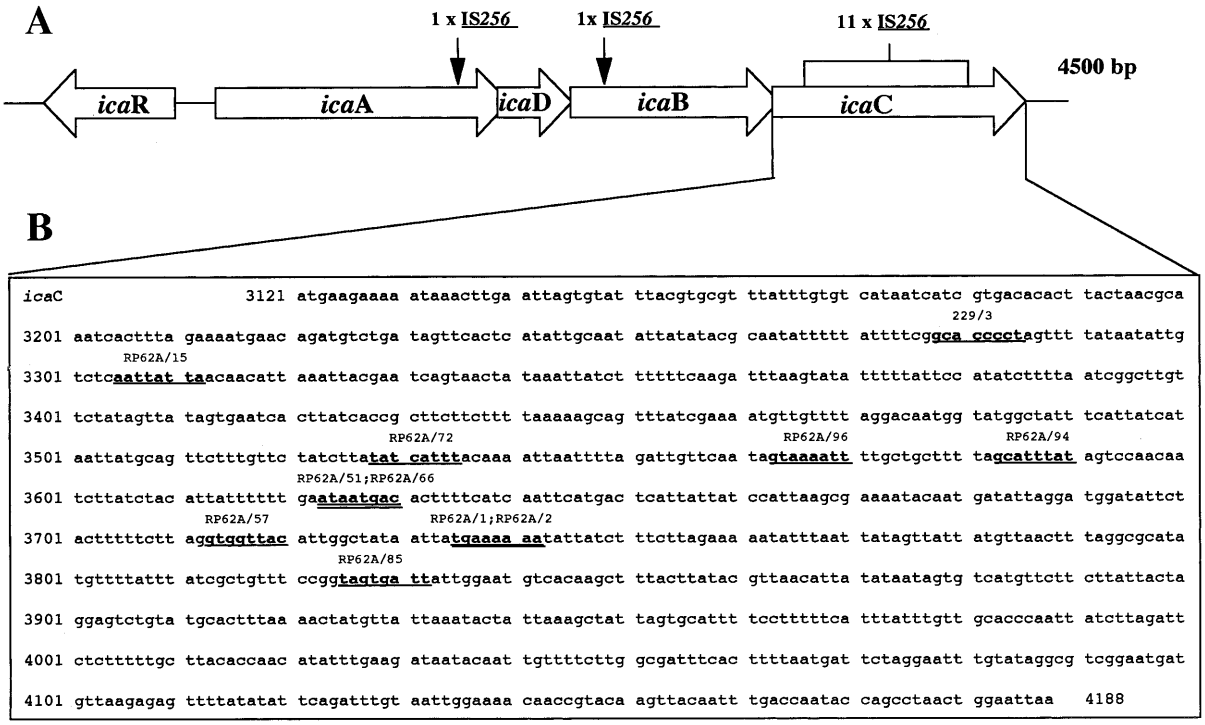

Figure 1. Phase variation of biofilm formation in *S. epidermidis* by insertion and precise excision of IS*256* in the *ica* operon [54]. (*A*) Overview of the *ica* operon which mediates the production of a polysaccharide adhesin. The number and positions of IS*256* insertions detected in two *S. epidermidis* wild-type strains are marked by arrows. (*B*) Detailed representation of the IS*256* insertions in the *ica*C nucleotide sequence. IS*256* target sites which become duplicated during the insertion of the element are underlined. Double lines mark identical target sequences identified in different insertional mutants. The wild-type nucleotide sequence can be restored by the complete excision of IS*256*, including the initially duplicated 8-bp target sites. The designations above the targets indicate the individual names of the variants.

ments were previously able to spread among bacterial populations by horizontal gene transfer. Interestingly, in some species, Pais are closely associated with bacteriophages. It was recently shown that the Pai of *V. cholerae*, which encodes a type IV pilus, is located on a bacteriophage. This phage can be transferred between *V. cholerae* strains and the Pai-encoded pilus acts as a receptor for another phage carrying the cholera toxin gene [57]. In *S. aureus*, the Pai which encodes the toxic shock syndrome toxin-1 is not an integral part of a bacteriophage genome; however, the Pai is excised, circularized and mobilized to another strain by a particular transducing phage [58]. It is tempting to speculate that in other species also, Pais are linked to bacteriophages and it is conceivable that at least some of them represent parts of 'old' viruses which were adapted to the host genome during evolution.

In bacteria it is well established that plasmids, transposons and integrons contribute considerably to the spread of antibiotic resistance genes. However, bacteria can also evolve resistance by acquiring large chromosomal DNA fragments. Thus, in staphylococci, the emergence of methicillin resistance is due to the acquisition of the *mecA* gene that encodes an additional PBP with a low binding affinity for beta-lactam antibiotics [59]. The *mecA* determinant and its adjacent regulatory genes represent the core sequence of a 51.7-kb DNA element (*mec*) that is unique to methicillin-resistant staphylococci and absent in susceptible strains. The origin of the *mec* DNA is not clear, but it is suggested that a *mecA* precursor comes from a coagulase-negative species and the regulatory genes have evolved from beta-lactamase plasmids [60]. In different strains, *mec* was located on the same *Sma*I-generated chromosomal fragment. The entire element has been completely sequenced and analysed [61]. It contains terminal inverted repeats and is bounded by a characteristic pair of 15-bp direct repeats. Moreover, the *mec* determinant carries IS elements and clustered antibiotic resistance genes which are due to the integration of plasmid pUB110 and transposon Tn*554*. Additionally, two open reading frames encoding putative site-specific recombinases were identified and it is speculated that these enzymes mediate the spontaneous site-specific excision of the element. It is assumed that *mec* does not represent an independent mobile element. However, it was shown that it can be mobilized to plasmids and by phage transduction [62, 63]. These properties show a striking similarity to the Pai paradigm described above, and the *mec* determinant can therefore be considered as a 'resistance island'.

Horizontal gene transfer by plasmids is another important mechanism of bacterial evolution, and the spread of antibiotic resistance traits by these mobile elements has been extensively studied. However, in addition to resistance genes, plasmids of many bacterial pathogens can also carry important virulence factors. Virulence plasmids are harboured by the pathogenic *Yersinia* (*Y. pestis*, *Y. enterocolitica*, *Y. pseudotuberculosis*), *Shigella* and several groups of diarrhoeagenic *E. coli* where they contribute significantly to the virulence of these microorganisms. An impressive example is given by enterohaemorrhagic *E. coli* (EHEC) strains where virtually all isolates carry large plasmids about 75–100 kb in size [64, 65]. Nucleotide sequencing and expression studies of pO157, a prototype plasmid of this group, showed that it carries the genetic information for a pore-forming cytotoxin (i.e. EHEC-haemolysin), a bifunctional catalase-peroxidase (KatP), and a complex type II secretion system (Etp) which is apparently involved in the secretion of virulence factors [66–68]. Additionally, plasmid pO157 encodes a secreted protein of about 104 kDa (EspP) that shows strong homologies to autotransporter proteins [69]. These proteins possess a C-terminal domain responsible for their own transport through the outer membrane. It was shown that EspP acts as a serine protease with pepsin and the human coagulation factor V as possible substrates. Since deficiencies of factor V result in prolonged bleeding, EspP could increase the haemorrhage into the gastrointestinal tract observed in patients with EHEC infections. Accordingly, EspP might be an accessory virulence factor of EHEC. Moreover, on plasmid pO157, genes or groups of genes are flanked by complete and incomplete ISs. The ISs are suggested to have contributed to the evolution of the large virulence plasmids. They could transfer genes into the plasmid either by regular transposition events or by homologous recombination between similar ISs. Additionally, they could mediate the exchange of genes between the plasmid and the bacterial chromosome of this important pathogen.

Conclusions

Particular genetic mechanisms contribute to the evolution of microbes. These mechanisms include point mutations, DNA rearrangements and gene transfer processes [70]. The development of pathogenic bacteria is also directed by these mechanisms, which can act in short time intervals (microevolution) or over longer periods of time (macroevolution). It is interesting that the evolution of the resistance mechanisms of pathogenic bacteria shows features similar to the development of pathogenic traits. Thus, point mutations in genes contributing to virulence (e.g. adhesins, capsules) or resistance (e.g. topoisomerase) lead to new variants with new properties. Genetic rearrangements may result in new membrane proteins, important for pathogenic

Table 3. Examples of bacterial pathogenicity islands (Pais) according to Hacker et al. [6].

Pathogenic microorganism	Pai designation	Virulence genes encoded	Size (kb)	Boundary	Associated genes	Deletion frequency
Escherichia coli 536 (uropathogenic)	Pai I	*hly*	70	16 bp DR	*sel*C	10^{-4}–10^{-5}
	Pai II	*hly*, *prf*	190	18 bp DR	*leu*X	10^{-4}–10^{-5}
E. coli J96 (uropathogenic)	Pai IV	*hly*, *pap*	170	–	*phe*V	?
	Pai V	*hly*, *prs*, *cnf* I	110	135 bp DR	*phe*R	10^{-5}
E. coli E2348/69 (enteropathogenic)	LEE (Pai III)	*eae*, *esp*AB, *sep*A–I	35	–	*sel*C	stable
Yersinia pestis	HPI (*pgm* locus)	*hms* HFRS, *fyu*A, *irp*B–D	102	IS100	–	10^{-4}–10^{-5}
Y. enterocolitica	HPI	*fyu*A, *irp*2 (*irp*1)	45	–	*asn*T	10^{-5}
Salmonella typhimurium	SPI I	*inv*, *spa*, *hil*	40	–	–	stable/unstable
	SPI II	type III secretion system, two-component regulatory system	40	–	*val*V	stable
Helicobacter pylori	Cag Pai	*cag*A–T	40	31 bp DR	*glr*	unstable
Dichelobacter nodosus	Vap region	*vap*A–E	11.9	19 bp DR	*ser*V	?
	Vrl region	*vrl*	27	–	*ssr*A	?
Vibrio cholerae	VPI	*acf*, *tcp*, *tox*T, *int*	45	*att* sites	*ssr*A	stable
Staphylococcus aureus	SaPI, SaPII	*tst*	15.2	17 bp	*tyrB*, *trp*	transferable by phages

Abbreviations: DR, direct repeat; *hly*, haemolysin gene; *sel*C selenocysteine tRNA gene; *prf*, P-related fimbrial gene cluster; *leu*X, leucine tRNA gene; *pap*, pili associated with pyelonephritis genes; *phe*V phenylalanine tRNA gene; *prs*, P-related sequence; *cnf* I cytotoxic-necrotizing factor I gene; LEE, locus of enterocyte effacement; *eae*A, *E. coli* attaching and effacing gene; *esp*, enteropathogenic *E. coli* secreted protein gene; *sep*, secretion of *E. coli* protein genes; HPI, high-pathogenicity island; *pgm*, pigmentation locus; *hms* HFRS, hemin storage protein gene HFRS; *fyu*, ferric yersiniabactin uptake; *irp*, genes encoding iron-repressible proteins; IS, insertion element; *asn*T, asparagine tRNA gene; SPI, *Salmonella* pathogenicity island; *inv*, invasion genes; *spa*, secretion protein antigen gene; *hil*, hyperinvasion locus; *val*V valine tRNA gene; Cag, cytotoxin-associated antigen; *glr*, glutamate racemase; Vap, virulence-associated protein; *vrl*, virulence-related locus; *ser*V, serine tRNA; *ssr*A, small stable RNA; VPI, vibrio pathogenicity island; *acf*, accessory colonization factor; *tcp*, toxin-coregulated pilus; *tox*T, transcriptional activator gene; *int*, integrase gene; *att*, attachment; SaPI, *S. aureus* pathogenicity island; *tst*, toxic shock syndrome toxin gene.

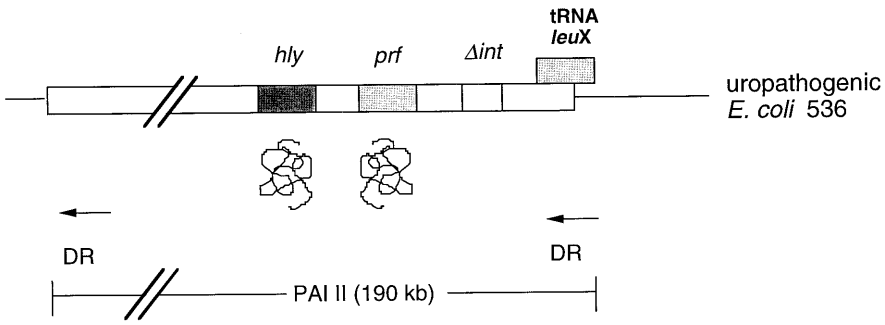

Figure 2. Genetic structure of pathogenicity island (Pai) II of uropathogenic *E. coli* 536. The Pai comprises representative genetic elements characteristic for Pais. These elements include direct repeats (DR), a tRNA gene (*leu*X), a cryptic integrase locus (*int*) and virulence gene clusters (*hly*, *prf*).

processes (e.g. in gonococci or *Mycoplasma*), and in new variants of PBPs conferring resistance to antibiotics (e.g. in pneumococci). The Pais are of particular importance for the evolution of virulence. These DNA fragments exhibit specific features and are found in pathogenic variants of many bacteria. Their genetic composition, however, represents a common theme of particular genome segments found in many pathogenic and non-pathogenic bacteria. The *mecA* region of pathogenic staphylococci, conferring methicillin resistance, shows similar features to Pais. In addition, non-pathogenic bacteria carry islands encoding factors important for symbiosis (e.g. *Rhizobia*), sugar metabolism (e.g. *Salmonella senftenberg*) or degradation of xenobiotic compounds (e.g. *Pseudomonas putida*). These structures were therefore termed genomic islands as they play an important role in the development of microbes. Pais may represent just one example of genomic islands, which have contributed to the evolution of pathogenesis in the past and will presumably do so in the future.

1 Kogoma T. (1997) Stable DNA replication: interplay between DNA replication, homologous recombination, and transcription. Microbiol. Mol. Biol. Rev. **61:** 212–238
2 Sniegowski P. (1998) Mismatch repair: origin of species? Curr. Biol. **8:** R59–R61
3 Seifert H. S. and So M. (1988) Genetic mechanisms of bacterial antigenic variation. Microbiol. Rev. **52:** 327–336
4 Arber W. (1993) Evolution of prokaryotic genomes. Gene **135:** 49–56
5 Groisman E. A. and Ochman H. (1996) Pathogenicity islands: bacterial evolution in quantum leaps. Cell **87:** 791–794
6 Hacker J., Blum-Oehler G., Mühldorfer I. and Tschäpe H. (1997) Pathogenicity islands of virulent bacteria: structure, function and impact on microbial evolution. Mol. Microbiol. **23:** 1089–1097
7 Sokurenko E. V., Hasty D. L. and Dykhuizen D. E. (1999) Pathoadaptive mutations: gene loss and variation in bacterial pathogens. Trends Microbiol. **7:** 191–195
8 Perry R. D. and Fetherston J. D. (1997) *Yersinia pestis* – etiologic agent of plague. Clin. Microbiol. Rev. **10:** 35–66
9 Paton J. C. and Paton A. W. (1998) Pathogenesis and diagnosis of Shiga toxin-producing *Escherichia coli* infections. Clin. Microbiol. Rev. **11:** 450–479
10 Winn W. C. Jr (1988) Legionnaires disease: historical perspective. Clin. Microbiol. Rev. **1:** 60–81
11 Grimm D., Merkert H., Ludwig W., Schleifer K. H., Hacker J. and Brand B. C. (1998) Specific detection of *Legionella pneumophila*: construction of a new 16S rRNA-targeted oligonucleotide probe. Appl. Environ. Microbiol. **64:** 2686–2690
12 Rupp M. E. (1997) Coagulase-negative staphylococcal infections: an update regarding recognition and management. Curr. Clin. Top. Infect. Dis. **17:** 51–87
13 Erickson J. W. and Burt S. K. (1996) Structural mechanisms of HIV drug resistance. Annu. Rev. Pharmacol. Toxicol. **36:** 545–571
14 Dybvig K. and Voelker L. L. (1996) Molecular biology of mycoplasmas. Annu. Rev. Microbiol. **50:** 25–57
15 Achtman M. (1995) Epidemic spread and antigenic variability of *Neisseria meningitidis*. Trends Microbiol. **3:** 186–192
16 Meyer T. F., Pohlner J. and Putten J. P. van (1994) Biology of the pathogenic Neisseriae. Curr. Top. Microbiol. Immunol. **192:** 283–317
17 Leathart J. B. and Gally D. L. (1998) Regulation of type 1 fimbrial expression in uropathogenic *Escherichia coli*: heterogeneity of expression through sequence changes in the *fim* switch region. Mol. Microbiol. **28:** 371–381
18 Loewen P. C. and Hengge-Aronis R. (1994) The role of the sigma factor sigma S (KatF) in bacterial global regulation. Annu. Rev. Microbiol. **48:** 53–80
19 Haldenwang W. G. (1995) The sigma factors of *Bacillus subtilis*. Microbiol. Rev. **59:** 1–30
20 Hecker M. and Volker U. (1998) Non-specific, general and multiple stress resistance of growth-restricted *Bacillus subtilis* cells by the expression of the sigmaB regulon. Mol. Microbiol. **29:** 1129–1136
21 Losick R. and Stragier P. (1992) Crisscross regulation of cell-type-specific gene expression during development in *B. subtilis*. Nature **355:** 601–604
22 Hengge-Aronis R. (1999) Interplay of global regulators and cell physiology in the general stress response of *Escherichia coli*. Curr. Opin. Microbiol. **2:** 148–152
23 Wu S., Lencastre H. de and Tomasz A. (1996) Sigma-B, a putative operon encoding alternate sigma factor of *Staphylococcus aureus* RNA polymerase: molecular cloning and DNA sequencing. J. Bacteriol. **178:** 6036–6042

24 Chen C. Y., Buchmeier N. A., Libby S., Fang F. C., Krause M. and Guiney D. G. (1995) Central regulatory role for the RpoS sigma factor in expression of *Salmonella dublin* plasmid virulence genes. J. Bacteriol. **177:** 5303–5309

25 Kullik I., Giachino P. and Fuchs T. (1998) Deletion of the alternative sigma factor sigmaB in *Staphylococcus aureus* reveals its function as a global regulator of virulence genes. J. Bacteriol. **180:** 4814–4820

26 Gertz S., Engelmann S., Schmid R., Ohlsen K., Hacker J. and Hecker M. (1999) Regulation of sigmaB-dependent transcription of *sig*B and *asp*23 in two different *Staphylococcus aureus* strains. Mol. Gen. Genet. **261:** 558–566

27 Sokurenko E. V., Chesnokova V., Dykhuizen D. E., Ofek I., Wu X. R., Krogfelt K. A. et al. (1998) Pathogenic adaptation of *Escherichia coli* by natural variation of the FimH adhesin. Proc. Natl. Acad. Sci. USA **95:** 8922–8926

28 Pouttu R., Puustinen T., Virkola R., Hacker J., Klemm P. and Korhonen T. K. (1999) Amino acid residue Ala-62 in the FimH fimbrial adhesin is critical for the adhesiveness of meningitis-associated *Escherichia coli* to collagens. Mol. Microbiol. **31:** 1747–1757

29 HooperD. C. (1988) Bacterial topoisomerases, anti-topoisomerases, and anti-topoisomerase resistance. Clin. Infect. Dis. **27** (Suppl 1)**:** S54–S63

30 Musser J. M. (1995) Antimicrobial agent resistance in mycobacteria: molecular genetic insights. Clin. Microbiol. Rev. **8:** 496–514

31 Birch A., Hausler A., Ruttener C. and Hutter R. (1991) Chromosomal deletion and rearrangement in *Streptomyces glaucescens*. J. Bacteriol. **173:** 3531–3538

32 Haselkorn R. (1992) Developmentally regulated gene rearrangements in prokaryotes. Annu. Rev. Genet. **26:** 113–130

33 Stragier P., Kunkel B., Kroos L. and Losick R. (1989) Chromosomal rearrangement generating a composite gene for a developmental transcription factor. Science **243:** 507–512

34 Shinagawa H. and Iwasaki H. (1996) Processing the holliday junction in homologous recombination. Trends Biochem. Sci. **21:** 107–111

35 Clark A. J. and Sandler S. J. (1994) Homologous genetic recombination: the pieces begin to fall into place. Crit. Rev. Microbiol. **20:** 125–142

36 Seifert H. S. (1996) Questions about gonococcal pilus phase and antigenic variation. Mol. Microbiol. **21:** 433–440

37 Putten J. P. van and Robertson B. D. (1995) Molecular mechanisms and implications for infection of lipopolysaccharide variation in *Neisseria*. Mol. Microbiol. **16:** 847–853

38 Coffey T. J., Enright M. C., Daniels M., Morona J. K., Morona R., Hryniewicz W. et al. (1998) Recombinational exchanges at the capsular polysaccharide biosynthetic locus lead to frequent serotype changes among natural isolates of *Streptococcus pneumoniae*. Mol. Microbiol. **27:** 73–83

39 Tomasz A. (1997) Antibiotic resistance in *Streptococcus pneumoniae*. Clin. Infect. Dis. **24** (Suppl 1)**:** S85–S88

40 Hakenbeck R., Grebe T., Guenzi E., Konig A., Krauss J. and Reichmann P. (1995) The role of penicillin-binding proteins in penicillin resistance of *Streptococcus pneumoniae*. Dev. Biol. Standards **85:** 115–123

41 Hakenbeck R. and Coyette J. (1998) Resistant penicillin-binding proteins. Cell. Mol. Life Sci. **54:** 332–340

42 Hallet B. and Sherratt D. J. (1997) Transposition and site-specific recombination: adapting DNA cut-and-paste mechanisms to a variety of genetic rearrangements. FEMS Microbiol. Rev. **21:** 157–178

43 Abraham J. M., Freitag C. S., Clements J. R. and Eisenstein B. I. (1985) An invertible element of DNA controls phase variation of type 1 fimbriae of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **82:** 5724–5727

44 Zieg J., Silverman M., Hilmen M. and Simon M. (1977) Recombinational switch for gene expression. Science **196:** 170–172

45 Klemm P. (1986) Two regulatory fim genes, fimB and fimE, control the phase variation of type 1 fimbriae in *Escherichia coli*. EMBO J. **5:** 1389–1393

46 Gally D. L., Leathart J. and Blomfield I. C. (1996) Interaction of FimB and FimE with the *fim* switch that controls the phase variation of type 1 fimbriae in *Escherichia coli* K-12. Mol. Microbiol. **21:** 725–738

47 Friedman D. I. (1992) Interaction between bacteriophage lambda and its *Escherichia coli* host. Curr. Opin. Genet. Dev. **2:** 727–738

48 Hall R. M. and Collis C. M. (1995) Mobile gene cassettes and integrons: capture and spread of genes by site-specific recombination. Mol. Microbiol. **15:** 593–600

49 Mazel D., Dychinco B., Webb V. A. and Davies J. (1998) A distinctive class of integron in the *Vibrio cholerae* genome. Science **280:** 605–608

50 Mahillon J. and Chandler M. (1998) Insertion sequences. Microbiol. Mol. Biol. Rev. **62:** 725–774

51 Lewis L. A., Gopaul S. and Marsh C. (1994) The non-random pattern of insertion of IS2 into the *hem*B gene of *Escherichia coli*. Microbiol. Immunol. **38:** 461–465

52 Craig N. L. (1991) Tn7: a target site-specific transposon. Mol. Microbiol. **5:** 2569–2573

53 Hammerschmidt S., Hilse R., Putten J. P. van, Gerardy-Schahn R., Unkmeir A. and Frosch M. (1996) Modulation of cell surface sialic acid expression in *Neisseria meningitidis* via a transposable genetic element. EMBO J. **15:** 192–198

54 Ziebuhr W., Krimmer V., Rachid S., Lößner I., Götz F. and Hacker J. (1999) A novel mechanism of phase variation of virulence in *Staphylococcus epidermidis*: evidence for control of the polysaccharide intercellular adhesin synthesis by alternating insertion and excision of the insertion sequence element IS*256*. Mol. Microbiol. **32:** 345–356

55 Bonafede M. E., Carias L. L. and Rice L. B. (1997) Enterococcal transposon Tn*5384*: evolution of a composite transposon through cointegration of enterococcal and staphylococcal plasmids. Antimicrob. Agents. Chemother. **41:** 1854–1858

56 Rice L. B. and Carias L. L. (1998) Transfer of Tn*5385*, a composite, multiresistance chromosomal element from *Enterococcus faecalis*. J. Bacteriol. **180:** 714–721

57 Karaolis D. K., Somara S., Maneval D. R. Jr, Johnson J. A. and Kaper J. B. (1999) A bacteriophage encoding a pathogenicity island, a type-IV pilus and a phage receptor in cholera bacteria. Nature **399:** 375–379

58 Lindsay J. A., Ruzin A., Ross H. F., Kurepina N. and Novick R. P. (1998) The gene for toxic shock toxin is carried by a family of mobile pathogenicity islands in *Staphylococcus aureus*. Mol. Microbiol. **29:** 527–543

59 Chambers H. F. (1997) Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. Clin. Microbiol. Rev. **10:** 781–791

60 Wu S., Piscitelli C., Lencastre H. de and Tomasz A. (1996) Tracking the evolutionary origin of the methicillin resistance gene: cloning and sequencing of a homologue of *mec*A from a methicillin susceptible strain of *Staphylococcus sciuri*. Microb. Drug Resist. **2:** 435–441

61 Ito T., Katayama Y. and Hiramatsu K. (1999) Cloning and nucleotide sequence determination of the entire *mec* DNA of pre-methicillin-resistant *Staphylococcus aureus* N315. Antimicrob. Agents. Chemother. **43:** 1449–1458

62 Cohen S. and Sweeney H. M. (1973) Effect of the prophage and penicillinase plasmid of the recipient strain upon the transduction and the stability of methicillin resistance in *Staphylococcus aureus*. J. Bacteriol. **116:** 803–811

63 Trees D. L. and Iandolo J. J. (1988) Identification of a *Staphylococcus aureus* transposon (Tn*4291*) that carries the methicillin resistance gene(s). J. Bacteriol. **170:** 149–154

64 Karch H., Heesemann J., Laufs R., O'Brien A. D., Tacket C. O. and Levine M. M. (1987) A plasmid of enterohemorrhagic *Escherichia coli* O157:H7 is required for expression of a new fimbrial antigen and for adhesion to epithelial cells. Infect. Immun. **55:** 455–461

65 Tzipori S., Karch H., Wachsmuth K. I., Robins-Browne R. M., O'Brien A. D., Lior H. et al. (1987) Role of a 60-megadalton plasmid and Shiga-like toxins in the pathogenesis of infection caused by enterohemorrhagic *Escherichia coli*

O157:H7 in gnotobiotic piglets. Infect. Immun. **55:** 3117–3125

66 Schmidt H., Beutin L. and Karch H. (1995) Molecular analysis of the plasmid-encoded hemolysin of *Escherichia coli* O157:H7 strain EDL 933. Infect. Immun. **63:** 1055–1061

67 Brunder W., Schmidt H. and Karch H. (1996) KatP, a novel catalase-peroxidase encoded by the large plasmid of enterohaemorrhagic *Escherichia coli* O157:H7. Microbiology **142:** 3305–3315

68 Schmidt H., Henkel B. and Karch H. (1997) A gene cluster closely related to type II secretion pathway operons of gram-negative bacteria is located on the large plasmid of enterohemorrhagic *Escherichia coli* O157 strains. FEMS Microbiol. Lett. **148:** 265–272

69 Brunder W., Schmidt H. and Karch H. (1997) EspP, a novel extracellular serine protease of enterohaemorrhagic *Escherichia coli* O157:H7 cleaves human coagulation factor V. Mol. Microbiol. **24:** 767–778

70 Hacker J. and Kaper J. (in press) The concept of pathogenicity island. In: Pathogenicity islands and other mobile virulence elements, Kaper J. and Hacker J. (eds.), ASM-Press, Washington, DC