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Micro-Review

Challenges for molecular plant pathology over the next ten years

Horizontal gene transfer: sustaining pathogenicity and optimizing host–pathogen interactions

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SUMMARY

Successful host–pathogen interactions require the presence, maintenance and expression of gene cassettes called 'pathogenicity islands' (PAIs) and 'metabolic islands' (MAIs) in the respective pathogen. The products of these genes confer on the pathogen the means to recognize their host(s) and to efficiently evade host defences in order to colonize, propagate within the host and eventually disseminate from the host. Virulence effectors secreted by type III and type IV secretion systems, among others, play vital roles in sustaining pathogenicity and optimizing host–pathogen interactions. Complete genome sequences of plant pathogenic bacteria have revealed the presence of PAIs and MAIs. The genes of these islands possess mosaic structures with regions displaying differences in nucleotide composition and codon usage in relation to adjacent genome structures, features that are highly suggestive of their acquisition from a foreign donor. These donors can be other bacteria, as well as lower members of the Archaea and Eukarya. Genes that have moved from the domains Archaea and Eukarya to the domain Bacteria are true cases of horizontal gene transfer. They represent interdomain genetic transfer. Genetic exchange between distinct members of the domain Bacteria, however, represents lateral gene transfer, an intradomain event. Both horizontal and lateral gene transfer events have been used to facilitate survival fitness of the pathogen.

INTRODUCTION

Bacteria that colonize and infect their hosts are clearly equipped with sets of genes that confer on them the capacity for virulence and pathogenicity. These gene sets, often clustered as cassettes, are located either on plasmids or chromosomes. The expression of these genes is regulated via sensory response regulators commonly mediated through signal transduction. The clustered feature of these closely grouped genes, referred to as pathogenicity islands (PAIs) (Hacker *et al.*, 1997), suggests that they have been acquired through lateral gene transfer events. The transferred genetic element can range in size from large (40 kb) PAIs, such as SP1 and SP2 of *Salmonella typhimurium* (Mills *et al.*, 1995; Shea *et al.*, 1996), to single genes inserted in an operon (Guphna *et al.*, 2004; Saunders *et al.*, 2001; Stein *et al.*, 1996). Analyses of complete genome sequences have revealed mosaic structures with regions displaying differences in nucleotide composition and codon usage in relation to adjacent genome structures. Such mosaics add further evidence for the acquisition of genes through lateral gene transfer (Dufraigne *et al.*, 2005; Guidot *et al.*, 2007; Koonin *et al.*, 2001; Lawrence, 2002; Ochman *et al.*, 2000). Equipped with PAIs, recent pathogens continue to evolve to increase host pathogen fitness through reductive evolution (Hacker and Carniel, 2001). For example, the human pathogen *Mycobacterium ulcerans* was found to have originated from *Mycobacterium marinum*, a frog, fish and reptile pathogen (Stinear and Johnson, 2007). The presence of PAIs alone may be insufficient for host pathogen fitness. The concomitant horizontal gene transfer of 'metabolic islands' (MAIs) may be necessary for optimal fitness (Chen, 2006; Chiu *et al.*, 2005; Karch *et al.*, 1999; Tumapa *et al.*, 2008). Hence, the persistence and maintenance of laterally transferred genes reflect their need to provide selective advantages on the recipient bacterium in relationship with its host.

The ongoing analysis of numerous bacterial genomes has revealed how horizontal gene transfer has been instrumental in shaping genome structures (Nakamura *et al.*, 2004). The detection of recently acquired genes in the analysed genomes of plant pathogens is possible because the laterally transferred genes have compositional features that distinguish them from vertically inherited genes (Dufraigne *et al.*, 2005; Guidot *et al.*, 2007; Lawrence, 2002; Lawrence and Ochman, 2002). Previously, the reconstruction of the evolution of prokaryotic and eukaryotic organisms has been based on comparative analyses of 16S and

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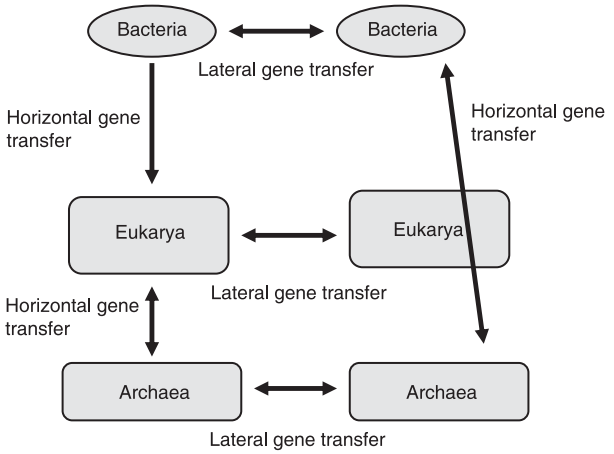

Fig. 1 Diagram showing delineation of the terms 'horizontal gene transfer' and 'lateral gene transfer' for genetic interchange between and within domains, respectively. Arrows depict directions of interdomain and intradomain genetic interchanges as documented herein and elsewhere (Almeida *et al.*, 2008; Bouhouche *et al.*, 2000; Choi and Kim, 2007; Dohm *et al.*, 2006; Lage *et al.*, 2004; Nembaware *et al.*, 2004; Syvanen, 2002).

18S ribosomal RNA (rRNA) genes, respectively. Comparative analysis of complete genomes reveals a different picture of microbial evolution, that is, there has been horizontal (and lateral) movement of genes across vast phylogenetic distances (Jain *et al.*, 2002).

HORIZONTAL VERSUS LATERAL GENE TRANSFER?

In reviewing the current literature, there remains a certain level of confusion with the use of the terms 'horizontal gene transfer' and 'lateral gene transfer'. These terms have been used interchangeably in the literature. To obviate confusion in their current cross usage, lateral gene transfer should be used *senso stricto* in the intergenetic transfer between organisms within a domain, i.e. within Bacteria, Archaea or Eukarya (eukaryotes), whereas horizontal gene transfer should be used for interdomain transfer of genes between Bacteria and Archaea, Archaea and Eukarya, and Eukarya and Bacteria or Archaea (Fig. 1) (Kado, 2002a).

Both lateral gene transfer and horizontal gene transfer events between bacteria and between bacteria and their eukaryotic hosts, respectively, have been documented extensively (Almeida *et al.*, 2008; Bouhouche *et al.*, 2000; Choi and Kim, 2007; Dohm *et al.*, 2006; Lage *et al.*, 2004; Syvanen, 2002). The genes required for successful pathogenic interactions with eukaryotic hosts have been acquired through lateral transfer events, and can be accessory genes clustered on mobile elements such as plasmids. Long-term associations of pathogenic or symbiotic genes as clusters bearing shufflons (Komano, 1999) and integrons (Mazel, 2007) on plasmids can result in their insertion and fixation into the bacterial chromosomes, and obviate the need for an extrachromosomal element to bear them (Hacker and Kaper, 2000). Eventually, the gene transfer machinery of PAIs can result in their loss through reductive evolution (Hacker and Carniel, 2001). When the host–pathogen interactions are a recent evolutionary event, the gene transfer machinery remains functional as an important component for lateral and horizontal gene transfer. Such transfer events are essential for the first step in survival of the set of pathogenicity-conferring genes. On transfer into the bacterial cell, the foreign DNA must escape restriction nucleases. The integration of the DNA into the host genome by illegitimate recombination provides an escape mechanism. Accessory genes clustered as MAIs to best establish the pathogen with its host are equally important, as they ensure colonization and perpetuation of the bacterial pathogen. Overall fitness stabilizes the newly acquired trait.

MECHANISMS OF LATERAL GENE TRANSFER

The lateral transfer of genes can occur through transformation, transduction and conjugation. *Ralstonia solanacearum* is naturally transformable and uses natural transformation to exchange genes at significant frequencies *in planta* (Bertolla *et al.*, 1999). With human pathogens, transformation was found to be the mechanism of plasmid transfer between *Streptomyces* and *Mycobacterium* (Bhatt *et al.*, 2002). Evidence for the *in planta* transfer of a 37 kb plasmid, pXcB, between *Xanthomonas citri* pv. aurantifolii and *X. citri* pv. citri via a type IV secretion system (T4SS) has been demonstrated (El Yacoubi *et al.*, 2007). The *in planta* transfer of virulence genes between virulent *Agrobacterium tumefaciens* and avirulent *A. radiobacter* was observed in 1969 by Kerr (Kerr, 1969, 1971). Likewise, transfer of a large (*c.* 70 kb) plasmid *in vivo* between *Shewanella algae* and *Kluyvera* and *Klebsiella* has been observed (Lascols *et al.*, 2008).

Both natural transformation and conjugation mechanisms and their respective barriers to gene flow have been described in detail (Thomas and Nielsen, 2005). With certain human pathogens, virulence determinants have been transferred by lysogenic conversion by bacteriophages. The transduction of DNA is highly efficient between *Staphylococcus* and *Streptococcus* (Miao and Miller, 1999). However, large gene clusters in PAIs are mainly transferred by conjugation (Hacker and Kaper, 2000). Genes that encode the conjugative transfer machinery are found on many conjugative plasmids. PAIs located on conjugative plasmids ensure their transfer into either related or unrelated bacterial recipients. As described below, PAIs encode virulence effectors and secretion systems. In many cases, these pathogens harbour more than one secretion system that is used to deliver virulence effectors. Of these, the type III secretion system (T3SS) is well known to be transferred by lateral gene transfer (Aizawa, 2001;

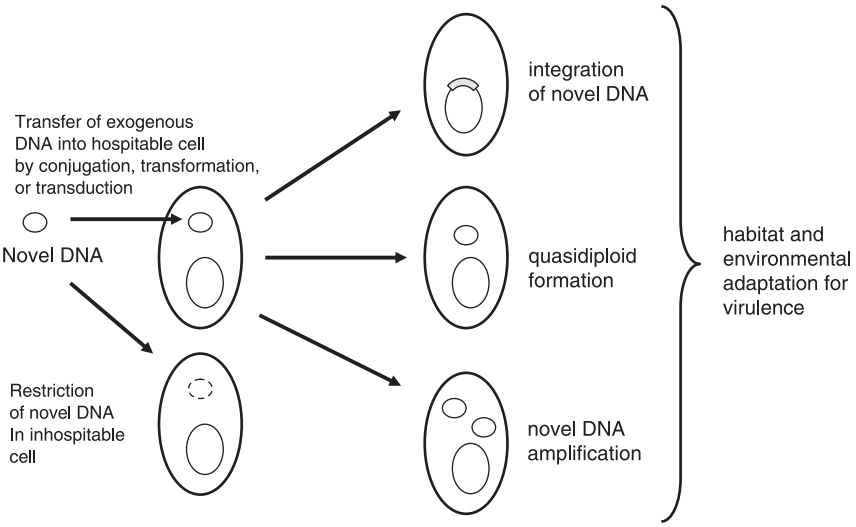

Fig. 2 Fate of the acquisition of novel DNA from a variety of sources. Stable acquisition of novel DNA can culminate in its insertion into the genome of the recipient cell, in it remaining as an independent replicon (if there are replication determinants carried on the novel DNA) or in it forming multiple copies of the replicon. Multiple copies of the replicon aid in conferring the cellular phenotype, such as, for example, increased virulence on the pathogen. With members of the Eukarya, rearrangements and reduction in the size of the inserted foreign DNA are the direction towards germ-line inherited status that normally occurs through vertical transfer of genes.

Francis *et al.*, 2004; Gosh, 2004; Hueck, 1998; Mudgett, 2005). T3SSs are activated on perception of environmental factors, such as low calcium, pH, temperature and physical contact with host cells (Bergman *et al.*, 1994; Deakin *et al.*, 2005; Linares *et al.*, 2005; Van Dijk *et al.*, 1999; Xu *et al.*, 2008). Virulence effectors are secreted via a needle-like structure (for animal/human pathogens) or long pilus-like structures (for plant pathogens) into the host cells during colonization and infection.

The functions of the majority of type III effectors in plant pathogens remain unknown. In *R. solanacearum*, more than 40 effector proteins (possibly as many as 95) are translocated via the type III secretion pathway (Cunnac *et al.*, 2004a,b). Likewise, *Pseudomonas syringae* pv. tomato harbours at least 48 type III effectors (Alfano and Collmer, 2004; Buell *et al.*, 2003; Collmer *et al.*, 2002; Schechter *et al.*, 2004). A number of effectors, known as avirulence (avr) proteins, appear to suppress basal resistance of the plant host. How these effectors cause suppression remains unknown, but some may contribute to pathogenicity by interfering with plant defence signal transduction (Mudgett, 2005; Tang *et al.*, 2006). For example, the avr protein AvrXv4 in *Xanthomonas campestris* pv. vesicatoria is secreted and translocated into host plants via a type III pathway, and is a member of the YopJ family of *Yersinia pestis*. This protein mimics Ulp1 protease activity in eukaryotic cells (Orth *et al.*, 2000). AvrXv4 encodes an active small ubiquitin-like modifier (SUMO) protein that localizes in the plant cytoplasm (Roden *et al.*, 2004). SUMO is a protein of 97 amino acids that is structurally similar to ubiquitin, and therefore covalently attaches to certain lysine residues of specific target proteins. In *R. solanacearum*, the RipT effector belongs to the YopT family of cysteine proteases (Cunnac *et al.*, 2004b). With *P. syringae* pv. tomato, AvrPphB cysteine protease specifically cleaves a PBS1 kinase of *Arabidopsis thaliana* (Shao *et al.*, 2003). Moreover, HopPtoN of *P. syringae* pv. tomato is a cysteine protease that is thought to suppress the hypersensitive response in both compatible and incompatible interactions (López-Solanilla *et al.*, 2004). In addition to cysteine proteases, HopPtoD2 produced by *P. syringae* pv. tomato appears to possess tyrosine phosphatase activity that is needed for virulence (Bretz *et al.*, 2003; Espinosa *et al.*, 2003). With *Yersinia* spp., tyrosine phosphatase disrupts host signal transduction processes involved in bacterial killing (Anderson *et al.*, 1996; Bliska *et al.*, 1991). This type of disruptive activity may be associated with HopPtoD2 and related proteins in plant pathogenic bacteria.

The acquisition of novel DNA via transformation, transduction or conjugation can result in either its stable existence in compatible host cells or its destruction in inhospitable recipient cell conditions (Fig. 2).

MECHANISMS OF HORIZONTAL GENE TRANSFER

In addition to T3SSs, which are critical in sustaining successful host–pathogen interactions, virulence effectors are also secreted by other secretion machineries, such as those classified as type I, type II, type IV, type V and type VI secretion systems (T1SS, T2SS, T4SS, T5SS, T6SS). Of these secretion systems, T4SSs play an important role in both lateral and horizontal gene transfer. Like T3SS, T4SSs transfer various substrates required for effective colonization, leading to biofilm formation and disrupting the normal physiology of the host cell. However, unlike T3SS, T4SSs can transfer from the bacterium high-molecular-weight substrates, such as DNA molecules, into members of the Eukarya, i.e. eukaryotic cells. T4SSs are therefore an established basic mechanism for horizontal gene transfer. T4SSs have adapted a plasmid-based bacterial conjugation machinery that is composed of a conjugal pilus for establishing contact with recipient bacteria and providing

a conduit for DNA transfer into them (Kado, 2000; Lai and Kado, 2000). Recent studies have clearly shown that a DNA–protein (e.g. relaxase) substrate is transferred via the conjugative F pilus of *Escherichia coli* (Babic *et al*., 2008; Lanka and Wilkins, 1995). Both plant and human/animal pathogens, including obligate intracellular *Wolbachia* species, have adapted T4SSs to transfer large effector molecules to their eukaryotic host cells (Christie and Vogel, 2000; Rancès *et al*., 2008; Seubert *et al*., 2003).

So far, the only well-documented natural transfer of genes between a prokaryote and a eukaryote is by *Agrobacterium* species (*A. tumefaciens*, *A. rhizogenes*, *A. vitis* and *A. rubi*) that have adapted T4SS to deliver DNA and proteins into eukaryotic cells. These substrates are delivered via the T pilus encoded by T4SS (Kado, 2000; Lai and Kado, 2000). *Agrobacterium tumefaciens* is the prototypical species that uses T4SS to transfer a specific sector (T-DNA) of a large plasmid, known as the Ti (for tumour-inducing) or Ri (for root-inducing) plasmid, into plant cells, culminating with the integration and fixation of the T-DNA into the plant genome. The T-DNAs bear oncogenes which, when expressed, lead to the formation of tumours or hairy roots. With the *A. tumefaciens* horizontal gene transfer system, most plant cells are transformed by only one or a few bacterial cells (Depicker *et al*., 1985).

Under laboratory conditions, *A. tumefaciens* has been reported to transform filamentous fungi, such as *Aspergillus niger*, *Agaricus bisporus*, *Colletotrichum gloeosporoides*, *Fusarium venenatum* and *Neurospora crassa* (deGroot *et al*., 1998), and the air-borne conidia of the brown rot pathogen *Monilinia fructicola* (Lee and Bostock, 2006). Hence, the T4SS-based horizontal gene transfer system of *A. tumefaciens* is highly promiscuous and non-selective. This degree of promiscuity extends even to animal cells. *Agrobacterium tumefaciens*-mediated transformation of HeLa cells (Kunik *et al*., 2001), sea urchin embryos (Bulgakov *et al*., 2006) and animal cells in general (Hohn *et al*., 2002) has been reported. Interestingly, components of the T4SS pilus-like structure are encoded by the *cag* PAI of *Helicobacter pylori* (Andrzejewska *et al*., 2006). The pilus-like structure is required for pathogenicity, but its precise role remains to be determined. It is tempting to speculate here that its role may be similar to that of the T pilus in delivering a DNA–protein substrate to the host cell.

Although the frequency of naturally occurring, stable, germ-line gene transfers by Ti and Ri plasmids is unknown, the presence of T-DNA hairy root oncogene homologues in wild tobacco (*Nicotiana tabacum* L.), tree tobacco (*Nicotiana glauca* Graham), carrots (*Dacus carota* L. cv. sativa) and petunia (*Petunia hybrida* Vilm.) strongly suggests that stable germ-lines evolved long ago through *A. rhizogenes* transformations of plants (Aoki and Syono, 1999; Frűndt *et al*., 1998; Furner *et al*., 1986; Kado, 2002b; Meyer *et al*., 1995; Nagata *et al*., 1995). With legume symbionts, such as *Mesorhizobium loti* and *Sinorhizobium meliloti*, their T4SS does not function as a critical determinant in nodulation, and appears to be dispensable for plant symbiosis (Jones *et al*., 2007). However, with mammalian pathogens, such as *Brucella suis* and *Bartonella henselae*, T4SS is required for persistence within their respective hosts (O'Callaghan *et al*., 1999; Schulein and Dehio, 2002).

LATERAL GENE TRANSFER OF VIRULENCE DETERMINANTS

The *in planta* transfer of the Ti plasmid between *Agrobacterium* species is the classical example of plasmid-mediated lateral transfer of virulence determinants between virulent and avirulent bacteria under natural conditions (Kerr, 1969, 1971). The virulence determinants are arranged in six operons (*virA*, *B*, *G*, *C*, *D* and *E*), clustered as a '*vir*' regulon on the Ti plasmid (Rogowsky *et al*., 1990). The *vir* regulon contains all the information required for virulence, i.e. the processing and transfer of oncogenic T-DNA from *Agrobacterium* species to its host plant cell. The *vir* regulon is the PAI of *Agrobacterium* species. The *virB* operon in this regulon encodes the T4SS that is dedicated to conjugal DNA transfer. This operon is highly conserved usually on plasmids in both plant and animal bacterial pathogens. The historical experiment of transforming avirulent pneumococci into a virulent strain with a substance shown to be DNA was the first clear demonstration that lateral gene transfer of virulence determinants was possible (Avery *et al*., 1944). Since its original discovery (Ochia *et al*., 1959), plasmid-mediated transfer of antibiotic resistance between bacterial genera has been widely documented. Following the plasmid-mediated transfer of virulence determinants between *Agrobacterium* species, it soon became apparent that virulence determinants of other plant pathogenic bacteria (e.g. *P. syringae* and *X. campestris* pathovars), as well as mammalian bacterial pathogens (e.g. *Shigella* and *Yersinia* species), are transferred via plasmids. Recent studies on 32 plasmids from 10 *P. syringae* pv. savastanoi strains showed the widespread presence of T4SSs and genes involved in the biosynthesis of cytokinins (Pérez-Martinez *et al*., 2008).

Genes encoding the surface components of bacteria, such as the six genes of a 12.2 kb virulence locus whose products catalyse the formation of lipopolysaccharides of *X. oryzae* pv. oryzae, show atypical G + C content and altered codon usage with respect to the chromosomal genes. This suggests that *X. oryzae* pv. oryzae acquired the virulence locus through a lateral gene transfer event from a foreign donor. The locus is flanked by *metB*, which encodes cystathionine gamma lyase, and *etfA*, which encodes an electron transport flavoprotein. Two different sets of lipopolysaccharide-encoding genes are present at this virulence locus in *X. campestris* pv. campestris and *X. axonopodis* pv. citri (Patil and Sonti, 2004). The conservation of lipopolysaccharide and lipoprotein genes has long been documented among members of the Enterobacteriaceae, including *Erwinia amylovora*

(Yamagata *et al.*, 1981) and *X. campestris* pv. campestris (Dow *et al.*, 1995; Newman *et al.*, 1995).

Lateral gene transfer of PAIs has been observed in *Streptomyces* spp. It has been postulated that saprophytic *Streptomyces* species evolved into plant pathogens through the acquisition of PAIs by *Streptomyces acidiscabies* and *S. turgidiscabies*. These species are phylogenetically distinct from those classified in the Diastatochromogenes group of *Streptomyces* (Bukhalid *et al.*, 2002). The mechanism by which PAIs were transferred remains unknown. However, it has been shown that lateral transfer of plasmids occurs between *Streptomyces* and *Mycobacterium* through transformation rather than conjugative mechanisms (Bhatt *et al.*, 2002). Conjugative gene transfer of T-DNA was demonstrated between *A. tumefaciens* and *S. lividans* mediated by the type IV secretion/conjugation system (Kelly and Kado, 2002).

Gene cassettes that bear the biosynthetic pathway for the synthesis of aminoglycocide antibiotics, such as rhodostreptomycins, appear to have been transferred from *S. padanus* to the plant pathogen *Rhodococcus fascians* (Kurosawa *et al.*, 2008). The need for rhodostreptomycin by *Rh. fascians* remains unclear. Rhodostreptomycin is effective against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Helicobacter pylori*. Resistance to antibiotics is an important survival mechanism associated with animal/human pathogens.

Between eukaryotes, lateral gene transfers via transposons, such as the *P* element and *Mariner*, have been noted in *Drosophila* species (Clark *et al.*, 2002) and in other insects (Robertson *et al.*, 2002), respectively. Incongruencies revealed by phylogenetic analyses of various related species support the hypothesis that lateral gene transfer of these transposons occurred in the evolutionary history of these insects. The fungal pathogen of wheat, *Pyrenophora tritici-repentis*, may have acquired a toxin-encoding gene (*ToxA*) from another fungus, *Stagonospora nodorum* (Friesen *et al.*, 2006). Here, one or more lateral gene transfer events have occurred. With fungi, vegetative hyphae can fuse (anastomosis) and exchange genetic elements. Conidia of these fungi form anastomosis tubes, which fuse with each other (Roca *et al.*, 2005).

CONCLUSIONS

Successful host–pathogen interactions require the presence, maintenance and expression of genes carried on PAIs and MAIs in the respective pathogen. Current studies of complete genome sequences of plant pathogenic bacteria have revealed the presence of a number of genes involved in virulence. These virulence genes exhibit mosaic structures with regions displaying differences in nucleotide composition and codon usage in relation to adjacent genome structures. These features are highly suggestive of their acquisition from a foreign donor. Such donors can be other members of the domain Bacteria or members of the domains Eukarya or Archaea. Although the transfer of PAIs has not been shown to occur via horizontal gene transfer to Bacteria from Eukarya, one PAI gene, *ros*, whose product is involved in T-DNA *ipt* gene expression in *Agrobacterium* (Chou *et al.*, 1998), has been proposed to have originated from *Fugu rupripes* (Bouhouche *et al.*, 2000). How *ros* was acquired by *A. tumefaciens* is unknown. Its acquisition may have originated from marine bacteria, such as *A. sanguineum* and *A. luteum*, as *ros*-like sequences are present in these species (Kado, 2002c). In addition, genes of MAIs have been shown to be transferred from Archaea and Eukarya to Bacteria (Almeida *et al.*, 2008; Dohm *et al.*, 2006; Lage *et al.*, 2004; Syvanen, 2002). Genes acquired from the latter two donors would be true cases of horizontal gene transfer because they represent interdomain genetic transfer. However, genetic exchange between distinct members of the domain Bacteria would represent lateral gene transfer, an intradomain event. So far, the mechanisms of lateral and horizontal gene transfer have been confined to transformation, transduction and conjugative transfer. Artificially introduced DNA has been achieved through microinjection, electroporation, bombardment with projectiles laced with DNA and forced endocytosis mediated by dextran, calcium phosphate and lipids. Such procedures result in quasi-horizontally transferred DNA, often limited by low transfection efficiency and high cytotoxicity.

In nature, both lateral gene transfer and horizontal gene transfer continue throughout the life cycles of bacteria. Circumstances and conditions that alter survival requirements help to promote the acquisition of novel genes that confer beneficial features on the organism. The discovery of novel genes in the analyses of genomic sequences is therefore highly probable, and represents one of many ongoing natural events in microbial evolution.

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