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# Modulation of Horizontal Gene Transfer in Pathogenic Bacteria by In Vivo Signals

Minireview

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The transfer of DNA from one bacterium to another is one of several fundamental processes driving the evolution of microorganisms. In addition to the exchange of chromosomal DNA through bacterial conjugation, transformation, and transduction, accessory genetic elements such as plasmids, lysogenic bacteriophages, and conjugative transposons are also able to move between bacterial strains, leading to increased genetic diversity of bacterial populations. Horizontal DNA transfer plays a critical role in the emergence of new pathogenic organisms by the dissemination of genes encoding virulence factors (e.g., toxins, adhesins, capsules, invasion properties, etc.) and antibiotic resistance. It is becoming clear that horizontal DNA transfer between bacterial pathogens can be regulated by host factors unique to the in vivo environment of an infection. This suggests that genetic elements that are thought to be nontransmissible may in fact be transmissible if given the appropriate signals within a host environment in vivo.

Pathogenic bacteria establish infections in a wide variety of host environments and specific host in vivo signals may play an important role in modulating bacterial genetic exchange. While the microenvironment of an infection is often difficult to study, it is clear that DNA transfer can occur within the host milieu. Indeed, the discovery of interbacterial gene transfer by Griffith in 1928 relied on the pneumococcus mouse infection model in which transformation was first demonstrated (Griffith, 1928). As early as 1961, evidence for conjugative transfer of DNA in vivo came in a study in which a mixture of E. coli and Salmonella typhimurium strains were fed orally to mice (Schneider et al., 1961). Within 24 hours, hybrid strains were recovered from feces and importantly, some of these recombinants were unlike any that had been observed in in vitro matings in the laboratory. This observation suggested that certain DNA transfer events may be specifically regulated and mechanistically controlled by factors found only in the host environment and not in the laboratory. Other examples of in vivo DNA transfer have been documented, such as the transmission of ENT (enterotoxin) plasmids between two E.coli strains in the intestines of newly weaned guinea pigs (Gyles et al., 1978), the transfer of Tn*916*-type conjugative transposons between different genera of Gram-positive bacteria in the gastrointestinal tracts of germ-free mice (Doucet-Populaire et al., 1991), and the apparent lysogenic conversion of Corynebacterium diphtheriae in humans (Pappenheimer and Murphy, 1983). While it is possible that specific host factors are regulating DNA transfer in each of these systems, such factors have not yet been identified.

Here we briefly review two examples in diverse pathogen–host systems, one well-studied and one newly-discovered, in which bacterial DNA transfer is intimately coupled to eukaryotic host signals occurring locally at the site of an infection. In addition to elucidating the signaling process necessary for DNA transfer in these individual systems, the results of these studies suggest a new conceptual framework for thinking about the transfer of other genetic elements in bacterial populations.

### *Agrobacterium–Host Plant Interaction*

Perhaps the best understood example of in vivo regulation of DNA transfer is found in the interaction of Agrobacterium species with plants, in which factors synthesized in the eukaryotic host plant closely regulate two distinct forms of bacterial DNA transfer. Agrobacterium species are Gram-negative soil bacteria that infect a variety of plants, leading to root proliferation (A. rhizogenes) or to the formation of crown gall tumors (A. tumefaciens) (reviewed by Farrand, 1993; Winans, 1992). Virulent Agrobacterium species harbor Ti (tumor inducing) and Ri (root inducing) virulence plasmids in A. tumefaciens and A. rhizogenes, respectively. Two entirely different but related sets of genes on these virulence plasmids control two distinct forms of bacterial DNA transfer, *vir* genes regulating the transfer of a 20 kb fragment of plasmid DNA from the Agrobacterium to the plant and *tra* genes regulating the conjugal transfer of the entire Ti plasmid to other Agrobacteria. Both of these bacterial DNA transfer systems are regulated by specific factors synthesized in the eukaryotic host.

In response to chemoattractants, Agrobacteria are drawn to the site of plant wounds where they bind to target plant cells and activate genes necessary for the unique interkingdom transfer of DNA from bacteria to plant. Sugars and phenolic compounds released from the plant wound site (Figure 1, "1") activate the bacteria's VirAG two component regulatory system, which in turn activates the transcription of a number of plasmid encoded *vir* genes. A subset of *vir* genes encode proteins responsible for the processing of the plasmid DNA. The end result is the excision and transfer of a discrete fragment of plasmid DNA (called T-DNA or transferred DNA; Figure 1, "2") into the host plant cell where it becomes integrated into the host plant genomic DNA (Figure 1, "3"). It was very recently shown that, as in bacterial conjugation, a pilus is necessary for the transfer of T-DNA to plants (Fullner et al., 1996). One of the outcomes of T-DNA gene expression is the disruption of the normal balance of plant hormones, leading to abnormal root proliferation or crown gall tumors, depending on the Agrobacterium species.

In addition to carrying genes responsible for plant oncogenesis, the integrated T-DNA also directs the synthesis of small organic compounds (amino acid and sugar derivatives) called opines, which play key roles in several closely linked bacterial functions including bacterial conjugation and opine catabolism. Conjugal

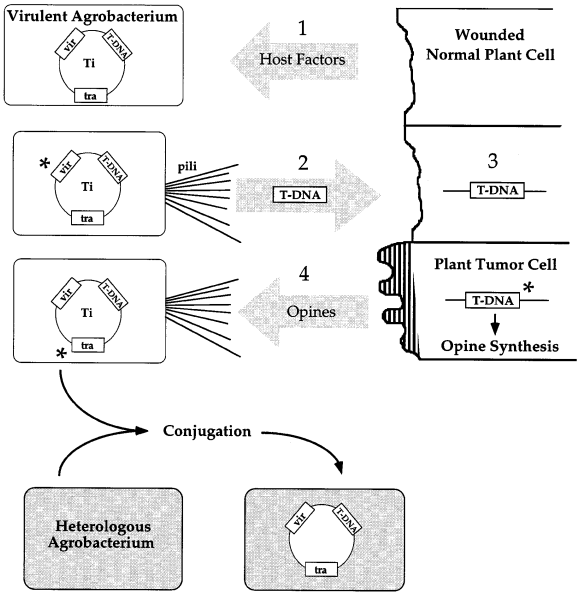


Figure 1. Model for Host Plant Induced Agrobacterium-Horizontal Virulence Gene Transfer

See text for details.

* refers to gene activation.

opines synthesized by the T-DNA in the host plant feed back to the bacterial cell (Figure 1, "4") and lead to the activation of conjugal transfer genes (*tra*) and opine catabolic genes. The activation of *tra* genes by opines occurs indirectly through a LuxR/LuxI type quorum-sensing regulatory system, homologous to that found in Vibrio fischeri (reviewed by Salmond et al., 1995; Fuqua et al., 1996). Conjugal opines activate the transcription of the plasmid-borne gene *traR*. Coupled with an acyl homoserine lactone autoinducer, TraR activates the expression of *tra* genes necessary for bacterial conjugation. TraR also activates the expression of *traI*, which is itself required for autoinducer synthesis. Depending on the type of Ti plasmid, different conjugal opines stimulate bacterial conjugation through either positive or negative regulatory cascades. The quorum-dependent expression of *tra* genes ensures that conjugal DNA transfer will occur only when large numbers of bacteria are present. Other density dependent systems regulate virulence factor expression and horizontal gene transfer in several pathogenic microorganisms but this topic will not be covered here as it is the focus of recent reviews (Solomon and Grossman, 1996; also, see quorum-sensing reviews above).

A picture emerges of an intricate dialogue between the Agrobacteria and their plant hosts, in which bacterial DNA transfer is closely regulated by host factors synthesized in the microenvironment of the infection. Because the opines produced by a crown gall tumor are catabolized by the resident plasmid-containing Agrobacteria at the site of infection, this population of bacteria can expand selectively within the tumor environment. In the case of conjugal DNA transfer, it has been proposed that the opines synthesized by the host can lead to horizontal spread of Ti plasmids within a mixed population of Agrobacteria, increasing the fitness of the transconjugants by conferring on them a competitive growth advantage within the milieu of the plant tumor (Farrand, 1993). Ti plasmids transferred between different Agrobacterium strains and species in turn gain access to other chromosomal backgrounds, thus increasing the genetic diversity of the entire bacterial population. Thus, in this pathogenic system, the wound site on the plant provides the necessary regulatory signals for mediating not only critical interactions between the bacteria and the plant host but also between different bacterial strains.

### *Vibrio cholerae in the Mammalian Intestine*

The stimulation of horizontal gene transfer in a pathogen by host signals is a theme also found in the interaction of Vibrio cholerae with a mammalian host. In this pathogenic system, the mammalian gastrointestinal tract provides the necessary signals for the in vivo transduction of a newly discovered bacteriophage of the Gram-negative bacterium V. cholerae. This phage encodes the principal virulence factor of V. cholerae, cholera toxin (CT), a protein enterotoxin responsible for the severe diarrheal symptoms of cholera. The genes encoding CT, *ctxAB*, are located within a chromosomal "CTX genetic element," found only in toxigenic strains of V. cholerae (reviewed by Mekalanos, 1995). In addition to the genes encoding CT, this element harbors several other genes potentially encoding two other toxins, a colonization factor, a repressor, and a recombinase; flanking this entire element are sites which promote its integration into the chromosome by a site-specific mechanism. This cluster of genes is reminiscent of other groups of genes which have apparently been transferred horizontally from one strain to another by unknown mechanisms (see minireview on pathogenicity islands by Groisman and Ochman, 1996 [this issue of *Cell*]). Recently, it was reported that the CTX genetic element of V. cholerae actually corresponds to the genome of a filamentous bacteriophage called CTX$\Phi$ (Waldor and Mekalanos, 1996). The mechanism by which this bacteriophage infects V. cholerae strains has shed new light on the strategies that bacteria use to move DNA encoding virulence determinants between strains, particularly during infection.

After ingestion of V. cholerae by a host, bacterial colonization of the small intestinal mucosa occurs by a process that is critically dependent on specific surface bacterial appendages called toxin co-regulated pili (TCP). Both TCP and CT are coordinately expressed by V. cholerae through the activity of three transcriptional regulatory proteins ToxR, ToxS, and ToxT. Colonization of mouse and human intestine is dependent on the ToxRST regulatory system, suggesting that these regulators respond either directly or indirectly to host environmental signals present in the gastrointestinal tract. There is strong evidence to suggest that TCP pili are the receptor for the CTX$\Phi$; the phage will not infect mutants deficient in ToxR or TCP and infection of bacterial cells is blocked by anti-TCP antiserum. V. cholerae strains of the El Tor biotype are notoriously poor producers of TCP under in vitro laboratory conditions but still require TCP for intestinal colonization (Thelin and Taylor, 1996). If mice

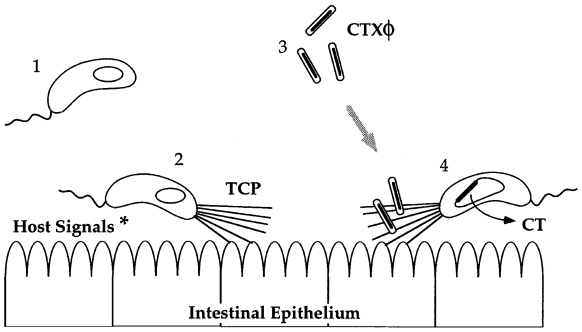


Figure 2. Model for Emergence of New Toxigenic V. cholerae
See text for details.

are co-infected with a CTXΦ donor strain and a TCP-positive, CTX-negative El Tor recipient strain, it is possible to demonstrate within hours that the El Tor strain can be readily infected by CTXΦ within the gastrointestinal tract (Waldor and Mekalanos, 1996). This in vivo lysogenic conversion of an El Tor strain occurrs $10^5$–$10^6$ times more efficiently within the host than is seen under in vitro conditions. It is likely that this increase in transduction efficiency reflects dramatically increased in vivo expression of the TCP phage receptors. Thus, CTXΦ has evolved to be a highly transmissible agent within the host gastrointestinal environment, the same environment which provides the signals for coordinate expression of both CT (a phage encoded toxin) and TCP (the phage receptor).

This leads to a model for the emergence of new strains of toxigenic V. cholerae within the gastrointestinal tract (Figure 2). Non-toxigenic V. cholerae swimming freely in the gastrointestinal lumen (Figure 2, "1") encounter host intestinal signals (*) which turn on the expression of TCP (Figure 2, "2"). CTXΦ phage (Figure 2, "3") produced by a bystander bacterium (i.e., a toxigenic V. cholerae or other bacterium) bind TCP and infect recipient bacteria, converting them to CT producers (Figure 2, "4"). As in the Agrobacterium system, host environmental signals in the intestine modulate the expression of gene products required for V. cholerae pathogenesis as well as gene products that are critical for horizontal gene transfer between bacterial strains within this environment.

### *Type IV Pili and DNA Transfer*

TCP pili are members of a large family of bacterial fimbriae that have been shown to be important virulence factors in a number of prokaryotic pathogens. These Type IV pili typically contribute to adherence of bacteria to host epithelial cells in species belonging to the genera Neisseria, Pseudomonas, Moraxella, Vibrio, and Escherichia (reviewed by Strom and Lory, 1993, and references therein). As in Vibrio, type IV pili act as receptors for filamentous phages of P. aeruginosa. Type IV pili have also been implicated in other DNA uptake or transfer events such as transformation competence in Neisseria species. In addition, proteins with homology to type IV pili assembly genes have been shown to be required for competence in Bacillus and extracellular secretion of bacterial virulence factors in several species including P. aeruginosa and V. cholerae.

Is it just coincidental that type IV pili and their related virulence factor secretion gene products are so frequently associated with both bacterial pathogenesis *and* DNA uptake events? Perhaps, the mechanistic assembly of these pili and secretion systems (e.g., involving steps such as protein export, chaperone-interactions, subunit polymerization-depolymerization, filament retraction, etc.) may, in some way, be compatible with the transport of DNA into bacterial cells. Alternatively, a link between pathogenic function and DNA transfer may have co-evolved because both require bacterial surface structures and both occur within the same host environment. Thus, expression of these pili and pili-related gene products and the acquisition of new virulence genes via horizontal gene transfer may occur far more frequently within host tissues than outside the host. For bacterial products like type IV pili or related molecules to function as virulence factors, they must be expressed by bacteria interacting with the host and are therefore likely to be under the control of regulatory systems that detect host environmental signals. It follows that a variety of horizontal gene transfer events dependent on this class of proteins are probably enhanced within the host milieu and are modulated by host environmental signals.

It is worth noting that some genetic elements may have an ill-defined host range if they use virulence factors as their receptors. For example, the genes that encode TCP are present only in certain strains of Vibrio cholerae. In these strains, the TCP genes are part of a "pathogenicity island" whose origin is uncertain (Kovach et al., 1996). If the CTXΦ's receptor is moving horizontally between bacteria, then clearly the phage too may gain access to new bacterial species. It is intriguing that the major subunits of type IV pili such as TCP are characterized by 20–30 residue conserved region located at their mature N-terminus. It is possible that this region may be recognized by a variety of bacteriophages, or may in some way be linked to DNA uptake. Thus, a variety of "broad host range" horizontal DNA transfer events may be occurring in vivo, particularly on host mucosal surfaces where type IV pili adhesins play their functional role. For example, the heat-labile enterotoxin (LT) of E. coli is highly related to cholera toxin but is encoded by a plasmid and not a bacteriophage (reviewed by Betley et al., 1986). Since some enteropathogenic strains of E. coli produce a type IV pilus termed the bundle-forming pilus (BFP) (Donnenberg et al., 1992), it is possible that a phage related to CTXΦ might have utilized the BFP as a receptor and in that way disseminated a cholera toxin-like gene into E. coli. Such DNA transfer events have undoubtedly contributed to the emergence of new pathogenic clones but are today difficult to reconstruct after so many years of subsequent divergent evolution.

### *Concluding Remarks*

In conclusion, the regulation of bacterial DNA transfer by the eukaryotic host as illustrated in the above examples leads us to reconsider our notions about the sites where horizontal gene transfer usually occurs, the designation of genetic elements as non-transmissible, and the meaning of genetic host-range. A number of bacterial pathogens including Yersinia, Shigella, and pathogenic

E. coli species harbor virulence plasmids or chromosomal pathogenicity "islands" which often encode multiple virulence determinants. While the homology of the gene products encoded by these genetic elements suggests that they have been acquired by horizontal spread, many of these plasmid and chromosomal inserts do not appear to be self-transmissible under laboratory conditions. If horizontal transfer of these genetic elements is intimately tied to local host environments, then their transmission between strains by conjugation, transformation, or transduction may not be observable except under in vivo conditions. Similarly, elements that encode antibiotic resistance may also be limited in their transmission between strains under laboratory conditions but be readily transferred under in vivo conditions in response to undefined host signals. An increased understanding of the host signals that control both the expression of bacterial virulence factors and the transfer of DNA elements encoding virulence and antibiotic resistance genes in vivo may offer new opportunities in the therapeutic arena. Antimicrobial drugs that block virulence gene expression, secretion and assembly of virulence factors, or DNA uptake and transfer, may provide the additional benefit of decreasing the rate of emergence of new bacterial pathogens as well as drug resistant versions of old pathogens.

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