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COMMENTARIES

A Variety of Bacterial Pili Involved in Horizontal Gene Transfer[▽]

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Horizontal gene transfer (HGT) is central to microbial evolution. It contributes to the diversity of acquired traits, which are otherwise not available through gene transfer from parent to progeny. Occurrence of HGT is seen when comparing genomes of related bacteria, or of different isolates from the same species, in which homologous genes show low sequence divergence whereas gene content varies enormously. Those genes that are present or absent are mostly horizontally transferred. Pathogenicity islands are such mobile genetic elements that contribute to rapid changes in virulence potential in numerous bacterial pathogens.

Studies in the laboratory of Stephen Lory have previously shown that the *Pseudomonas aeruginosa* pathogenicity island PAPI-1, initially identified on the genome of the PA14 isolate (10), could be exchanged between *Pseudomonas* strains (19). In this issue of *Journal of Bacteriology*, the same group demonstrates that type IV pili are the components of the PAPI-1 conjugation machinery (3), thus adding to the list of diverse biological functions associated with these surface organelles.

The genes carried on the PAPI-1 island are largely of unknown function but include fimbrial *cup* genes (15) as well as genes encoding toxins, pyocins, and regulatory elements. A gene cluster encoding type IV pilus components is also found on the island and is essential for the horizontal transmission of PAPI-1. This is a unique observation, since type IV pili are usually not involved in conjugation-like processes but contribute to DNA uptake from an extracellular source (1, 17). It is thus important to understand the mechanisms that mediate DNA movement between bacteria, i.e., conjugation, transformation, and phage transduction.

Bacterial conjugation involves conjugative plasmids that spread autonomously and, for example, disseminate antibiotic resistance genes. These mobile elements carry genes that are required for processing the plasmid into a transfer-competent form (*mob* genes) and genes that are involved in the formation of a *trans*-envelope machinery and a pilus structure (*tra* or *trb* genes) (20). It has not been clearly demonstrated that DNA exchange takes place through the pilus, but at least the pilus brings the bacterial cells into close contact. Because the pilus initiates mating and favors DNA exchange between bacteria, it is also known as the sex pilus.

The sex pilus assembly machine shares extensive similarities with the so-called type IV secretion system (T4SS). The T4SS archetype was initially described for *Agrobacterium tumefaciens* (VirB system), in which the assembly of a T pilus contributes to the transfer of bacterial DNA into the genomes of plants. Since then, T4SSs have been described for many bacterial pathogens, and their role has been associated with the injection of effector proteins into host cell targets (4). In *A. tumefaciens* the T-DNA is not delivered as naked DNA but is bound to at least one protein, the VirD2 transesterase (4). Thus, the T4SS transports either proteins or nucleoprotein complexes. The conjugative pilus machinery encoded by the *tra* genes is a true T4SS involved in the transport of DNA bound to the relaxase protein (14).

There are essentially two types of sex pili, the F-like pili (mainly IncF plasmids) and the P-like pili (IncP, -N, and -W). The VirB system in *A. tumefaciens* is most similar to the P-like pilus assembly machine and is also known as the T4SSa. The T4SSb shares some components with the T4SSa but is very much related to the Tra system found in IncI plasmids such as R64 or ColIb-P9 (13). The T4SSb is also best known as the Dot/Icm system associated with virulence in *Legionella pneumophila*, whose function is to inject effectors such as RalF in host cell targets (16).

This brief overview of conjugative plasmids and the T4SS reveals the variety of mechanisms and structures involved in the transport of DNA or DNA-protein complexes. Nevertheless, all of these rely on the assembly of a pilus, be it an F, P, I, or T pilus, which brings cells together and provides an interface to exchange macromolecules directly from cell to cell. Interestingly, a different function has been shown for the T4SS in *Neisseria gonorrhoeae*, which is encoded on the gonococcal genetic island (GGI) (9). Many of the genes on this island show similarity to *tra* genes found on the *Escherichia coli* F plasmid. What is remarkable is that this T4SS is involved in neither conjugation nor effector secretion but is needed for secretion of chromosomal DNA into the extracellular milieu.

DNA uptake from the milieu is known as transformation. The DNA from bacterial donor can be released by cell lysis or, as mentioned above, by specific secretion. In many bacteria the uptake of extracellular DNA is achieved by binding of the DNA to type IV pili assembled at the surface of the recipient cell (1, 7, 17). Type IV pili are different from the previously described sex pili. They are retractile and can thus bring the genetic material in contact with the cell surface. The role of

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type IV pili in transformation has been well documented in several bacterial species, both Gram positive, such as *Bacillus subtilis* or *Streptococcus pneumoniae*, and Gram negative, such as *Neisseria gonorrhoeae* and *Haemophilus influenzae* (7).

In the study by Carter et al. (3), the type IV pili are assigned a novel role that is related to conjugation of the PAPI-1 DNA between *Pseudomonas* species. The only other example of type IV pili being involved in conjugation was described in previous studies on the IncI1 plasmid R64 in the laboratory of Teruya Komano (11, 13, 23). The *pil* genes carried on the R64 plasmid produce a thin pilus, which belongs to the type IV family. The type IV pilin family is divided into two groups. The most classical type IV pilins are known as type IVa and are those found in *Neisseria* or the archetype *P. aeruginosa* system. These pili are retractile and are involved in DNA uptake, but they are also required for many other functions, such as twitching motility and attachment (17). The type IVb class is exemplified by bundle pili such as the Bfp pilus from enteropathogenic *E. coli* (6). The type IVb pilins are distinguished by their N-terminal leader sequence, which is longer than that of the type IVa pilin (six or seven residues for type IVa). A further subclass of the type IVb pilin is known as Flp pilin, which is exemplified by the system described for *Actinobacillus actinomycetemcomitans* (18). The R64-associated thin pilus is made with a type IVb pilin, PilS, which possesses a 23-amino-acid (aa)-long leader peptide (11).

The *pil* gene cluster carried on PAPI-1 is very similar to the *pil* cluster from R64. The leader peptide of the *pilS2* gene product is 14 aa long and therefore likely belongs to the type IVb family. The genetic organization of the *pilL* to *-V* genes is nearly identical in PAPI-1 and R64. The main difference is that the R64 *pilU* gene, encoding the prepilin peptidase, is missing in PAPI-1. Interestingly, the processing of the pilin subunit precursor is dependent on the PilD prepilin peptidase, which is encoded in the core genomes of all *P. aeruginosa* strains. This prepilin peptidase is involved in the maturation of all pilins or pseudopilins encoded in the genome of *P. aeruginosa* (2), with the exception of the Flp pilin, the cleavage of which is mediated by the FppA prepilin peptidase (5). As discussed by Carter et al. (3), the dependence of PAPI-1 transmission on a gene from the core genome is likely a mechanism that restricts the range of bacteria that can participate in the exchange of this genetic element.

The R64 conjugation system is complex and involves the *pil* genes as well as a large set of genes showing extensive homology to the *L. pneumophila* T4SSb genes, *dot*/*icm*. In classical T4SS-like conjugative systems, the competent nucleoprotein complex is targeted to the T4SS pore by coupling proteins. The archetype of such a protein is VirD4 or TraD. Although it has not been experimentally demonstrated, one cannot exclude the possibility that in R64 the T4SSb component TrbC, a VirD4 homolog (13), contributes to present the DNA to the channel formed by the type IV pilus assembly machine, thus combining the activities of both systems. In *P. aeruginosa* there is no complete T4SS gene cluster on PAPI-1 or on the core *Pseudomonas* genome. However, Stephen Lory's group highlights the presence of two genes on PAPI-1, *RL022* and *RL047*. *RL047* has the signature of the VirD4/TraD coupling ATPase. *RL022* has the signature of the VirB4/TraC ATPase, which is an interacting partner for VirD4. The third ATPase in the T4SS partnership is VirB11, which is also homologous to the ATPase involved in type IV pilus assembly (PilT2 and PilQ2 for PAPI-1-encoded system). PAPI-1 may have gained a unique conjugation machinery by acquiring a few components from the T4SS to recruit DNA to the pore formed by the machinery used for assembly of the type IV pili. The rest of this organelle would participate in fusions typically associated with conjugative pili, such as recognition of the recipient cell. Retraction of the type IV pilus will result in direct contact between the recipient and donor cells. This mechanism may represent a superb example of evolution of a machinery for a specialized process through a mix-and-match approach involving gene acquisition from multiple sources.

Finally, a T4SS, and thus not type IV pili, has been shown to be involved in the natural transformation in *Helicobacter pylori* (21). This T4SS (ComB) is distinct from the one encoded in the *cag* pathogenicity island, which is involved in CagA secretion (4).

In conclusion, horizontal transfer of genetic material largely involves pili. Some pili, such as T4SS-related pili or sex pili, transfer DNA from one cell to the other, whereas type IV pili are needed for DNA uptake from the extracellular milieu. This is now more complex, since few examples of type IV pili involved in mating, such as those described by Stephen Lory's group, are known. The use of pili to move DNA in and out of cells seems to be the preferred route used by bacteria. There is obviously modulation/adaptation of systems from one bacterium to another, and one may think that evolution could deliver any possible combination. These modulations could produce machines involved in DNA, protein, or more generally macromolecule transport. We know of pili, such as those from the T4SSb in *L. pneumophila*, which are required both for effector secretion and for transfer of the RSF1010 plasmid (22). We even know of type IV pilus-like structures that are involved in protein secretion, e.g., the pseudopilus of the type II secretion system (8). Genuine type IV pili are also able to secrete proteins, as was seen with the *Vibrio cholerae* toxin-coregulated pili (TCP), which are required for secretion of a soluble colonization factor, TcpF (12). I believe that more surprises are to come.

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