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The Ins and Outs of DNA Transfer in Bacteria

Inês Chen[1], **Peter J. Christie**[2,*], and **David Dubnau**[1,*]

[1]Public Health Research Institute, 225 Warren Street, Newark, NJ 07103, USA

[2]Department of Microbiology and Molecular Genetics, University of Texas Medical School at Houston, Houston, TX 77030, USA

Abstract

Transformation and conjugation permit the passage of DNA through the bacterial membranes and represent dominant modes for the transfer of genetic information between bacterial cells or between bacterial and eukaryotic cells. As such, they are responsible for the spread of fitness-enhancing traits, including antibiotic resistance. Both processes usually involve the recognition of double-stranded DNA, followed by the transfer of single strands. Elaborate molecular machines are responsible for negotiating the passage of macromolecular DNA through the layers of the cell surface. All or nearly all the machine components involved in transformation and conjugation have been identified, and here we present models for their roles in DNA transport.

In bacteria, transformation and conjugation usually mediate the transport of single-stranded DNA (ssDNA) across one or more membranes. Transformation involves the uptake of environmental DNA, whereas conjugation permits the direct transfer of DNA between cells (Fig. 1). Other DNA-transport phenomena in bacteria, such as the passage of DNA through the bacterial division septa and those carried out by many bacteriophages (1), involve the movement of double-stranded DNA (dsDNA) and will not be discussed here. Transformation and conjugation probably evolved for the acquisition of fitness-enhancing genetic information, but other mutually nonexclusive theories posit that transformation might have evolved to provide templates for DNA repair or to supply nutrition for bacteria (2). Today, both processes are recognized as important mechanisms for horizontal gene transfer and genome plasticity over evolutionary history, and they are largely responsible for the rapid spread of antibiotic resistance among pathogenic bacteria (3, 4).

Bacterial Transformation

Naturally transformable bacteria acquire a physiological state known as “competence” through the regulated expression of genes for protein components of the uptake machinery. Natural transformation has been most studied in *Bacillus subtilis*, *Streptococcus pneumoniae*, *Neisseria gonorrhoeae*, and *Haemophilus influenzae*. These and other competent bacteria use similar proteins for DNA uptake, with few differences between species. An interesting exception is *Helicobacter pylori*, which uses a conjugation-like system for transformation (5). Here, we will discuss the DNA uptake systems of *B. subtilis* and *N. gonorrhoeae* as representative of those in Gram-positive and -negative bacteria, respectively (Fig. 1A). The main distinction between these cell types is that Gram-negative bacteria are enclosed by cytoplasmic and outer membranes, with an intervening periplasmic space and thin layer of peptidoglycan (~3 to 7 nm) (6). Gram-positive bacteria lack an outer membrane, and their cytoplasmic membrane is surrounded by a ~22-nm periplasmic space and a thick layer of peptidoglycan (~33 nm) (7).

*To whom correspondence should be addressed. Peter.J.Christie@uth.tmc.edu (P.J.C.); dubnau@phri.org (D.D.).

Initial interactions with the bacterial surface

In both Gram-positive and Gram-negative bacteria, dsDNA interacts with the competent cell surface by a process that is not completely understood. DNA binds to competent *B. subtilis* cells in a state that is resistant to centrifugal washing but susceptible to added nucleases. ComEA, a membrane-bound dsDNA binding protein, is required for transformation (8, 9). In the absence of ComEA, 20% residual DNA binding still occurs in a competence-dependent manner (8). Similar results were observed in *S. pneumoniae* (10), but the proteins responsible for this residual binding remain unidentified in both species. In Gram-negative bacteria, dsDNA enters the periplasm, but in both Gram-negative and -positive systems, a single strand of DNA passes across the cytoplasmic membrane while its complement is degraded (Fig. 1A). DNA is taken up into the cytosolic space linearly (11), and a free end is presumably required to initiate the transport process. In *B. subtilis*, new termini are provided by random cleavage events on the cell surface, catalyzed by the integral membrane nuclease NucA (12).

Efficient DNA uptake in *Neisseria* and *H. influenzae* requires a species-specific DNA uptake sequence about 10 nucleotides long (13, 14). The genomes of these bacteria are enriched for their respective uptake sequences, favoring the uptake of homospecific DNA (15). However, sequence-specific binding receptors have not yet been identified.

Secretins and uptake into the periplasm

In Gram-negative bacteria, dsDNA becomes nuclease-resistant as it passes through the outer membrane (Fig. 1A). This step requires the presence of a secretin protein (16). Secretins form stable, donut-like multimers in the outer membrane, with an aqueous central cavity (17). Secretins are also components of type-4 pilus, filamentous phage-extrusion systems, and dedicated protein-secretion systems, and they are also likely required for conjugation. For transformation, DNA probably enters the periplasm through the secretin channel, although direct evidence is lacking. The central cavity of the PilQ dodecamer is 6.5 nm in diameter at its widest point (17), adequate for the passage of dsDNA (2.4 nm) or of a DNA-protein complex.

The competence pseudopilus

Transformation systems of Gram-negative and -positive bacteria are made up of subunits with striking similarities to those needed for assembly of type-4 pili and type-2 secretion systems. Type-4 pili are long and thin appendages that mediate a form of locomotion known as twitching motility, which is powered by the extension and retraction of the pilus through assembly and disassembly. Type-2 secretion systems export folded-protein substrates across the outer membrane through a secretin channel. The conserved proteins for all three systems include a cytoplasmic adenosine triphosphatase (ATPase) of the AAA^+ ATPase superfamily (ATPases associated with various cellular activities), a polytopic membrane protein, a pre-pilin peptidase, and several pilins or pilin-like proteins (18). In type-4 pilus systems, these proteins mediate the assembly of the major pilin into the pilus fibers. Genetic manipulation, e.g., pilin overproduction, of a number of type-2 secretion systems also results in the production of pilus-like structures, termed pseudopili (Ψ-pili), that extend through the periplasm and in some cases beyond the cell surface (19–22).

In *B. subtilis*, the ComG proteins necessary for DNA binding (23) include the AAA^+ ATPase (ComGA), polytopic membrane protein (ComGB), major pre-pilin–like protein (ComGC), and three minor pre-pilin proteins (ComGD, ComGE, and ComGG) (Fig. 2). The pre-pilin proteins integrate into the cytoplasmic membrane, and when processed by the peptidase ComC, these subunits translocate to the exterior of the membrane (24). Recently, a polymeric complex dependent on the ComG proteins has been detected on the exterior of the

membrane (25). This structure, termed a competence Ψ-pilus, consists of processed ComGC molecules joined to one another by disulfide bonds and by additional noncovalent interactions. The competence Ψ-pilus ranges in sizes corresponding to 40 to 100 subunits and, on the basis of length estimates for a secretion Ψ-pilus (22) and type IV pili (26), the competence Ψ-pilus is long enough to traverse the periplasm and cell wall (~55 nm) (7).

N. gonorrhoeae produces type-4 pili, and many proteins needed for pilus formation are also required for DNA uptake and transformation, leading to the assumption that pili participate in DNA uptake. However, there is evidence that two distinct structures exist in *Neisseria*, the type-4 pilus and a competence Ψ-pilus, and that these structures apparently compete for common components and morphogenetic proteins (27, 28).

The growing secretion Ψ-pilus may act as a piston, pushing substrate proteins through the secretin channel in the outer membrane (18, 29, 30). Analogously, assembly and disassembly of the competence Ψ-pilus may contribute to DNA uptake by pulling DNA to the translocation machine in the cytoplasmic membrane (Fig. 2). Repeated cycles of assembly and disassembly would result in a low concentration of maximal-length Ψ-pilus and a broad size distribution, as observed for the *B. subtilis* competence Ψ-pili and the secretion Ψ-pili of *Xanthomonas campestris* (21). In single-molecule studies of DNA uptake in *B. subtilis* (31), the rate of uptake (~80 base pairs s^{-1}) was relatively constant with forces up to 40 pN, without detectable pauses or reversals. These features, unusual for molecular motors that move along DNA, are similar to the force characteristics of type-4 pilus retraction in *N. gonorrhoeae* (32). The proton motive force may be a source of energy for DNA uptake; the rate of uptake decreases sharply with the addition of uncoupling agents before any detectable decline in the ATP pool (31). Thus, the proton motive force might directly drive the movement of the Ψ-pilus subunits into the membrane, causing Ψ-pilus disassembly and retraction.

Transport across the cytoplasmic membrane and DNA processing

In both *B. subtilis* and *N. gonorrhoeae*, similar poly-topic membrane proteins (ComEC and ComA, respectively) are required for DNA transport into the cytosol (8, 33). These large proteins (ComEC contains 776 residues) are proposed to form channels for the passage of DNA (34). In addition, the Gram-positive systems encode a membrane-bound ATPase, ComFA, that functions in DNA uptake (35). ComFA resembles the family of Asp-Glu-Ala-Asp (DEAD) box helicases, and may assist the translocation of DNA through the membrane or carry out strand separation. In *S. pneumoniae*, the membrane-associated EndA nuclease degrades the nontransforming strand, even when the ComEC equivalent is absent (10). In *B. subtilis*, the identity of the corresponding nuclease is unknown, but degradation of the nontransforming strand seems dependent on passage through or interaction with ComEC (12).

Cellular location of DNA uptake and the role of cytosolic proteins

In *B. subtilis*, which is a rod-shaped bacterium, DNA binding and uptake take place preferentially at the cell poles, where the membrane-associated proteins ComGA and ComFA and the cytosolic ssDNA binding protein YwpH colocalize (36). Several additional cytosolic proteins participate in transformation, and some have been shown to associate with ssDNA entering the cell. In *S. pneumoniae*, the Smf protein protects transforming DNA from degradation in the cytosol (37). The repair/recombination proteins RecN and RecA also localize to the poles of competent *B. subtilis* (38). RecN oscillates from pole to pole, but becomes static at one pole when transforming DNA is added. RecA localization depends on ComGA; when DNA is added, RecA forms a filament extending from the pole to the

centrally located nuclear body, perhaps facilitating the search for a homologous site on the chromosome.

A transformation model

We propose that repeated cycles of Ψ-pilus assembly and disassembly drive a DNA molecule through the cytoplasmic membrane channel formed by ComEC and that an unidentified DNA binding protein anchors DNA to the Ψ-pilus. ComEA may ensure processivity by maintaining contact with DNA as these cycles push DNA through the channel (8). The proton motive force might drive disassembly of the Ψ-pilus. Finally, the binding energy of cytosolic ssDNA binding proteins might provide a pulling force by a Brownian ratchet mechanism (39), and the helicase/translocase ortholog ComFA may also assist uptake.

Conjugation

Most bacterial and some archaeal species encode conjugation systems, and several classes of mobile elements exist, including self-transmissible and mobilizable plasmids, conjugative transposons, and integrative conjugative elements (40). We will restrict the discussion to a few of the better-characterized, plasmid-encoded conjugation systems of Gram-negative bacteria and draw on examples from the Gram-positive bacteria where information is available.

The conjugation apparatus is composed of a cell-envelope–spanning translocation channel and either a pilus for Gram-negative bacteria or surface-localized protein adhesins for Gram-positive bacteria (Fig. 1B) (41, 42). The mating pair formation (Mpf) proteins elaborate the extracellular pilus, and these subunits plus the coupling protein, here termed the substrate receptor, mediate substrate transfer across the cell envelope (43, 44). *Agrobacterium tumefaciens* elaborates a model conjugation machine from 11 Mpf subunits, VirB1 to VirB11, and the VirD4 substrate receptor, to deliver oncogenic transferred DNA (T-DNA) to susceptible plant species (45). Many plasmid conjugation systems, exemplified by transfer systems of plasmids R388, F, and RP4, are built from nearly complete sets of VirB/D4-like subunits, whereas other systems have only one or two discernible homologs (41, 42, 44–47). Conjugation and ancestrally-related translocation machines make up the large and functionally versatile family of type-IV secretion systems (46–48).

Processing of the conjugative-transfer intermediate

The processing of substrate DNA for conjugative transfer is a widely conserved reaction among Gram-negative bacteria and unicellular Gram-positive bacteria (Fig. 1B) (49). A relaxase plus one or more auxiliary factors initiate processing by binding the origin-of-transfer (*oriT*) sequence and cleaving the DNA strand destined for transfer (T-strand). The relaxase remains covalently bound to the 5′ end of the T-strand, resulting in the formation of the relaxase–T-strand transfer intermediate. This processing reaction clearly is distinct from the strand-specific degradation pathways operating during transformation. Also in contrast to the competence systems, signals conferring substrate recognition are carried not by the DNA but by the relaxase; these minimally consist of positively charged or hydrophobic clusters of C-terminal residues and are found in other protein substrates as well (50–52). Conjugation systems thus are currently viewed as protein-trafficking systems that have evolved the capacity to recognize and translocate relaxases and, only coincidentally, “hitchhiker DNA” (53).

Definition of a DNA substrate translocation route

The processed DNA substrates are recruited to the cognate conjugation apparatus by VirD4-like receptors. These receptors are multimeric ATPases (54), and they are defining components of Gram-negative and -positive conjugation systems (43, 55–59). Members of this protein family might also function as cytoplasmic membrane translocases, which is suggested by a structure of the TrwB receptor of plasmid R388 presenting as a spherical homohexamer with an N-terminal transmembrane stem and a central 2-nm channel (43, 59). However, VirD4 receptors cannot mediate transport independently of the Mpf proteins, e.g., VirB components. In *A. tumefaciens*, the VirD2 relaxase–T-strand intermediate forms a series of spatially and temporally ordered close contacts with six VirB/D4 machine subunits during translocation (Fig. 3) (58). Upon substrate docking, VirD4 delivers the transfer intermediate to the VirB11 ATPase, a member of the AAA^+ superfamily positioned at the inner face of the cytoplasmic membrane (60, 61). This reaction proceeds in the absence of ATP use by VirD4 and VirB11, but requires several other subunits distributed across the cell envelope that probably contribute to the structural integrity of the cytoplasmic membrane translocase (62, 63).

Next, the relaxase–T-strand is delivered sequentially to the integral cytoplasmic membrane components VirB6 and VirB8 by mechanisms dependent on ATP energy consumption by VirD4, VirB11, and a third ATPase of this system, VirB4 (Fig. 3) (62). VirB6 is a polytopic membrane protein and might function as a water-filled channel through which the substrate passes, reminiscent of *B. subtilis* ComEC discussed above (64). Finally, the substrate is delivered to two periplasmic/outer membrane–bound subunits, VirB2 pilin and VirB9. On the basis of the demonstrated substrate contacts, VirD4, VirB11, VirB6, VirB8, VirB2, and VirB9 are postulated to make up the mating channel for DNA transfer across the *A. tumefaciens* cell envelope (58). Gram-positive systems possess VirD4- and VirB4-like subunits that probably also form part of the membrane translocase (42).

Structural and energetic requirements for substrate translocation through the periplasm and outer membrane

The mating channel extending through the periplasm has been depicted as a rudimentary pilus (Fig. 3) (40, 44, 45). Alternative models exist, most notably one postulating that the VirB2 pilin undergoes cycles of assembly and disassembly to form a dynamic piston (47). This model is reminiscent of that proposed above for the competence Ψ-pilus, but here a VirB2 piston would supply the force needed for passage of DNA across the outer rather than the cytoplasmic membrane. VirB9-like subunits presently are the best candidates among the VirB components for forming an outer-membrane pore or channel (41, 45). These outer-membrane components share sequence similarities with the pore-forming secretins and, like secretins, they often form stabilizing interactions with cognate lipoproteins (41, 45, 65). *A. tumefaciens* VirB9 confers selective trafficking of different relaxase–T-strand substrates through the distal portion of the secretion channel, also reminiscent of substrate-specifying activities reported for secretins (66, 67). VirB2 pilin and VirB9 secretin-like components are found in nearly all conjugation systems of Gram-negative bacteria but not Gram-positive bacteria, suggesting that mechanistic differences probably exist for translocation to the surfaces of these cell types (42, 45).

Both ATP energy and proton motive force are needed for conjugative DNA transfer (68). In *A. tumefaciens*, the VirD4 and VirB11 ATPases convert ATP energy to a mechanical force by inducing a structural transition in the cytoplasmic membrane subunit VirB10 (Fig. 3) (69). In turn, energized VirB10 forms a stable complex with secretin-like VirB9 at the outer membrane. VirB9-VirB10 complex formation is a prerequisite for the passage of DNA substrates from the portion of the channel composed of VirB6 and VirB8 at the cytoplasmic

membrane to that composed of VirB2 and VirB9 (69). Energized VirB10 might physically bridge machine subassemblies at the two membranes or, alternatively, trigger gate opening at the distal portion of the secretion channel. Intriguing structural and functional similarities exist between VirB10-like subunits of conjugation systems and the TonB family of energy transducers, although the former sense ATP energy and the latter sense the proton motive force (69).

Roles of extracellular structures and the nature of the donor-recipient cell contact

In Gram-negative bacteria, the pilus mediates initial attachment of donor cells with recipient cells. In the *Eschericia coli* F plasmid system, the pilus retracts and is postulated to function dynamically to bring donor and recipient cells into contact to form the mating junction (41). In contrast, the *A. tumefaciens* VirB/D4 system and related plasmid transfer systems, e.g., RP4, R388, and pKM101, lack the Mpf subunits dedicated to F pilus retraction (70), and these systems most probably release their pili from the cell surface either by breakage or an active sloughing mechanism (Fig. 3) (44, 45). Such pili probably function as adhesive structures, resembling the surface adhesins of Gram-positive conjugation systems, e.g., *E. faecalis* pCF10-encoded aggregation substance, by promoting aggregation of donor and recipient cells (71).

Conjugative pili extending from the cell surface induce the formation of mating pairs but probably play no direct role in substrate transfer. Conjugative junctions visualized by electron microscopy appear as tightly apposed outer membranes devoid of structures, e.g., pili, and they typically exceed 100 nm along the cell length (72). These findings, plus new evidence for interactions between membrane proteins of donor and recipient cells (73), suggest that donor and recipient cell membranes might undergo extensive remodeling during the formation of mating junctions. Additionally, mutations in certain Mpf subunits of the plasmid RP4 and *A. tumefaciens* VirB/D4 machines genetically “uncouple” two pathways, one leading to the formation of the pilus, the other to a functional secretion channel (45, 74). Thus, reminiscent of the neisserial competence and type-4 pilus systems discussed above (28), the Mpf subunits might assemble alternatively as a secretion channel or an extracellular pilus (45).

Spatial positioning of the conjugative transfer apparatus

Conjugation components and pili display both distributed and polar patterns of localization. Conjugation components and pili of the plasmid R27 system localize at many sites around the cell surface (75). In contrast, VirB subunits and pili of the *A. tumefaciens* VirB/D4 assemble at the cell poles (76). The VirD4 T4CP also is polar-localized where it recruits a green fluorescent protein (GFP)–tagged protein substrate, strongly suggesting that this is the site for translocation (57). In this plant pathogen, a polar-localized conjugation machine might have evolved as a specialized adaptation for substrate transfer to susceptible hosts.

Summary

The early reactions mediating processing of dsDNA to translocation-competent ssDNA substrates clearly are strikingly different for transformation and conjugation systems. Yet for both systems, the actual process of ssDNA transport across bacterial membranes might be more mechanistically conserved than previously envisioned. Both systems probably use similar strategies for substrate passage through the following: (i) the outer membranes of Gram-negative bacteria (via secretin complexes), (ii) the periplasm or cell wall (pilus-or Ψ-pilus–mediated), and (iii) the cytoplasmic membrane (at least in part through a water-filled channel composed of a polytopic membrane protein). Both systems also appear to use AAA^+ ATPases and proton motive force to induce dynamic structural changes for

translocation. Finally, at least one conjugation-like machine, the *H. pylori* Com system, has evolved for DNA acquisition (5).

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References and Notes

1. Errington J, Bath J, Wu LJ. Nat Rev Mol Cell Biol. 2001; 2:538. [PubMed: 11433368]
2. Redfield RJ. J Hered. 1993; 84:400. [PubMed: 8409360]
3. Burrus V, Waldor MK. Res Microbiol. 2004; 155:376. [PubMed: 15207870]
4. Krause S, et al. J Bacteriol. 2000; 182:2761. [PubMed: 10781544]
5. Hofreuter D, Odenbreit S, Haas R. Mol Microbiol. 2001; 41:379. [PubMed: 11489125]
6. Yao X, et al. J Bacteriol. 1999; 181:6865. [PubMed: 10559150]
7. Matias VR, Beveridge TJ. Mol Microbiol. 2005; 56:240. [PubMed: 15773993]
8. Inamine GS, Dubnau D. J Bacteriol. 1995; 177:3045. [PubMed: 7768800]
9. Provvedi R, Dubnau D. Mol Microbiol. 1999; 31:271. [PubMed: 9987128]
10. Berge M, et al. Mol Microbiol. 2002; 45:411. [PubMed: 12123453]
11. Gabor M, Hotchkiss R. Proc Natl Acad Sci USA. 1966; 56:1441. [PubMed: 16591391]
12. Provvedi R, Chen I, Dubnau D. Mol Microbiol. 2001; 40:634. [PubMed: 11359569]
13. Danner DB, et al. Gene. 1980; 11:311. [PubMed: 6260577]
14. Elkins C, et al. J Bacteriol. 1991; 173:3911. [PubMed: 1904861]
15. Smith HO, et al. Science. 1995; 269:538. [PubMed: 7542802]
16. Tomb JF, El-Hajj H, Smith HO. Gene. 1991; 104:1. [PubMed: 1916268]
17. Collins RF, et al. J Biol Chem. 2004; 279:39750. [PubMed: 15254043]
18. Hobbs M, Mattick JS. Mol Microbiol. 1993; 10:233. [PubMed: 7934814]
19. Sauvonnet N, et al. EMBO J. 2000; 19:2221. [PubMed: 10811613]
20. Durand E, et al. J Bacteriol. 2003; 185:2749. [PubMed: 12700254]
21. Hu NT, et al. Biochem J. 2002; 365:205. [PubMed: 11931643]
22. Kohler R, et al. Mol Microbiol. 2004; 54:647. [PubMed: 15491357]
23. Chung YS, Dubnau D. J Bacteriol. 1998; 180:41. [PubMed: 9422590]
24. Chung YS, Breidt F, Dubnau D. Mol Microbiol. 1998; 29:905. [PubMed: 9723928]
25. Chen I, Provvedi R, Dubnau D. unpublished data.
26. Craig L, Pique ME, Tainer JA. Nat Rev Microbiol. 2004; 2:363. [PubMed: 15100690]
27. Chen I, Dubnau D. Nat Rev Microbiol. 2004; 2:241. [PubMed: 15083159]
28. Koomey, M. personal communication.
29. Nunn D. Trends Cell Biol. 1999; 9:402. [PubMed: 10481178]
30. Burrows LL. Mol Microbiol. 2005; 57:878. [PubMed: 16091031]
31. Maier B, et al. Nat Struct Mol Biol. 2004; 11:643. [PubMed: 15184891]
32. Maier B, et al. Proc Natl Acad Sci USA. 2002; 99:16012. [PubMed: 12446837]
33. Facius D, Fussenegger M, Meyer TF. FEMS Microbiol Lett. 1996; 137:159. [PubMed: 8998979]
34. Draskovic I, Dubnau D. Mol Microbiol. 2005; 55:881. [PubMed: 15661011]
35. Londono-Vallejo JA, Dubnau D. Mol Microbiol. 1994; 13:197. [PubMed: 7984101]
36. Hahn J, et al. Cell. 2005; 122:59. [PubMed: 16009133]
37. Berge M, et al. Mol Microbiol. 2003; 50:527. [PubMed: 14617176]
38. Kidane D, Graumann PL. Cell. 2005; 122:73. [PubMed: 16009134]
39. Peskin CS, Odell GM, Oster GF. Biophys J. 1993; 65:316. [PubMed: 8369439]

40. Frost L, Laplae R, Summer AO, Toussaint A. Nat Rev Microbiol. 2005; 3:722. [PubMed: 16138100]
41. Lawley TD, Klimke WA, Gubbins MJ, Frost LS. FEMS Microbiol Lett. 2003; 224:1. [PubMed: 12855161]
42. Grohmann E, Muth G, Espinosa M. Microbiol Mol Biol Rev. 2003; 67:277. [PubMed: 12794193]
43. Gomis-Ruth FX, Sola M, de la Cruz F, Coll M. Curr Pharm Des. 2004; 10:1551. [PubMed: 15134575]
44. Schroder G, Lanka E. Plasmid. 2005; 54:1. [PubMed: 15907535]
45. Christie PJ, Atmakuri K, Krishnamoorthy V, Jakubowski S, Cascales E. Annu Rev Microbiol. 2005; 59:451. [PubMed: 16153176]
46. Baron C. FEMS Microbiol Lett. 2005 10.1016/j.femsle. 2005.09.030.
47. Cascales E, Christie PJ. Nat Rev Microbiol. 2003; 1:137. [PubMed: 15035043]
48. Hamilton HL, Dominguez NM, Schwartz KJ, Hackett KT, Dillard JP. Mol Microbiol. 2005; 55:1704. [PubMed: 15752195]
49. Guasch A, et al. Nat Struct Biol. 2003; 10:1002. [PubMed: 14625590]
50. Simone M, McCullen CA, Stahl LE, Binns AN. Mol Microbiol. 2001; 41:1283. [PubMed: 11580834]
51. Vergunst AC, et al. Proc Natl Acad Sci USA. 2005; 102:832. [PubMed: 15644442]
52. Nagai H, et al. Proc Natl Acad Sci USA. 2005; 102:826. [PubMed: 15613486]
53. Lybarger SR, Sandkvist M. Science. 2005; 304:1122. [PubMed: 15155939]
54. Tato I, Zunzunegui S, de la Cruz F, Cabezon E. Proc Natl Acad Sci USA. 2005; 102:8156. [PubMed: 15919815]
55. Schroder G, Lanka E. J Bacteriol. 2003; 185:4371. [PubMed: 12867445]
56. Hormaeche I, et al. J Biol Chem. 2002; 277:46456. [PubMed: 12244053]
57. Atmakuri K, Ding Z, Christie PJ. Mol Microbiol. 2003; 49:1699. [PubMed: 12950931]
58. Cascales E, Christie PJ. Science. 2004; 304:1170. [PubMed: 15155952]
59. Gomis-Ruth FX, et al. Nature. 2001; 409:637. [PubMed: 11214325]
60. Savvides SN, et al. EMBO J. 2003; 22:1969. [PubMed: 12727865]
61. Krause S, Pansegrau W, Lurz R, de la Cruz F, Lanka E. J Bacteriol. 2000; 182:2761. [PubMed: 10781544]
62. Atmakuri K, Cascales E, Christie PJ. Mol Microbiol. 2004; 54:1199. [PubMed: 15554962]
63. Yuan Q, et al. J Biol Chem. 2005; 280:26349. [PubMed: 15901731]
64. Jakubowski SJ, Krishnamoorthy V, Cascales E, Christie PJ. J Mol Biol. 2004; 341:961. [PubMed: 15328612]
65. Cao TB, Saier MH Jr. Microbiology. 2001; 147:3201. [PubMed: 11739753]
66. Thanassi DG. J Mol Microbiol Biotechnol. 2002; 4:11. [PubMed: 11763968]
67. Jakubowski S, Cascales E, Krishnamoorthy V, Christie PJ. J Bacteriol. 2005; 187:3486. [PubMed: 15866936]
68. Palmen R, Driessen AJ, Hellingwerf KJ. Biochim Biophys Acta. 1994; 1183:417. [PubMed: 8286395]
69. Cascales E, Christie PJ. Proc Natl Acad Sci U SA. 2004; 101:17228.
70. Harris RL, Silverman PM. J Bacteriol. 2004; 186:5480. [PubMed: 15292150]
71. Dunny GM, Leonard BA. Annu Rev Microbiol. 1997; 1997:527. [PubMed: 9343359]
72. Samuels AL, Lanka E, Davies JE. J Bacteriol. 2000; 182:2709. [PubMed: 10781537]
73. Marrero J, Waldor MK. Dev Cell. 2005; 8:963. [PubMed: 15935784]
74. Eisenbrandt R, Kalkum M, Lurz R, Lanka E. J Bacteriol. 2000; 182:6751. [PubMed: 11073921]
75. Gilmour MW, Taylor DE. J Bacteriol. 2004; 186:1606. [PubMed: 14996790]
76. Judd PK, Kumar RB, Das A. Proc Natl Acad Sci USA. 2005; 102:11498. [PubMed: 16076948]
77. Bates S, Cashmore AM, Wilkins BM. J Bacteriol. 1998; 180:6538. [PubMed: 9851996]

78. Bundock P, den Dulk-Ras A, Beijersbergen A, Hooykaas PJ. EMBO J. 1995; 14:3206. [PubMed: 7621833]
79. Kunik T, et al. Proc Natl Acad Sci USA. 2001; 98:1871. [PubMed: 11172043]
80. Waters VL. Nat Genet. 2001; 29:375. [PubMed: 11726922]

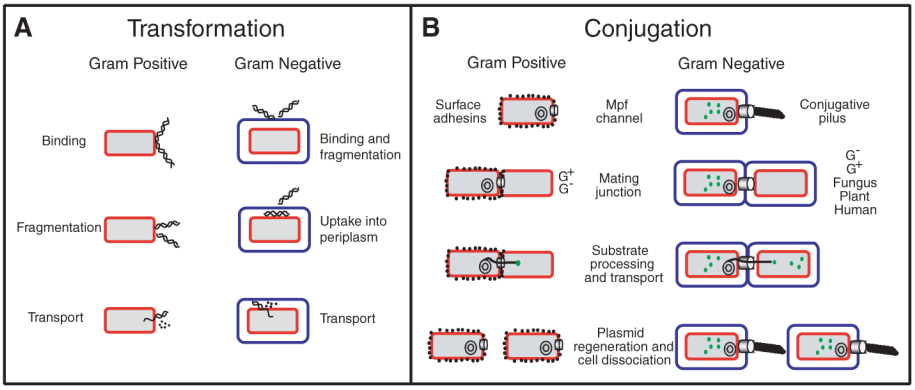

Fig. 1.

Comparison of DNA processing and transfer during transformation and conjugation. (**A**) In transformation, dsDNA substrates are converted to single-stranded transfer intermediates for transport across the cytoplasmic membrane. (**B**) For conjugation, surface adhesins or conjugative pili mediate donor-target cell contacts. Initial reactions involve the formation of a relaxase–T-DNA transfer intermediate (green dot joined to black line) and tight mating junctions. Substrate transfer is probably mechanistically conserved in bacteria, although Gram-negative systems can deliver substrates, including proteins (green dots), to phylogenetically diverse target cells (77–80).

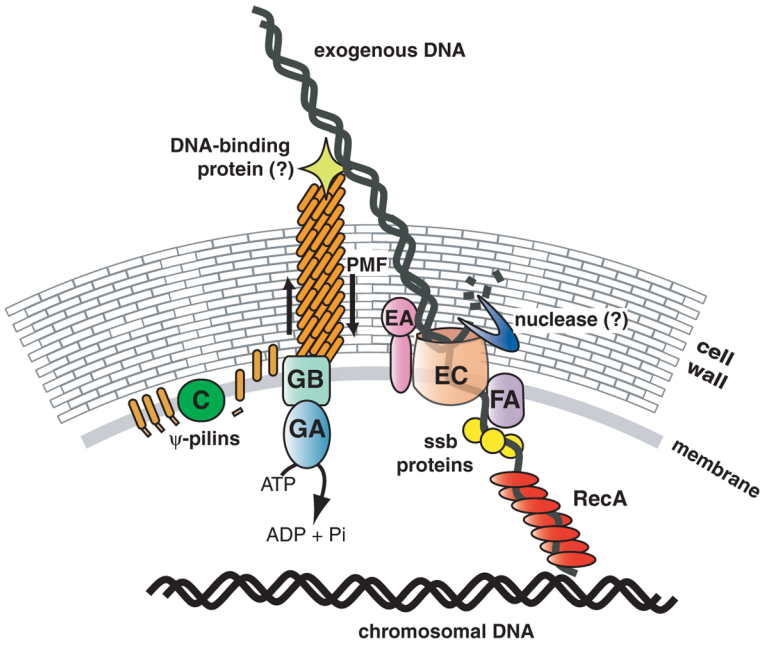

Fig. 2.

DNA uptake during transformation in *B. subtilis*. The uptake machinery is preferentially located at the cell poles. The Ψ-prepilins are processed by the peptidase and translocate to the outer face of the membrane. With the aid of the other ComG proteins, the major Ψ-pilin ComGC assembles into the Ψ-pilus, which attaches exogenous DNA via a hypothetical DNA binding protein. Retraction of the Ψ-pilus, driven by the proton motive force, and DNA binding to the receptor (ComEA) are required to transport one strand of DNA through the membrane channel (ComEC) while the other is degraded by an unidentified nuclease. The helicase/DNA translocase (ComFA) assists the process, along with ssDNA binding proteins that interact with the incoming DNA. RecA forms a filament around the ssDNA, and mediates a search for homology with chromosomal DNA. ADP, adenosine diphosphate; Pi, inorganic phosphate; PMF, proton motive force; ssb, single-stranded DNA binding protein.

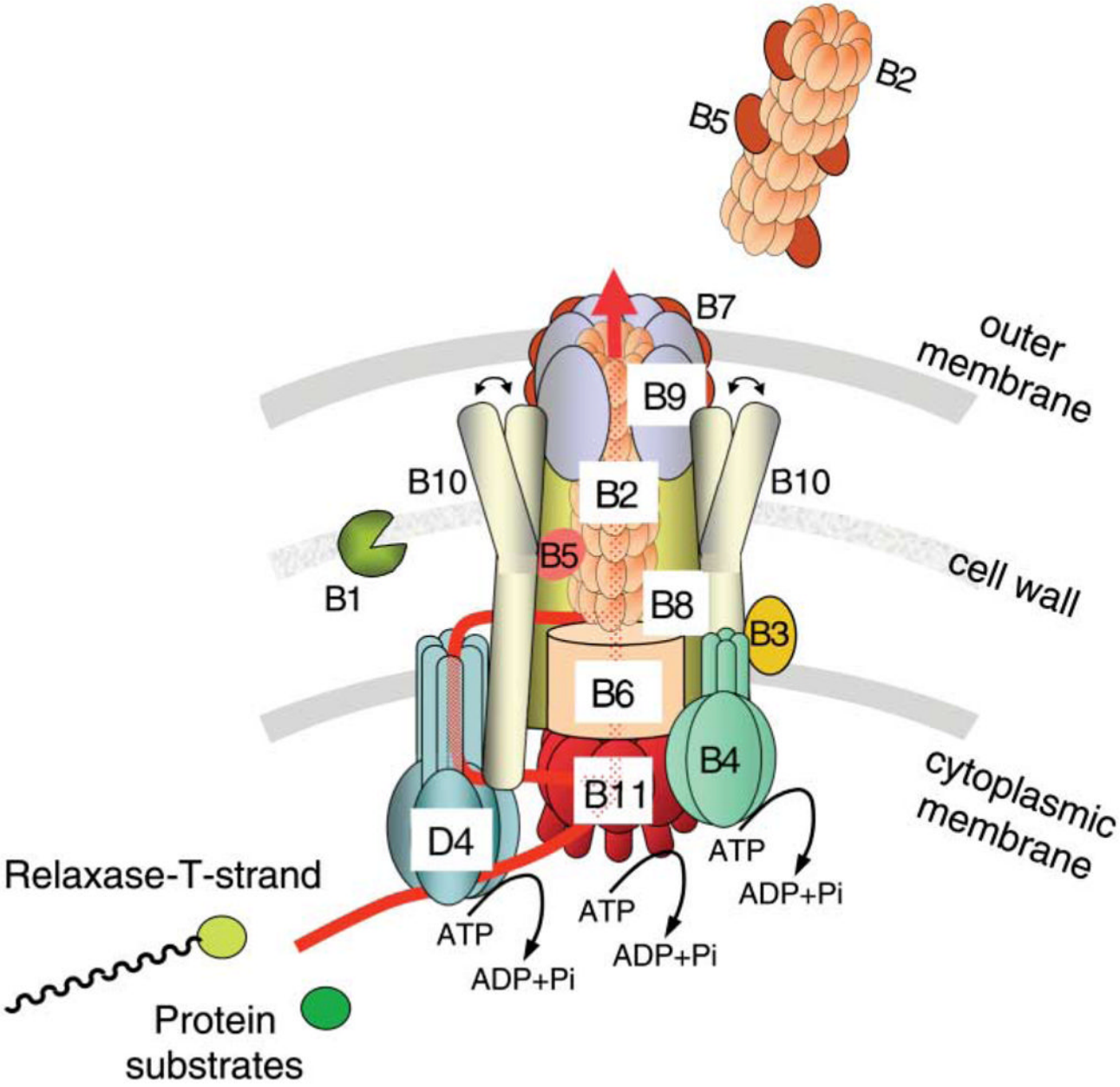

Fig. 3.

Conjugative DNA transfer through the *A. tumefaciens* VirB/D4 system. DNA and protein substrates dock initially at the VirD4 receptor, then transfer in succession to the channel components VirB11 ATPase, VirB6, and VirB8, and finally VirB2 and VirB9. Three ATPases (VirD4, VirB4, VirB11) energize DNA substrate transfer through the membrane translocase comprised of either or both VirD4 and VirB6. The DNA substrate translocates to the cell surface via a channel comprised of VirB2 pilin and secretin-like VirB9. ATP energy also induces a structural transition (double-ended arrow) in VirB10 to mediate substrate transfer to the distal portion of the secretion channel.