DNA UPTAKE DURING BACTERIAL TRANSFORMATION

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Naturally competent bacteria are able to take up exogenous DNA and undergo genetic transformation. The transport of DNA from the extracellular milieu into the cytoplasm is a complex process, and requires proteins that are related to those involved in the assembly of type IV pili and type II secretion systems, as well as a DNA translocase complex at the cytoplasmic membrane. Here, we will review the current knowledge of DNA transport during transformation.

Bacteria can acquire new genetic information by three means: conjugation, transduction and transformation. During conjugation, DNA is transferred directly from one organism to another, whereas in transduction, the DNA is carried by bacteriophages. Transformation involves the acquisition of naked DNA from the extracellular environment (BOX 1), and genetic competence is the ability to undergo transformation. Early experiments on transformation showed that DNA is the genetic material (BOX 2).

Since the advent of recombinant DNA technology, biologists have transformed *Escherichia coli*, the 'workhorse' of molecular biology, using procedures that alter the permeability of the cell membrane (for example, using calcium or electroporation), such that DNA can be introduced to the bacterial cell. By contrast, in this review we will discuss natural transformation, in which specialized bacterial proteins are responsible for the uptake and processing of DNA. At least 40 bacterial species, distributed through all taxonomic groups, are known to be naturally transformable[1]. In most of these species, genetic competence is a transient physiological state, the development of which is tightly controlled by organism-specific processes, including quorum sensing and nutritional signals[2–4]. It is therefore possible that many more species are competent for transformation, but the conditions in which they develop competence are still unknown[5].

Transporting DNA from the extracellular milieu into the cytosolic compartment is a complex task. The incoming DNA must cross the outer membrane (in Gram-negative bacteria), the cell wall and the cytoplasmic membrane. The outer membrane of Gram-negative bacteria represents an extra barrier for the DNA that is absent from Gram-positive bacteria, and this lends confusion to the term 'uptake'. Uptake is defined operationally as the conversion of exogenous, DNase-sensitive DNA into a DNase-protected state. In Gram-negative bacteria, this protection can be achieved by crossing the outer membrane; by contrast, in Gram-positive bacteria, DNA uptake is synonymous with passage across the cytoplasmic membrane. Only one strand of the DNA molecule is effectively transported into the cytoplasm; the other strand is degraded into nucleotides, which are released into the extracellular environment (in Gram-positive bacteria) or presumably into the periplasmic space (in Gram-negative bacteria).

Gram-positive and Gram-negative microorganisms use related proteins to import DNA (the only known partial exception, *Helicobacter pylori*, will be discussed later). Parts of this common competence system share homology with proteins that are involved in the assembly of type IV pili (T4P) and type II secretion systems (T2SSs), and form a structure that partially spans the cell envelope. This structure is functionally coupled to a DNA translocation complex at the cytoplasmic membrane.

We will review each of the steps of the DNA transport process and the components involved therein. Given the structural differences in the cell envelope of Gram-positive and Gram-negative bacteria, and the consequent distinct characteristics in the DNA-uptake pathways, the initial steps will be described separately. We will use *Bacillus subtilis* and *Neisseria gonorrhoeae* as prototypes for DNA transport in Gram-positive and Gram-negative

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Box 1 | Why do bacteria take up DNA and where does it come from?

Three non-mutually exclusive models can account for the evolution of DNA uptake systems[72]:

- DNA for genetic diversity — the acquisition of potential useful genetic information, such as novel metabolic functions, virulence traits or antibiotic resistance.
- DNA repair — environmental DNA from closely related bacteria might serve as templates for the repair of DNA damage.
- DNA as food — DNA can be used as a source of carbon, nitrogen and phosphorous[90,91].

Free DNA is abundant in the environment[1], as it is released from dead organisms. The development of competence in *Streptococcus pneumoniae* leads to lysis and DNA release from a subpopulation, providing DNA for transformation[92,93]. DNA can also be actively secreted by viable organisms: some strains of *Neisseria gonorrhoeae* use a type IV secretion system to release DNA into the medium[94].

microorganisms, respectively, as these systems are the best characterized, but we will also refer to other bacteria.

DNA binding and fragmentation in Gram-positives

The binding of exogenous DNA to the surface of competent cells is the first event during transformation. ComEA, a protein with DNA-binding activity, was identified as a DNA receptor in *B. subtilis* (see below). Mutants lacking ComEA have a reduced ability to bind DNA[6]. A close orthologue of ComEA is present in *Streptococcus pneumoniae*, although residual DNA attachment independent of ComEA was observed in this organism[7], indicating that other DNA receptor(s) might be present on the bacterial surface.

As DNA is transported linearly into the cytoplasm[8], a free end must be present in the DNA molecule for transport to begin. In *B. subtilis*, the rate of uptake is increased by the presence of a surface endonuclease (NucA) that introduces double-stranded cleavages in the bound DNA[9]. In *S. pneumoniae*, DNA transport is preceded by the initial introduction of single-strand nicks, followed by double-strand breaks[10,11], but the endonuclease that is involved in these events has not been identified.

DNA binding and uptake in Gram-negatives

DNA uptake in most systems is not sequence-specific. However, in some Gram-negative microorganisms, such as *Haemophilus influenzae*[12] and *Neisseria* species[13], efficient uptake occurs only if a specific sequence is present, although the DNA binding is nonspecific. The sequence motifs that are required for efficient uptake, called DUS (DNA uptake sequences; also known as USS, for uptake signal sequences), have been identified for *Neisseria* sp. (5′-GCCGTCTGAA-3′)[14] and *H. influenzae* (5′-AAGTGCGGT-3′)[15,16]. The latter shares this DUS with the related bacterium *Actinobacillus actinomycetemcomitans*[17]. As the genomes of these bacteria are enriched in their respective DUS[18–20], uptake of homospecific DNA is favoured. Specific DUS receptors on the bacterial surface have not yet been identified. In *N. gonorrhoeae*, DUS-specific binding of DNA to the cell depends on the presence of the major pilin, and can be modulated by the expression of different minor pilins[21,22], which indicates that a DUS-binding activity is associated with the pilus or a related structure (see below). The putative receptor presumably recognizes the DUS and triggers the uptake process — the transport of DNA across the outer membrane — into either the periplasmic space or specialized vesicle structures (transformasomes) that have been described in *H. influenzae*[23].

Crossing the outer membrane: secretins. Secretins are outer-membrane proteins that are involved in the extrusion of T4P and filamentous phages, type II and type III secretion, and transformation in Gram-negative microorganisms. Secretins form stable multimeric structures, with 12 or 14 subunits, whose correct assembly and insertion into the outer membrane can require the presence of a specific lipoprotein, known as the pilot protein[24,25]. Electron microscopy shows that secretins form doughnut-like structures, with the diameter of the central cavity ranging from 6 to 8.8 nm[26–28]. That secretins can indeed form aqueous channels has been shown electrophysiologically[29]. Clearly, such large channels must be gated to preserve the integrity of the outer membrane and periplasmic compartment, and a different domain of the secretin itself probably occludes the cavity[27,28].

Secretins that are involved in DNA uptake have been identified in several microorganisms. Among these, the best characterized is PilQ from *N. gonorrhoeae*, which also functions in pilus biogenesis[30]. Although PilQ is not required for pilus assembly, it is required to extrude the pilus filament across the outer membrane. The central cavity in the PilQ 12-mer, with a diameter of 6.5 nm, fits the proposed pilus fibre model (~6-nm diameter)[26,31], and could easily accommodate the DNA double helix (~2.4 nm), either by itself or in a nucleoprotein complex. DNA uptake in *N. gonorrhoeae* requires the presence of a DUS, so the putative DUS receptor could participate in signalling the opening of the PilQ channel during transformation; the pilus or the putative pseudopilus (see below) might also be involved. However, there is still no direct evidence that DNA passes through the secretin channel.

The type IV pilus and type II secretion

To describe DNA transport during competence, we must present two closely related machines, the T4P and the T2SS. The sequence and structural similarities among the components of these three systems indicate their common origin. However, their genetic organization is not conserved, nor is there complete correspondence among their components, and even machines within the same class can show distinct elements.

Box 2 | Transformation and the discovery of DNA

In 1928, Griffith reported that mixing heat-killed, virulent (capsulated, serum-resistant) pneumococci with live, non-virulent (non-capsulated, serum-sensitive) bacteria gave rise to virulent organisms, which were able to cause septicaemia in mice[95]. The subsequent identification by Avery, MacLeod and McCarthy of the 'transforming principle' — that a substance from the dead bacteria carried the information to the live, non-virulent ones — provided evidence that the genetic material is DNA[96].

Clearly, these systems have diverged and acquired unique characteristics to perform distinct tasks. The differences in these machines and their comparative analysis could prove to be as informative as their similarities. The nomenclature of the components of these systems is confusing, and we will refer only to the constituents from the prototypical systems.

The type IV pilus. T4P are long, thin appendages that are present on the surface of many Gram-negative microorganisms. T4P function in bacterial cell-to-cell interactions, adhesion to host cells and twitching motility — a form of locomotion that is powered by extension and retraction of the pilus filament[32,33]. Owing to their importance in virulence, T4P have been studied in detail. Several, if not all, proteins that participate in pilus assembly are known, but the biogenesis process itself is not yet clearly understood[34]. The main structural constituent of the pilus is type 4 pilin, which is assembled to form the pilus fibre, while a few minor pilins participate in the biogenesis process. Type 4 pilins are small proteins (usually <20 kDa), which are made as precursors (prepilins) that comprise a short, positively charged amino-terminal leader peptide, a hydrophobic stretch and a carboxy-terminal domain. The leader peptide and hydrophobic region sequences are relatively conserved, whereas the C-terminal region is hypervariable. The prepilins are found associated with the cytoplasmic membrane through their hydrophobic domains. They are processed by a specific enzyme that resides in the cytoplasmic membrane — the prepilin peptidase, an aspartic acid protease that cleaves the leader peptide[35]. Some prepilin peptidases also *N*-methylate the new N-terminal residue of the mature pilin, which is almost always a phenylalanine. After processing by the prepilin peptidase, which probably facilitates the translocation of mature pilins across the membrane[36], the pilins are assembled into the pilus filament, which protrudes from the membrane. Two models for pilus fibres have been proposed on the basis of the X-ray crystallographic structures of two different pilins[31,37]. In both cases, the fibre is a helix with five subunits per turn. The core is formed by the conserved hydrophobic N-terminal domains of the mature pilins, whereas the C-terminal domains are exposed on the surface.

In *N. gonorrhoeae* T4P[38] (FIG. 1; TABLE 1), the major pilin is encoded by the *pilE* locus. The prepilin is processed by its peptidase (PilD). A putative ATPase (PilF) is required to assemble the pilin subunits into a filament, and might provide the energy that is necessary for polymerization. A polytopic membrane protein (PilG) also participates in pilus biogenesis. As it is assembled, the pilus filament extends outwards from the cytoplasmic membrane, across the peptidoglycan layer and periplasmic space, and crosses the outer membrane through a channel formed by the secretin (PilQ), assisted by its pilot protein (PilP). It has been proposed that the force from the protuding pilus fibre could be enough to open the secretin pore[39]. A second ATPase (PilT) is needed for twitching motility — which is caused by depolymerization and consequent retraction of the pilus filament — but is dispensable for pilus assembly[40]. PilF and PilT are closely related, and belong to a large family of proteins that are involved in several transport systems, called the traffic NTPases. A tip-located adhesin (PilC) seems to stabilize the pilus filament, and can also be found associated with the outer membrane.

The type II secretion system. The T2SS, also known as the secreton or main terminal branch of the general secretory pathway, mediates the translocation of proteins from the periplasm across the outer membrane of Gram-negative bacteria[41,42]. The prototypic T2SS is the pullulanase secretion apparatus of *Klebsiella oxytoca*, which is composed of 15 components, several of which are similar to T4P proteins (FIG. 1; TABLE 1): a traffic NTPase (PulE); a polytopic membrane protein (PulF); a secretin (PulD); a prepilin-peptidase (PulO); and several type 4 pilins (the major pilin, PulG, and the minor pilins, PulH, PulI, PulJ and PulK). The proteins from the T2SS were postulated to form a pilus-like structure, known as the pseudopilus[43]. Some pseudopilins can form pilus-like filaments when overexpressed[44–46]. These structures might correspond to abnormally long, overgrown pseudopili, and their assembly requires the other components of the T2SS.

How does the pseudopilus participate in the secretion of proteins across the outer membrane? It is thought that the pseudopilus spans the cell envelope, and might work as a piston, pushing specific substrates from the periplasm through the secretin pore in the outer membrane. It has been proposed that the thrusting pseudopilus could use the force from its elongation, which is promoted by the traffic NTPase involved in assembly (PulE in *K. oxytoca*). However, a second traffic NTPase (equivalent to PilT in T4P), which would mediate retraction of the pseudopilus, is absent from T2SSs. Perhaps pseudopilus retraction is driven by the proton motive force, which is known to be necessary for the secretion of some T2SS substrates[41].

The competence pseudopilus

Most competent bacteria use components that are similar to those of T4P and T2SSs to take up DNA. In competent organisms that also have T4P, there is a correlation between the presence of pili and competence[47–51]. However, whether pili themselves have a direct role in transformation is unclear. No interaction between DNA and the pilus or pilins has been detected[52]. The models for pilus structure show a cavity in the middle of the filament, but its narrow diameter (1.2 nm) and hydrophobicity argue against its use as a conduit for

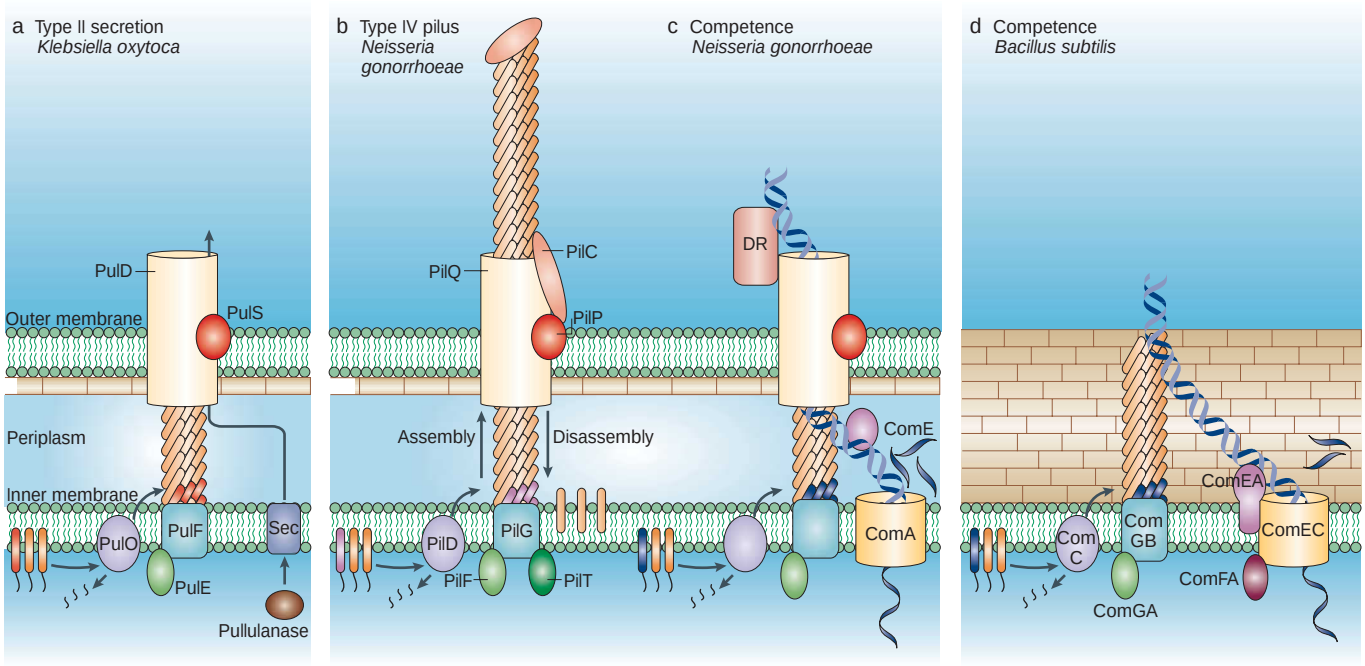

Figure 1 | **Comparison of machinery required for type II secretion, type IV pilus formation and transformation in Gram-negative and Gram-positive bacteria. a** | A schematic model for type II secretion, based on the pullulanase secretion system (Pul) from *Klebsiella oxytoca*. Not all components are represented. The pseudopilins, both major (PulG; orange) and minor (PulH,-I,-J and -K; red), are processed by the prepilin peptidase (PulO), and assembled into the pseudopilus. The polytopic membrane protein (PulF) and the traffic NTPase (PulE) participate in the process. Pullulanase (brown) is secreted into the periplasm by the Sec system, and crosses the outer membrane through a channel that is formed by the secretin (PulD), with the assistance of its pilot protein (PulS). **b** | A schematic model for type IV pilus formation, based on the *Neisseria gonorrhoeae* pilus. The major pilin (PilE; orange) and minor pilin (PilV; magenta) are processed by the prepilin peptidase (PilD), and assembled into the pilus fibre. The polytopic membrane protein (PilG) and the traffic NTPase (PilF) participate in this process. The outer-membrane/tip-located protein (PilC) stabilizes the assembled filament. The pilus crosses the outer membrane through a channel that is formed by the secretin (PilQ), with the assistance of its pilot protein (PilP). A second traffic NTPase (PilT) mediates the depolymerization of the pilus into pilin monomers and consequent retraction of the pilus. **c** | A schematic model for the competence pseudopilus and DNA translocase in *N. gonorrhoeae*. Assembly of the pseudopilus requires the same components as the type IV pilus (shown in part **b**). The major pilin (PilE; orange) and minor pilin (ComP; blue) are processed by the prepilin peptidase (PilD), and assembled into the pseudopilus. The polytopic membrane protein (PilG) and the traffic NTPase (PilF) participate in this process, as well as PilC (not shown). The specific sequence in the exogenous DNA that is required for efficient uptake is recognized by its postulated, but as-yet-unidentified, receptor (DR). The incoming DNA is transported across the outer membrane through a channel that is formed by the secretin (PilQ), with the assistance of its pilot protein (PilP). The periplasmic DNA-binding protein (ComE) is involved in uptake, and delivers the DNA to the channel at the cytoplasmic membrane (ComA). One strand enters the cytosol; the other is degraded and the degradation products are released into the periplasmic space. **d** | A schematic model for the competence pseudopilus and DNA translocase in *Bacillus subtilis*. The major pseudopilin (ComGC; orange) and minor pseudopilins (ComGD, -GE and -GG; blue) are processed by the prepilin peptidase (ComC), and assembled into the pseudopilus. The polytopic membrane protein (ComGB) and the traffic NTPase (ComGA) participate in this process. The pseudopilus allows the exogenous DNA to access its membrane-bound receptor (ComEA), which delivers the bound DNA to the channel at the cytoplasmic membrane (ComEC). An ATP-binding protein (ComFA) is involved in DNA transport across the membrane. One strand enters the cytosol, while the other is degraded and the degradation products are released into the extracellular milieu.

DNA[53]. In fact, there is considerable evidence that pili are not necessary for transformation in *N. gonorrhoeae*, although the expression of pilin is absolutely required[54–56]. Other competent organisms, such as *H. influenzae* or the Gram-positive bacteria *B. subtilis* and *S. pneumoniae*, require similar genes for DNA uptake, but do not possess filamentous structures that extend from the cell surface.

It has been proposed that a competence pseudopilus — a structure similar to T4P — participates in the transport of DNA during transformation[57]. This putative structure would be present in both Gram-negative and Gram-positive bacteria. The proposed function of the competence pseudopilus is to bring exogenous DNA to the transport machinery that is located at the cytoplasmic membrane. In organisms with T4P, the pseudopilus would be assembled using the same components as the pilus, thereby accounting for the correlation between competence and piliation. In *N. gonorrhoeae* (FIG. 1; TABLE 1), these would include: the major pilin (PilE), the prepilin peptidase (PilD), the traffic NTPase (PilF), the polytopic membrane protein (PilG), the secretin (PilQ) with its pilot protein (PilP), and the pilus-stabilizing protein (PilC). We favour the existence of a competence pseudopilus as a structure that is distinct from the T4P, albeit closely related, for several reasons. Various observations indicate that the presence of functional T4P and the ability to take up DNA have different requirements — for example, the existence of pilin variants that cannot assemble efficiently into a pilus fibre, but can still support transformation, and the requirement for the presence of minor pilins for competence but not for T4P formation[58–60]. The major pilin should be a structural component of both T4P and the competence pseudopilus; the minor pilins could be important in determining which structure is formed.

Supporting this hypothesis, two minor pilins have antagonistic effects in competence in *N. gonorrhoeae*: the expression of PilV inhibits transformation[22], whereas transformation is enhanced by ComP[58]. A minor pilin with a negative effect on transformation (PilAII) has also been identified in *Pseudomonas stutzeri*[61].

Microorganisms that lack T4P have an apparatus that is presumably devoted to assembly of the pseudopilus. In *B. subtilis*[62] (FIG. 1; TABLE 1), this apparatus includes the ComG proteins: a traffic NTPase (ComGA), a polytopic membrane protein (ComGB), four pseudopilins (one major, ComGC, and 3 minor, ComGD, ComGE and ComGG), as well as a protein with no known orthologues in other systems (ComGF). Other proteins are involved in the processing of the pseudopilins in *B. subtilis*: the prepilin peptidase (ComC) cleaves their N-terminal leader peptides, and a protein oxidoreductase pair (BdbD and BdbC) introduces an intramolecular disulphide bond into the major pseudopilin, ComGC, which is essential for its stability[63]. The ComG proteins are required for DNA binding to the competent cell, but the pseudopilins themselves do not show DNA-binding activity[64]. The ComG proteins are thought to modify the cell wall, perhaps by creating a channel, which allows the exogenous DNA to gain access to the membrane-anchored receptor ComEA. In fact, the ComG proteins are not required for DNA binding if the cell wall is removed[64]. Furthermore, the major pseudopilin (ComGC) can be found in the membrane fraction, as well as associated with the cell wall. Like other traffic NTPases, ComGA contains a nucleotide-binding site with Walker A and B boxes, and other conserved motifs; a mutation in the Walker A box leaves the cells incapable of binding DNA. ComGA might provide the energy that is necessary for pseudopilus assembly through ATP hydrolysis[62].

In Gram-positive bacteria, the competence pseudopilus would span the thick cell wall, whereas in Gram-negative bacteria it would extend across the periplasmic space and peptidoglycan layer, bridging the outer and inner membranes. How would it participate in DNA uptake? As stated above, no interaction between DNA and pilins or pseudopilins has been detected. In *B. subtilis*, the pseudopilus could have a passive role, forming a structure across the cell wall that allows exogenous DNA to contact its membrane-bound receptor. However, in organisms with T4P, the traffic NTPase that mediates pilus retraction (PilT) is required for DNA uptake[40,65], which indicates that the pseudopilus could retract and pull DNA into the cell from the bacterial surface, facilitating the interaction of the incoming DNA with its receptor (see below). In Gram-negative bacteria, the retraction of the pseudopilus would mediate passage of the DNA across the secretin channel.

Despite its appeal, this hypothesis has several shortcomings. First, it would involve DNA binding to the pseudopilus itself or to a protein that is associated with it, neither of which have been documented. However, in *N. gonorrhoeae*, this protein could be the elusive DUS receptor. Second, PilT orthologues are only present in bacteria with T4P; other organisms have a single competence traffic NTPase, which is presumably involved in assembly of the pseudopilus. It is conceivable that this single traffic NTPase could function in both assembly and disassembly of the pseudopilus; another possibility is that the retraction of the pseudopilus is powered by other means, such as the proton motive force, which participates in T2SS function and has also been implicated in DNA uptake in *B. subtilis*[66]. However, in a *P. stutzeri* strain expressing a pilin mutant that could not be assembled into a fibre, PilT was not required for transformation[65]. This indicates that PilT, which has a negative effect on pilus assembly[39,67], might not be directly involved in the uptake process; instead, PilT might be required for competence only because it allows the pseudopilus to compete for the common assembly machinery that is shared with T4P. In the absence of PilT, the formation of T4P would be favoured, to the detriment of pseudopilus assembly, and competence would be abolished.

The membrane translocation machinery

The translocation of DNA across the cytoplasmic membrane has been studied most in *B. subtilis*, where it requires three components: the DNA receptor ComEA, the permease/channel protein ComEC and the ATP-binding protein ComFA. Other organisms seem to use the same basic components (TABLE 1). The putative complex in *B. subtilis* is classified by the Transport Protein Database as a DNA translocase (TC 3.A.11.1.1)[68]. It has recently been proposed that the DNA translocase is actually an atypical ATP-binding cassette (ABC) transporter, the substrate of which is the incoming DNA during transformation (I. Draskovic and D.D., unpublished observations).

ABC transporters constitute one of the largest families of proteins and are present in all organisms, both the prokaryotes and the eukaryotes[69]. They can be exporters or importers, using energy from ATP hydrolysis to translocate solutes across the cytoplasmic membrane. The translocation component consists of two intracellular nucleotide-binding domains and two transmembrane domains, which can assume different arrangements, but which contain characteristic signature motifs. The substrate-binding protein, a component from ABC transporters that is involved in solute uptake, is located in the periplasm (in Gram-negative bacteria) or is anchored to the membrane (in Gram-positive bacteria and Archaea).

The DNA receptor. ComEA is a protein that is required for *B. subtilis* transformation, and mutants that lack ComEA are impaired in their ability to bind DNA[6]. The ComG proteins, which form the putative competence pseudopilus (see above), are required to provide access for the incoming DNA to ComEA, which is bound to the membrane through its N-terminal region. The C-terminus of ComEA contains the DNA-binding domain, which is composed of helix–hairpin–helix motifs that interact with double-stranded DNA without

Table 1 | Proteins involved in DNA uptake and their orthologues

Protein	*B. subtilis* (Ref. 62)	*S. pneumoniae* (Ref. 7)	*H. influenzae* (Ref. 97)	*T. thermophilus* (Refs 59,98)	*P. stutzeri* (Refs 49,65)	*N. gonorrhoeae* competence (Ref. 99)	*N. gonorrhoeae* T4P (Ref. 38)	*K. oxytoca* T2SS (Ref. 42)	*H. pylori*
The competence pseudopilus*									
Traffic NTPase(s)	ComGA	ComGA	PilB	PilF	n.i., PilT, PilU	PilF, PilT	PilF, PilT	PulE	n.a.
Polytopic membrane protein	ComGB	ComGB	PilC	PilC	PilC	PilG	PilG	PulF	n.a.
Pilins or pseudopilins	ComGC, -GD, -GE, -GG	CglC, CglD[100]	PilA	PilA1, -A2, -A3, -A4	PilAI	PilE,ComP	PilE, PilV[22,101]	PulG, -H, -I, -J, -K	n.a.
Prepilin peptidase	ComC	CilC[102]	PilD	PilD	n.i.	PilD	PilD	PulO	n.a.
Secretin/ pilot	n.a.	n.a.	ComE/n.i.	PilQ/n.i.	n.i.	PilQ/PilP	PilQ/PilP	PulD/PulS	n.a.
DNA translocation machinery									
DNA receptor	ComEA	ComEA	n.i.	ComEA	n.i.	ComE[70]	n.a.	n.a.	n.i.
Membrane channel	ComEC	ComEC	n.i.	ComEC	ComA	ComA[75]	n.a.	n.a.	ComE3[87]
ATP-binding protein	ComFA	ComFA	n.i.	n.i.	n.i.	n.i.	n.a.	n.a.	n.i.

*For most bacteria, the components of the competence pseudopilus are orthologues of these involved in the formation of type IV pili or type II secretion systems. n.a., not applicable; n.i., not identified; T2SS, type II secretion system; T4P, type IV pilus.

sequence specificity. Immediately N-terminal to the DNA-binding domain of ComEA is a stretch of residues that could form a flexible hinge; mutants in which this region is deleted can bind, but not transport, DNA. So, ComEA functions in both the binding and the transport of DNA in *B. subtilis*. The hinge region might allow ComEA to bend and deliver the bound DNA to the channel formed by ComEC. An interaction between ComEA and ComEC has been detected (I. Draskovic and D. D., unpublished observations), which supports this model.

Orthologues of *B. subtilis* ComEA can be found in the genomes of various competent Gram-negative organisms. In *N. gonorrhoeae*, the orthologue (ComE) is involved in competence[70], and contains a signal peptide and the DNA-binding domain. The mature ComE protein is localized to the periplasm, in contrast to the much larger, membrane-bound ComEA in *B. subtilis*. This difference in the arrangement of the ligand receptor in ABC transporters is common between Gram-positive and Gram-negative organisms[69]. An exception is the ComEA orthologue of the competent Gram-negative bacterium *Thermus thermophilus*, which contains a transmembrane domain[71].

The role of neisserial ComE in DNA transport has not been clearly established. ComE is required for DNA uptake in *N. gonorrhoeae*, and the protein binds DNA without sequence specificity. However, as described above, DNA uptake in this organism is dependent on the presence of the DUS. ComE might be a secondary DNA receptor in *N. gonorrhoeae*, and would therefore bind to DNA molecules that have already been selected by the presence of the DUS. The putative DUS receptor might recognize DUS-containing DNA and trigger the opening of the secretin channel, thereby allowing the passage of DNA — which could be mediated by ComE. So, ComE could link the competence pseudopilus (which also participates in uptake) with the transport machinery at the inner membrane.

The DNA channel across the cytoplasmic membrane. ComEC is a polytopic membrane protein that is necessary for *B. subtilis* transformation. ComEC is dispensable for DNA binding, but is essential for DNA transport, and it has been proposed that this protein forms an aqueous channel across the cytoplasmic membrane[72]. ComEC is present as a homodimer, with five transmembrane domains per subunit, and contains a signature motif that is characteristic of ABC transporter permeases — the binding-protein domain (BPD). Mutation of a residue in this region reduces the level of transformation (I. Draskovic and D.D., unpublished observations).

Orthologues of *B. subtilis* ComEC have been identified in other competent organisms and its role in transformation has been verified in several cases[7,71,73,74]. In *N. gonorrhoeae*, the orthologous protein (ComA) is not necessary for DNA uptake; mutants that lack ComA retained the incoming DNA as a double-stranded molecule in the periplasmic space[75].

The ATP-binding protein. *B. subtilis* ComFA is a membrane-associated protein that is required for competence; its mutation leads to a 1,000-fold reduction in transformation frequency, but does not affect DNA binding. ComFA has sequence homology to PriA of *E. coli*, which is an ATP-driven DNA translocase

Table 2 | Proteins involved in DNA uptake in *H. pylori* and orthologues*

Protein	*H. pylori*	*A. tumefaciens* Vir
ATPase	ComB4	VirB4
Channel subunits	ComB7, -B8, -B9, -B10	VirB7, -B8, -B9, -B10

*The *H. pylori* DNA uptake system is related to type IV secretion systems[103]. Orthologues in *A. tumefaciens* are shown.

that is related to DNA and RNA helicases. It has Walker A- and B-type ATP-binding consensus motifs, and point mutations in the Walker A motif result in transformation deficiency[72]. It is possible that ComFA-catalyzed ATP hydrolysis energizes the passage of single-stranded DNA through the ComEC channel; it could also have other roles, such as unwinding the double-stranded DNA or gating the ComEC channel.

Orthologues of ComFA have not been identified in Gram-negative bacteria. Perhaps other ATPases have been recruited in these organisms. For example, in *P. stutzeri*, an orthologue of *E. coli* ExbB (which is involved in energy-coupled transport across the outer membrane) might be involved in DNA transport[74].

Degradation of the non-transforming DNA strand

In Gram-positive microorganisms, only one strand of the DNA molecule enters the cytoplasm, while the other strand is degraded into acid-soluble products, which are released into the surrounding medium[72]. A nuclease activity might therefore be coupled to the transport process. In *S. pneumoniae*, a membrane-bound nuclease, EndA, is required for efficient transformation; however, degradation is still observed in mutants that are unable to transport DNA[7]. This is in contrast to *B. subtilis*, which does not have an EndA orthologue and in which degradation occurs only if there is transport[9].

In Gram-negative bacteria, the situation is less clear, although the proteins that are involved in DNA entry are relatively conserved, especially the channel protein. There is evidence that the transforming DNA can enter the cytoplasm as a single strand in *H. influenzae*, while the other strand is degraded[76]. A link between DNA transport to the cytoplasm and degradation was observed in *N. gonorrhoeae*, as no nucleolytic processing of DNA after uptake was observed in a strain that lacked the putative channel protein ComA[75]. However, low levels of single-stranded DNA are formed during transformation of *N. gonorrhoeae*, regardless of the presence of ComA[77].

The fate of incoming DNA in the cytoplasm

Incoming single-stranded DNA can be integrated into the bacterial chromosome by a RecA-dependent process that requires sequence homology between the incoming DNA and the bacterial chromosome. Plasmids can be reconstituted and stably maintained. The interaction of the incoming DNA with cytoplasmic proteins protects it from degradation and is essential for transformation[78].

The *H. pylori* exception

The human gastric pathogen *H. pylori* is naturally transformable[79]. There is controversy about the specificity of DNA uptake by this organism[80–82], but it is clear that uptake of DNA by *H. pylori* involves a distinct apparatus, which is related to type IV secretion systems (T4SSs) (TABLE 2). These systems function in the export of macromolecules, such as the conjugal transfer of DNA and the secretion of proteins, either to the extracellular milieu or into eukaryotic host cells. The prototype for type IV secretion is the Vir system used by *Agrobacterium tumefaciens* to transfer its T-DNA into plant cells[83]. The system is assembled from the products of the *virB* operon and the *virD4* gene, which form a channel for the translocation of the substrate, and an extracellular pilus (the T pilus, which is unrelated to T4P) that contacts the target cell. There are three putative ATPases involved in the process: VirD4, VirB4 and VirB11. The transfer channel itself is thought to be composed of the proteins VirB6–VirB10, the properties and interactions of which have been studied in detail. VirB6 is a polytopic inner-membrane protein, and might form the actual channel at the cytoplasmic membrane, whereas VirB7–VirB10 are thought to form a complex that spans the cell envelope.

The *comB* operon is involved in transformation in *H. pylori*[84]. It contains four open reading frames (ORFs), known as *comB7–comB10* (REF. 85) after their orthologues from the *A. tumefaciens* VirB system. ComB8, ComB9 and ComB10 are essential for transformation, whereas ComB7 is not, but seems to stabilize the complex. ComB7 is a lipoprotein that probably localizes to the outer membrane and forms a disulphide bond with ComB9; ComB8–ComB10 are tightly associated with the membrane, and might span the periplasmic space, forming a bridge between the outer and inner membranes[86]. A putative ATPase with similarity to *A. tumefaciens* VirB4 is also necessary for competence in *H. pylori*, and is called ComB4 (REF. 85). It is interesting to note that *H. pylori* has a *bona fide* T4SS, the Cag system, which is dedicated to the translocation of an effector protein (CagA) into eukaryotic cells. The Cag and the ComB systems are functionally independent[81,85].

A competence homologue of VirB6 (which might form a pore in the inner membrane) is missing from *H. pylori*. Instead, *H. pylori* seems to rely on an orthologue of *B. subtilis* ComEC for DNA translocation across the cytoplasmic membrane[87] (TABLE 1). So, *H. pylori* uses a different apparatus to bring DNA through the outer membrane, periplasmic space and cell wall, but the channel across the cytoplasmic membrane is similar to the one used by other competent bacteria.

Are there other organisms that use T4SS-related complexes to take up DNA? Orthologues of the *H. pylori* genes *comB8–comB10* have been identified in a plasmid from the closely related bacterium *Campylobacter jejuni*[88]. However, mutation of *comB10* in this organism led to only a fivefold reduction in transformation frequency. In fact, recently

identified genes involved in competence in *C. jejuni* show similarities to the T2SS/T4P-related proteins[89], so this organism could use both systems.

Concluding remarks

Competent bacteria possess highly specialized machines to transport DNA into their cytoplasm. These machines are assembled from components resembling those that form surface appendages (T4P) and transport systems as diverse as T2SS, T4SS and ABC transporters. Most of the proteins that are involved in the transport of DNA during transformation have been identified, although their precise functions remain obscure. A detailed analysis of protein–protein and protein–DNA interactions will provide us with a better understanding of the assembly of the various complexes, as well as of the DNA transport process itself. A further challenge is to characterize the energetics of DNA internalization.

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Competing interests statement

The authors declare that they have no competing financial interests.

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