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DNA Transport and Natural Transformation in Mesophilic and Thermophilic Bacteria

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Comparative genome analyses revealed a massive DNA exchange between microbes of distant evolutionary lineages. This phenomenon known as horizontal, or lateral, gene transfer has a tremendous impact in the evolution of prokaryotes. Here, the process of DNA transport via genetic transformation is discussed. This review will focuss on the process of DNA uptake mediated by type IV pilin-like proteins in Gram-positive and Gram-negative bacteria. Three tentative models of transformation machineries comprising components similar to proteins of type IV pili and type II secretion are presented. A comparative discussion of the structure of DNA translocators and the underlying mechanism of transfer of free DNA in mesophilic and extremely thermophilic bacteria highlights conserved and distinctive features of the DNA translocators in mesophilic and thermophilic bacteria.

KEY WORDS: DNA transport; natural transformation; competence proteins; type IV pili.

INTRODUCTION

Microorganisms are able to exploit very different environments and therefore must have evolved phenotypic traits allowing adaptation and survival under very different environmental conditions. Diversification of microorganisms can be achieved by gene mutations, differential gene loss, intramolecular recombination, and/or lateral gene transfer. Studies on the ecological requirements of gene transfer processes provide growing evidence that gene transfer is a major force for bacterial adaptation to changing environments. Genome-scale comparisons provide evidence that large portions of bacterial genomes have undergone gene transfer between major bacterial lineages and therefore corroborate this conclusion (Doolittle, 1999; Ochman *et al.*, 2000). Moreover genome-based quantitative assessments of interdomain DNA transfer revealed that 3% of the genes of most free-living bacteria have been acquired from archaea and eukaryotes. Thermophilic bacteria were found to clearly stand out in terms of interdomain DNA transfer, such as 24 and 16.2% of the genes in the hyperthermophilic bacteria *Thermotoga maritima* and *Aquifex aeolicus*, respectively, are suggested to be transferred from archaeal hyperthermophiles (Aravind *et al.*, 1998; Nelson *et al.*, 1999). These findings led to the conclusion that hyperthermophilic bacteria play a central role in interdomain DNA transfer and support the crucial importance of lateral gene transfer between hyperthermophiles for the evolution of microorganisms.

The mechanism of natural transformation is the oldest studied mechanism of DNA transfer and is of considerable historical importance, since it provided the first evidence that DNA is the genetic material (Griffith, 1928). The ability to bind and take up free DNA *via* natural transformation which is called competence is widely distributed among very different phylogenetic and trophic groups (Lorenz and Wackernagel, 1994). The growing evidence that natural transformation is not restricted to bacterial DNA but also mediates transfer of transgenic plant DNA to bacteria suggests that natural transformation is the most versatile mechanism of DNA transfer (de Vries *et al.*, 2001).

The process of natural transformation comprises five discrete steps: competence induction, DNA binding, DNA fragmentation, DNA uptake, and the heritable integration

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of incoming DNA *via* recombination with its homologous counterpart or reconstitution of plasmid DNA. The generality of the first two steps is questionable, such as *Thermus thermophilus* and *Helicobacter pylori* are always able to take up free DNA (Hidaka *et al.*, 1994; Israel *et al.*, 2000).

Both induction of competence and the physiology of natural transformation have been intensively studied in various Gram-positive and Gram-negative bacteria and have been extensively reviewed in some excellent reviews and these two topics will not be the focus of this review (Chen and Dubnau, 2003; Claverys and Havarstem, 2002; Lorenz and Wackernagel, 1994; Palmen and Hellingwerf, 1997). Much less is known with respect to the specific role of competence proteins in DNA transport, the mechanism of DNA transport, and the structure of DNA translocators. Moreover, until very recently nothing was known with respect to the components of DNA uptake systems in extremophilic bacteria.

This review will focus on a comparative discussion of the process of DNA uptake of mesophilic and extremely thermophilic bacteria hightlighting the similarities and the distinct features of the DNA translocators. However, since the available data concerning transformation machineries of mesophilic bacteria is huge and space is limited, we can here only give a comprehensive birds-eye view of the natural transformation in mesophilic bacteria.

NATURAL TRANSFORMATION IN MESOPHILIC BACTERIA

During transformation of Gram-positive bacteria DNA must be transported across the cell wall and the cytoplasmic membrane. Conversion to single-stranded DNA takes place during transport through the inner membrane which suggests the involvement of nucleases in DNA transport. So far, a competence-specific endonuclease has been identified only in the Gram-positive bacterium *S. pneumoniae* (Puyet *et al.*, 1990). In Gram-negative bacteria additional barriers have to be passed such as the outer membrane and the periplasmic space. The differences in the cell envelope call for differences in the DNA binding and uptake machineries.

In addition to these structural differences also physiological differences of natural transformation systems are known, such as different demands on transforming DNA. *B. subtilis*, *S. pneumoniae*, *Acinetobacter* sp. BD413, and *P. stutzeri* can take up any kind of DNA whereas DNA uptake in *Haemophilus* and *Neisseria* requires short binding motifs (DUS, termed for DNA uptake sequence) of 11 and 10 bp, respectively.

DNA Uptake Machineries, Type IV Pili and Type II Secretion Systems are Related Systems

Despite the distint features of DNA transformation systems in Gram-negative and Gram-positive bacteria there are significant similarities within the DNA uptake machineries. A common feature is the involvement of proteins that are similar to components of type IV pili (Tfp) and type II protein export systems (Chen and Dubnau, 2003; Hobbs and Mattick, 1993). So far the only known exceptions are the transformation machineries in *H. pylori* and *Campylobacter jejuni*. The DNA uptake machineries of these bacteria are related to type IV secretion systems and to the Vir system of the conjugation machinery in *Agrobacterium tumefaciens* (Hofreuter *et al.*, 2001; Smeets and Kusters, 2002). A model of the *H. pylori* transformation machinery based on these similarities was recently proposed by Smeets and Kusters (2002) and within this review the *H. pylori* system will not be subject of discussion.

Tfp are long dynamic filamentous surface structures of 6 nm in diameter. They can extend to several micrometers in length and are involved in adherence to host cells and binding of bacteriophages (Mattick, 2002). The correlation between Tfp and transformation was first observed in *N. gonorrhoeae*, by the finding that loss of piliation correlated with a loss of transformability (Sparling, 1966). Besides their role in infection such as adhesion on epithelial cells and activation of host cell response, Tfp mediate formation of biofilms, fruiting bodies, and surface translocation (Kaiser, 2000; Merz *et al.*, 2000; Strom and Lory, 1993). The latter, driven by the retraction of the dynamic pili structures, is termed twitching and social gliding motility. It has been shown that pilus retraction is achieved by a very fast depolymerization of pilin subunits with an observed rate of 1.2 μm per sec (about 1500 pilins per sec) (Kaiser, 2000). This depolymerization is mediated by traffic NTPases. The many data on Tfp structure and function are summarized in some excellent reviews (Kaiser, 2000; Mattick, 2002; Tonjum and Koomey, 1997).

Tfp are composed primarily of a single small protein subunit, termed pilin, which is arranged in helical conformation with 5 subunits per turn with a 41 Å pitch (Forest and Tainer, 1997). Due to similarities of pilins and pilin-like proteins of DNA translocators, it is tempting to speculate that pilin-like proteins of DNA translocators, the so called pseudopilins, exhibit a helical arrangement analogous to the pilins of Tfp. This suggestion is underlined by the finding that overexpression of pseudopilins of type II secretion machineries results in the assembly of pilus structures (Durand *et al.*, 2003; Sauvonnet *et al.*, 2000). Our recent finding that overexpression of the

Table I. Competence Proteins Related to Type IV Pili Proteins or Essential for Type IV Pili Biogenesis

Class[a]	*N.g.*[b]	Acinetobacter sp. BD413	*H.i.*[c]	*P.s.*[d]	*T.t.*[e]	*B.s.*[f]	*S.p.*[g]
1	PilE ComP	ComB, ComE ComF, ComB	PilA	PilAI PilAII	PilA1, PilA2 PilA3, PilA4	ComGC, ComGD ComGE, ComGG	CglC, CglD
2	PilD	ComD	PilD	PilD	PilD	ComC	CilC
3	PilT PilF	N.I.[h]	PilB	PilT	PilF	ComGA	CglA
4	PilG	N.I.	PilC	PilC	PilC	ComGB	CglB
5	PilQ	ComQ	ComE	N.I.	PilQ	N.I.	N.I.
6	PilC	ComC	N.I.	N.I.	N.I.	N.I.	N.I.

[a] Based on homology, the competence, the proteins are assigned to six groups 1) pseudopilins, 2) prepilin-processing leader peptidases, 3) traffic NTPases, 4) polytopic inner membrane proteins, 5) secretins, and 6) outer membrane/cell surface proteins.
[b] *Neisseria gonorrhoeae*.
[c] *Haemophilus influenzae*.
[d] *Pseudomonas stutzeri*.
[e] *Thermus thermophilus* HB27.
[f] *Bacillus subtilis*, and
[g] *Streptococcus pneumoniae*.
[h] N.I., not identified.

pilin-like competence protein ComP in a pilus-deficient *Acinetobacter* sp. BD413 mutant, resulted in pilus structures. also supports the hypothesis of a pilus-analogue helical arrangement of some pseudopilins (Averhoff, unpublished).

Taken together, the conserved proteins of Tfp, type II protein export, and natural transformation can be assigned to five distinct groups: 1) pseudopilins, 2) prepilin-processing leader peptidases, 3) traffic NTPases, 4) polytopic inner membrane proteins, and 5) secretins.

Proteins belonging to groups 1–4 are found in the transformation machineries of both, Gram-positive bacteria and Gram-negative bacteria (Chen and Dubnau, 2003) and the most prominent Tfp-related competence proteins are listed in Table I. Many transformation systems require multiple prepilins, some of which are implicated in transformation and pili biogenesis such as the pilin PilE in *N. gonorrhoeae* (Fig. 1) (Fussenegger *et al.*, 1997), others exhibit single function in DNA translocators such as ComP in *N. gonorrhoeae* (Wolfgang *et al.*, 1999), and in other cases no Tfp are present but prepilin-like components are essential for transformation. The latter is exemplified by the four ComG-proteins ComGC, ComGD, ComGE, and ComGG in *B. subtilis* (Chen and Dubnau, 2003) (Fig. 2), CglC and CglD in *S. pneumoniae* (Pestova and Morrison, 1998), and ComP, ComB, ComE, and ComF in *Acinetobacter* sp. BD413 (Busch *et al.*, 1999; Herzberg *et al.*, 2000; Porstendörfer *et al.*, 1997).

Two further competence proteins cannot be assigned to these five groups but are conserved in *N. gonorrhoeae* and *Acinetobacter* sp. BD413: PilC and ComC in *Neisseria gonorrhoeae* and *Acinetobacter*, respectively, that are required for DNA uptake to a DNase-resistant state (Fussenegger *et al.*, 1997; Link *et al.*, 1998).

Type IV Pili and DNA Uptake

The central question arising from the similarities of competence proteins and proteins of Tfp biogenesis machineries is whether Tfp themselves are implicated in DNA uptake. Although many data adressing this question have been accumulated a general answer to this question cannot be given. On one side several transformable bacteria such as *B. subtilis*, *S. pneumoniae*, and *H. influenzae* do not exhibit Tfp structures, but components of the transformation machineries exhibit significant similarities to components of Tfp systems. Furthermore, naturally transformable strains such as *Acinetobacter* sp. BD413, exhibit pilus structures, but mutant studies revealed that piliation is not linked to transformation (Porstendörfer *et al.*, 1997). Thus, the expression of Tfp is not a prerequisite for DNA uptake by these bacteria.

On the other side, in many competent bacteria exhibiting Tfp the ability to take up DNA is functionally linked to Tfp, such as piliation and transformation correlate in *N. gonorrhoeae*, *D. nodosus*, and *P. stutzeri. N. gonorrhoeae* and *P. stutzeri* mutants defective in traffic-NTPases, found to be implicated in pilus retraction, exhibited a morphological intact piliation

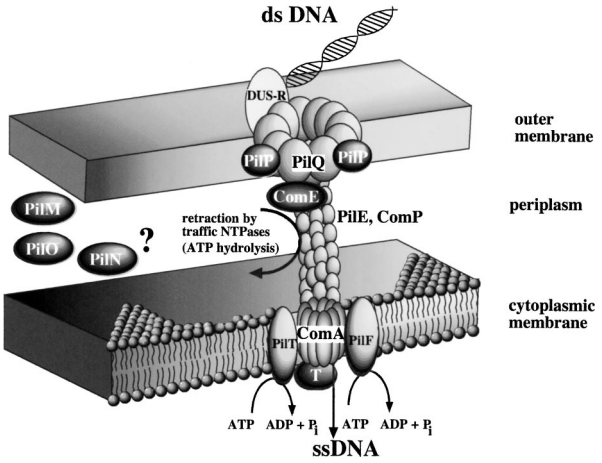

Fig. 1. Model for DNA uptake in *N. gonorrhoeae*. DNA is recognized by the DNA uptake sequence receptor (DUS-R) and subsequently transported through a secretin (PilQ) channel in the outer membrane. Retraction of the DNA translocator comprising of pilin-like proteins might pull the DNA through the outer membrane. After binding of the DNA to ComE the DNA is transported through the periplasmic space and peptidoglycan mediated by a pilin (PilE) and pilin-like (ComP) complex. Retraction of the DNA transporter or a ComE protein traversing the periplasmic space could mediate this step of DNA translocation. Whether Tfp itself are implicated in DNA uptake is still open. Traffic NTPases (PilF and PilT) could be involved in assembly and disassembly of the DNA translocator resulting in retraction of the DNA transporter. Subsequent translocation across the CM through a channel comprising of the membrane protein ComA is suggested to be catalyzed by an unknown translocase (T). dsDNA: double-stranded DNA; ssDNA: single-stranded DNA.

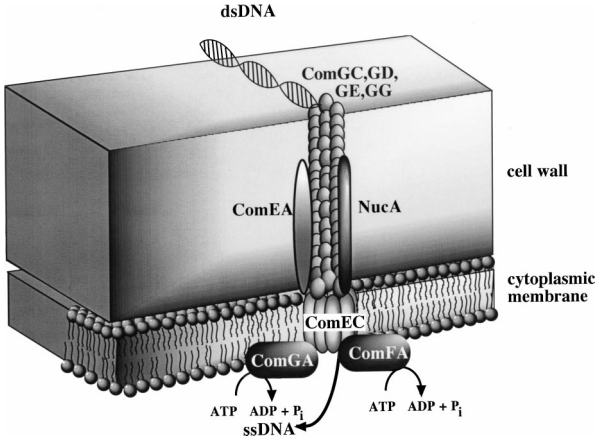

Fig. 2. Model for DNA uptake in *B. subtilis*. The pilin-like proteins, ComGC, ComGD, ComGE, and ComGG form a polymeric complex mediating the binding of exogenous DNA to the DNA binding protein ComEA. After binding to its receptor, ComEA, the DNA undergoes endonucleolytic cleavage by NucA. The DNA molecule is delivered to ComEC, which forms a channel traversing the cytoplasmic membrane. ComFA catalyzes the DNA transport through the cytoplamic membrane. ComGA which belongs to the traffic NTPases is also essential for natural transformation. dsDNA: double-stranded DNA; ssDNA: single-stranded DNA.

phenotype but were defective for DNA uptake. These findings suggest that DNA uptake requires retraction of the DNA translocator. Despite the many data on the link between transformation and piliation the role of Tfp in transformation remains ambiguous: It was demonstrated that only small amounts of the neisserial pilus subunit PilE rather than long pilus fibers were required for transformation of *N. gonorrhoeae* (Fussenegger *et al.*, 1997). Graupner *et al.* (2001) reported that in *P. stutzeri* the mutation in a *pilA1* gene resulted in transformation defective nonpiliated mutants. These mutants were complemented by a *pilAI* derivative encoding a prepilin-like protein with the six C-terminal amino acid residues being replaced by a His tag. These studies revealed that PilAI restored the transformation phenotype but not the piliation phenotype of the nontransformable, nonpiliated mutants. These findings suggest that pili structures are not required for transformation. Taken together the question whether Tfp are involved in transformation or whether proteins involved in Tfp assembly play a dual role in DNA translocators and Tfp is still open and perhaps has to be answered individually with respect to different transformation systems.

DNA Transport Through the Outer Membrane and the Periplasm

Secretins are integral outer membrane proteins which play a criticial role in Tfp biogenesis, protein secretion, phage extrusion, and natural transformation (Genin and Boucher, 1994). Some secretins have been found to interact with pilot lipoproteins, such as the gonococcal PilP, which probably facilitates correct insertion of the secretins into the outer membrane (OM) and confers secretin stability. Secretins, have been analyzed in a wide range of bacteria and these studies reported that secretins form multimeric ring structures in the outer membrane functioning as portal for guiding the pilus structures across the outer membrane. They are significantly conserved within a C-terminal domain, which is predicted to form a β-barrel structure in the OM and which is required for multimerization. The central N-terminal and parts are variable and the latter is thought to reside in the periplasm and function as the channel gate (for a review see Thanassi, 2002).

Recently, the first three-dimensional structure of a secretin, PilQ from *N. meningitides*, was presented (Collins *et al.*, 2001). PilQ was demonstrated to represent a dodecamer with a 12-fold rotational symmetry and a conical profile. The dominant feature of the ring structure is

a 10-nm deep cavity within the center of the complex. This cavitiy measures 6.5 nm in diameter at the top, and tapers to a closed point avoiding a continuosly open pore of the PilQ complex. These findings suggest that the PilQ ring structure undergoes conformational changes generating a plug preventing nonspecific release of periplasmic molecules and entry of harmful agents. Studies on PulD, the secretin of pullulanase secretion pathway in *Klebsiella oxytoca*, suggest that the ring could even interact with components located in the cytoplasmic membrane (Nouwen *et al.*, 2000).

The similarties of the secretins of DNA transformation systems to secretins of Tfp and protein export systems suggest that they might also form ring structures in the outer membrane with a central hole of 6–7 nm in diameter. The diameter is sufficient to accommodate incoming DNA or to guide a DNA translocator comprising of pilin-like proteins. The secretin channel could be a dynamic channel which opens by signals resulting from DNA binding to receptor proteins or interaction of with signaling proteins in the periplasm.

How do secretin-like and pilin-like proteins mediate DNA translocation in Gram-negative bacteria? A model of DNA uptake depicted for the Gram-negative bacterium, *N. gonorrhoeae*, is presented in Fig. 1. One possible scenario could be that the pilin PilE and the pseudopilin ComP of *N. gonorrhoeae* form retractable short pseudopili that span the periplasm. These structures might extend in length via polymerization and trigger opening of the OM channel and then could pull the DNA through the OM and the periplasm via depolymerization. This hypothesis is in favour with the finding that traffic NTPases implicated in pilus retraction in *P. stutzeri* and *N. gonorrhoeae* are also essential for DNA uptake. Also the data of Aas *et al.* (2002) indicating that DNA translocation in *N. gonorrhoeae* is physically linked to Tfp retraction are in favour with the hypothesis that pili retraction may facilitate DNA transfer from a primary receptor of the cell surface to the final DNA uptake machinery.

Chen and Dubnau (2003) speculate on an alternative mechanism of DNA transport through the periplasm of *N. gonorrhoeae* implicating the soluble DNA binding protein ComE. They suggest that after binding of DNA the neisserial ComE shuttles between the secretins and the DNA translocator in the cytoplasmic membrane through a putative pilin complex and subsequently delivers the DNA to the DNA translocator in the inner membrane (Fig. 1).

The ability of proteins and DNA to traverse the peptidoglycan is a prerequisite for DNA transport through the periplasm independent of the different models. Indeed there is evidence that DNA transport through the peptidoglycan is dependent on local changes in the peptidoglycan. In *Neisseria* two proteins, ComL and Tpc, were identified which facilitate DNA transport across the murein layer. The latter possibly acts as murein hydrolyase (Fussenegger *et al.*, 1997). Other cell wall remodeling proteins, such as penicillin binding proteins were identified in natural transformation in *H. influenzae* (Dougherthy and Smith, 1999).

DNA Transport Across the Cell Wall of Gram-Positive Bacteria

A working model for the DNA uptake through the cell wall of Gram-positive bacteria is based on the data from *B. subtilis* and *S. pneumoniae*. From the observation that the function of the ComG proteins (including the four prepilins) could be bypassed by cell wall-free systems (Provvedi and Dubnau, 1999) it is concluded that the *B. subtilis* ComG proteins (ComGC, GD, GE, GG) form a structure traversing the cell wall providing access of exogenous DNA to the binding domain of ComEA (Fig. 2). The assembly of this structure is suggested to require energy provided by the ATPase activity of the ComGA protein (Fig. 2). Due to the absence of an outer membrane in Gram-positive bacteria ComEA can act in interplay with ComG proteins directly as primary acceptor. After fragmentation of the DNA the binding protein ComEA is suggested to undergo conformational changes resulting in delivery of the DNA to the DNA transport complex in the cytoplasmic membrane (Chen and Dubnau, 2003).

A DNA Transporting Channel Across the Cytoplasmic Membrane

Several proteins unrelated to Tfp systems are involved in DNA transport across the cytoplasmic membrane. One of these competence proteins, designated ComEC or ComA, is conserved in different Gram-positive and Gram-negative bacteria (Chen and Dubnau, 2003). ComEC/ComA proteins are polytopic membrane proteins, which are suggested to form aqueous channels in the cytoplasmic membrane mediating the transport DNA. In Gram-positive bacteria, there is a second competence gene, *comEA*, downstream of *comEC*. ComEA contains a DNA binding helix-hairpin-helix motif at the C-terminus and has binding activity without any detectable sequence specificity (Chen and Gotschlich, 2001; Provvedi and Dubnau, 1999). ComEA orthologs have also been detected in Gram-negative bacteria. In contrast to the membrane localization of ComEA

in *B. subtilis*, the neisserial ortholog, ComE, is located in the periplasm. The involvement of ComEA orthologs in Gram-positive bacteria, Gram-negative proteobacteria (*N. gonorrhoeae*), and cyanobacteria (*Synechocystis*) indicates that DNA translocators mediating the DNA transport across the CM of phylogenetically distant bacteria exhibit structural and functional similaity.

Two additional conserved proteins, DprA and Smf, were found to be implicated in natural transformation of Gram-positive and Gram-negative bacteria (Dubnau, 1999). DprA (*D*NA *pr*ocessing) homologs are broadly distributed among different members of bacteria and even in archaea. They are found not only in different transformation systems, such as in type IV pili and type IV secretion related-transformation systems but are also present in nontransformable bacteria. The exact role of DprA in transformation is a still an open question. Currently two different potential functions are discussed, such as a function in DNA transport through the cytoplasmic membrane or a role in recombination. With respect to DNA transport DprA in *H. influenzae* has been found to be required for efficient chromosomal but not plasmid DNA transformation (Karudapuram *et al.*, 1995).

How Is the DNA Transported Across the Cytoplasmic Membrane?

DNA uptake requires energy and is driven either by ATP hydrolysis or proton motive force. Although the energetics of DNA uptake have been the subject of many studies the source of the required energy and the mechanism of energization of DNA transport is still not solved. ATP is required to drive DNA transport but it is not clear whether it is required to drive DNA transport directly or to guarantee an actice state of competence proteins.

In former studies with *B. subtilis* and *H. influenzae* (for a review see Palmen *et al.*, 1994) this question was addressed by studies on the correlation between components of the proton motive force and uptake of radiolabelled DNA. These studies indicated that in *H. influenzae* both components of the proton motive drive DNA uptake whereas in *B. subtilis* it was concluded that DNA was taken up in an electroneutral symport with protons. There are, on the other hand, genetic indications supporting the involvement of transport ATPases. In *B. subtilis* one competence protein, designated ComFA, is similar to the DEAD family of helicases and to an ATP-driven translocase in *E. coli* and therefore suggests that ComFA acts as a helicase/DNA translocator (Fig. 2). ComFA is probably fixed in the membrane adjacent to the DNA binding protein ComEA which is believed to deliver the DNA and uses the energy of ATP hydrolysis to transport DNA across the cytoplasmic membrane through a water-filled channel formed by the polytopic membrane protein ComEC (Fig. 2). A similar *comF*-gene cluster which is preceeded by a competence regulatory signal, was also detected in *S. pneumoniae*. So far ComFA orthologs have not been detected in transformation systems of Gram-negative bacteria, although it has to be noted that ComFA-like proteins are widely distributed in databases of Gram-negatives.

The Natural Transformation Machinery of the Extremely Thermophilic Bacterium *Thermus thermophilus* HB27: An Interplay of Conserved and Novel Proteins

Until recently nothing was known with respect to the components of the transformation machineries and the mechanism of DNA uptake in extremely thermophilic bacteria. The ability to take up free DNA *via* natural transformation in hot environments has so far only been described for representatives of the genus *Thermus*. Representatives of this Gram-negative genus grow at temperatures ranging from 50 to 82°C and are an important source of biotechnologically relevant enzymes. The physiology of natural transformation has been analyzed in *T. thermophilus* strain HB27, *T. thermophilus* HB8, *Thermus flavus* AT62, *Thermus caldophilus*, and *Thermus aquaticus* YT1. These studies revealed that the natural transformation process in these thermophilic bacteria is dependent on divalent cations and pH (Hidaka *et al.*, 1994; Koyama *et al.*, 1986).

A whole genome approach resulted in the detection of 31 potential competence genes in the genome of *T. thermophilus* HB27. No homologs to the type IV secretion system related transformation system in *H. pylori* have been detected. Gene disruptions and mutant studies revealed that 16 of these genes are competence genes: Three of the deduced proteins (ComEA, ComEC, DprA) are similar to proteins of DNA translocators in the cytoplasmic membranes, and nine are similiar Tfp proteins, such as four pilin-like proteins (PilA1, PilA2, PilA3, PilA4), a leader peptidase (PilD), a traffic-NTPase protein (PilF), an inner membrane protein (PilC), a PilM-homologue, and a secretin-like protein (PilQ). In addition to these conserved competence proteins four novel competence proteins, ComZ, PilN, PilO, and PilW, were identified. The significant similarities of several competence proteins to proteins of type IV pili systems again led to two fundamental questions: 1. Does *T. thermophilus* HB27

exhibit pilus structures on the surface? and if yes 2. are the *T. thermophilus* HB27 pili functionally linked to DNA uptake?

Thermus DNA Uptake Machinery and Pili: Two Functionally Related Systems

Electron microscopic studies revealed that *T. thermophilus* HB27 carries individual pilus structures with 6 nm in diameter and 1–3μm in length. These pilus structures were absent in mutants disrupted in the competence genes of the *pilMQ*-operon *PilD* encoding the prepilin-peptidase, *pilC*, and *pilA4* that encodes a pilin-like competence protein (Friedrich *et al.*, 2002, 2003). These findings suggest that the *Thermus* DNA uptake machinery and pili are functionally linked. Moreover these results together with the similarities of the *Thermus* competence proteins and proteins of Tfp biogensis strongly suggest that the *Thermus* pili structures represent Tfp. The question whether the *Thermus* pili themselves are implicated in DNA uptake or not cannot be answered yet. But since Tfp are thin structures of several μm in length without any long axial hole it is more likely that the long pilus structures themselves are not implicated in DNA uptake. Thus, it is more conceivable that either only the lower part of the pilus spanning the cell periphery or a distinct DNA translocator comprising of components playing a dual role in DNA uptake and Tfp mediate DNA uptake. Inactivation of the *Thermus* traffic ATPase PilF led to noncompetent mutants with morphologically intact pili. These findings suggest that the *Thermus* PilF is functionally similar to gonococcal PilT. Due to these findings it is tempting to speculate that DNA uptake in *Thermus* cells requires a dynamic DNA translocator comprising of pilin-like proteins and pulling the DNA through the cell wall and periplasm *via* retraction of the DNA translocator (Fig. 3).

Four prepilin-like proteins, PilA1-4, are involved in natural transformation of *Thermus*. Interestingly PilA4 is required for both, transformation and pilus biogenesis. Although these prepilin-like competence genes of *Thermus* are located in one locus they seem to exhibit distinct roles in natural transformation and piliation (Friedrich *et al.*, 2003). Pilin-like proteins with dual function in transformation and pili biogenesis, such as the *Thermus* PilA4, have been identified in several Gram-negative bacteria.

The identification of conserved competence proteins in *T. thermophilus* and the functional link to pili biogenesis underlines the structural similarities of the DNA uptake machineries of Gram-negative mesophilic and extremely thermophilic bacteria. On the other hand, the essential role of several novel competence genes in DNA transport

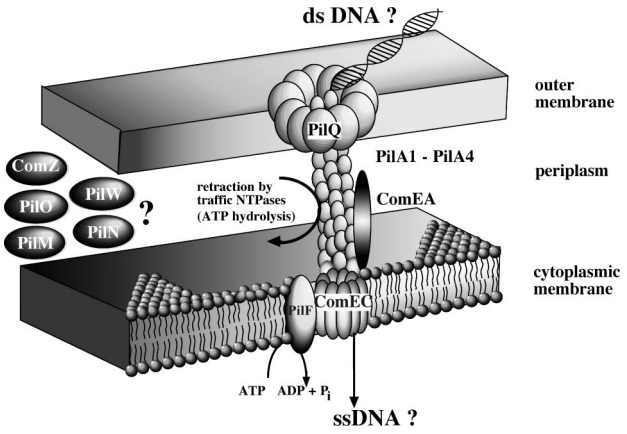

Fig. 3. Model for DNA uptake in *T. thermophilus* HB27. DNA is bound to a so far unknown DNA binding protein close to the potential ring-like structure of secretin-like subunits in the outermost layer, which comprises of S-layer and lipids and does not represent a classical outer membrane. After binding of the DNA to the ComEA protein the DNA is transported through the periplasmic space and peptidoglycan along or through a structure comprising of pilin-like proteins. Whether the Tfp structures themselves are implicated in DNA uptake is still open. The PilF traffic NTPase is essential for transformation and might be essential for pilus retraction. Subsequent translocation across the cytoplasmic membrane is performed through a ComEC comprising channel. dsDNA: double-stranded DNA; ssDNA: single-stranded DNA.

argues for unique features of the *T. thermophilus* HB27 DNA translocator.

Unique Features of the *Thermus* Transformation Machinery

The first set of nonconserved genes of the *Thermus* DNA translocator was detected within the competence gene cluster closely associated with the secretin gene. In Gram-negative mesophilic proteobacteria secretin genes are in a gene cluster of five genes, designated *pilM*, *pilN*, *pilO*, *pilP*, and *pilQ* in the case of the gonococcal gene cluster (Mattick, 2002). In contrast to the secretin, PilQ information on the function of PilMNOP is very limited (Table I, Fig. 1). In *N. gonorrhoeae* it was shown that the PilP repesents a lipoprotein potentially stabilizing PilQ. (Tonjum and Koomey, 1997).

The *Thermus pilQ* is also closely associated with four additional genes, but a *pilP* gene encoding a potentially PilQ stabilizing chaperon is substituted for a nonconserved competence gene *pilW* and no *pilP* homologue was detected in the *Thermus* genome. The absence of a lipoprorein potentially binding and stabilizing PilQ could be due to a higher stability of the DNA translocator in thermophilic bacteria. Such a potentially increased stability of the

T. thermophilus HB27 DNA translocator might faciliate purification of intact DNA translocators from *Thermus* cells. While sequence motifs of proteins encoded by *pilM* and *pilQ* are conserved, two potentially membrane-anchored proteins encoded by the *pilM*-flanking *pilN* and *pilO* are unique for the *Thermus* DNA uptake machinery.

The potential structural differences of the DNA transformation machinery in *Thermus* might be due to the unique structure of the cell envelope and the peptidoglycan of *T. thermophilus*. The murein from *Thermus* is lower in complexity compared to the murein of other Gram-negative bacteria, such as the composition of murein and peptide cross-bridges of *T. thermophilus* are typical for Gram-positive bacteria, whereas the murein content, degree of cross-bridges, and glycan chain length are more similar to those from Gram-negative bacteria (Quintela *et al.*, 1995). The outermost layer of the *Thermus* cell envelope is built by a S-layer, porins, and lipids covered by amorphous material (Caston *et al.*, 1993; Maier *et al.*, 2001). Taken together the distinct features of the *Thermus* cell envelope and the murein layer together with the extreme environment might have triggered the evolution of the *Thermus pilMNOWQ*-cluster.

The competence protein PilQ of *T. thermophilus* shows only limited similarities to secretins of mesophilic bacteria, restricted to the C-terminal 250 amino acid residues. The nonconserved N-terminal and central parts of PilQ and the missing *pilP* gene indicate that the *Thermus* PilQ may interact with other partners than the secretins of DNA translocators in mesophilic bacteria. This situtation is similar to that encountered in the nonproteobacterium *Synechocystis* sp. PC6803 which contains a *pilMQ* operon devoid of a PilP homolog (Yoshihara *et al.*, 2001).

Another competence gene unique for the *Thermus* DNA uptake machinery is *comZ* (Friedrich *et al.*, 2003). The *comZ* is located within a locus comprising of the four prepilin-like competence genes *pilA*, *pilA2*, *pilA3*, and *pilA4*. Although so far nothing is known with respect to the function of ComZ the hydrophobic N-terminal domain suggests a membrane association (Friedrich *et al.*, 2003). The question whether the presence of unique potentially membrane-associated proteins in the *Thermus* DNA translocator is due to the unique *Thermus* cell periphery or whether these proteins have been evolved to adapt to the extreme environment remains to be resolved in the future.

Model of DNA Uptake in *T. thermophilus* HB27

Based on results of the *Thermus* transformation machinery the first model of a DNA translocator in *T. thermophilus* HB27 is presented (Fig. 3). DNA binds to DNA binding proteins on the cell surface or to proteins associated with the DNA translocator structure comprising of pilin-like proteins. Subsequently the DNA is either transported along or through a DNA transformation shaft made up by the pilin PilA4 and some minor pilin-like proteins such as PilA1, PilA2, and PilA3. A ring-like structure comprising secretin-like PilQ-proteins is suggested to be required for guiding the DNA translocator through the cell envelope. The similarities of the C-terminal part of PilQ to members of the secretin family that are essential for secretin multimerization (Chen *et al.*, 1996) underline the suggestion that *Thermus* PilQ proteins form a homopolymeric ring structure. Retraction of the DNA translocator depends on the function of PilF, the putative traffic NTPase that may power DNA transport through the ring structures comprising of secretin-like proteins. In periplasmic space the DNA might bind to the putative binding protein ComEA which is probably anchored in the inner membrane and delivers the DNA to the inner membrane DNA transport complex. Finally the DNA is transported though an inner membrane channel generated by ComEC, a polytopic inner membrane protein.

CONCLUSIONS

The similarities of proteins of DNA translocators mediating transport of free DNA to Tfp biogenesis and type II protein secretion systems strongly suggest that these systems have evolved from a common ancestor, but differentiated into distinct structures with different functions. The common property of these different structures is an interaction with macromolecular components i. e. lipids during type IV pili-mediated adhesion, proteins during protein secretion, and/or DNA during transformation. The latter two systems have to aquire a structural basis for substrate selectivity for transport of proteins or DNA along or through the interior of the transporter. This specificity could be brought about by specific interactions of pilus-like structures with the macromolecules or by charge recognition. Further research is required to understand the structure of DNA translocators and the mechanism of uptake of free DNA. The study of DNA uptake systems from thermophiles will certainly facilitate the unravelling of the structure-function relationship of these fascinating systems.

The finding that *T. thermophilus* exhibits Tfp pili which are linked to transformation suggests that the connection “pili and transformation” is a widely distributed principle, not restricted to mesophilic bacteria. The implications of nonconserved proteins however, highlights the unique features of the *Thermus* transformation machinery. The question whether these features were triggered by the

extreme environment or are due to the phylogenetic position of *Thermus* and/or the distinct features of the cell envelope and the murein layer, is open and further research will focus on this important question.

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