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Role of pili and the phase-variable PilC protein in natural competence for transformation of *Neisseria gonorrhoeae*

(horizontal genetic exchange/type 4 pili/DNA uptake/membrane transport/recombination)

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ABSTRACT **The Gram-negative bacterial pathogen *Neisseria gonorrhoeae* is naturally competent for transformation with species-related DNA. We show here that two phase-variable pilus-associated proteins, the major pilus subunit (pilin, or PilE) and PilC, a factor known to function in the assembly and adherence of gonococcal pili, are essential for transformation competence. The PilE and PilC proteins are necessary for the conversion of linearized plasmid DNA carrying the *Neisseria*-specific DNA uptake signal into a DNase-resistant form. The biogenesis of typical pilus fibers is neither essential nor sufficient for this process. DNA uptake deficiency of defined piliated *pilC1,2* double mutants can be complemented by expression of a cloned *pilC2* gene in trans. The PilC defect can also be restored by the addition of purified PilC protein, or better, pili containing PilC protein, to the mutant gonococci. Our data suggest that the two phase-variable Pil proteins act on the bacterial cell surface and cooperate in DNA recognition and/or outer membrane translocation.**

Neisseria gonorrhoeae, a strictly human-specific Gram-negative bacterial pathogen, belongs to a group of microorganisms that are naturally competent for DNA transformation (1). Natural transformation competence is considered to play a role in the horizontal exchange of genetic information between species, aside from transduction and conjugation (2, 3). DNA transformation appears to be particularly relevant in the case of *N. gonorrhoeae*, which, based on current knowledge, is not infected by any transducing phage (or phage in general) and is devoid of genetic elements capable of mobilizing chromosomal determinants. Thus, transformation is the mechanism that most probably accounts for the evolutionary signs of horizontal exchange in *N. gonorrhoeae* and related species (3–9).

Transforming DNA is linearized during the uptake by *N. gonorrhoeae* (10). Rescue of linear plasmid DNA or chromosomal segments requires both a functional RecA protein and sufficient homology in the resident DNA pool; transformation is therefore limited to species-related DNA. Another restricting factor for species-related DNA is a specific nucleotide sequence required for the uptake of DNA (11, 12); such DNA uptake signals often represent part of the transcriptional terminators of neisserial genes (4, 13, 14). Other determinants required for DNA uptake (*dud*) and transformation competence (*ntr*) have been identified by chemical mutagenesis (15). One competence gene (*comA*) has recently been cloned in *Escherichia coli* (16).

Pioneering work by Sparling and coworkers (1, 17) suggests a central role of the gonococcal type 4 pili in transformation. Recent observations indicate that the synthesis of pilin (the major pilus subunit, or PilE), rather than long pilus fibers, is essential for gonococcal competence (8, 18). However, the biogenesis of gonococcal pili requires not only PilE but also additional accessory determinants. The recently identified PilC protein has been proposed to constitute such a biogenesis factor (19); however, assembly of morphologically intact pili can occur in the absence of PilC (20). In *N. gonorrhoeae* MS11, two variant forms of PilC protein exist, which are encoded by separate genes subjected to phase variation (19, 21). Here we demonstrate that the PilC proteins not only function in pilus biogenesis (19) and epithelial cell adherence (20, 22) but are also essential for transformation competence in *N. gonorrhoeae.*

MATERIALS AND METHODS

Construction of Strains and Plasmids. All gonococcal strains used in this study are derived from *N. gonorrhoeae* MS11 (ref. 23; see Table 1). Plasmid pHEMK40 carrying the inducible $pilE_{F3}$ gene (20) and pHTR93 encoding PilC (see Fig. 1) are derivatives of the conjugative *N. gonorrhoeae* plasmid ptetM25.2 constructed by gene replacement using the Hermes shuttle system (13, 20). Gene replacements in the gonococcal genome or ptetM25.2 were carried out via transformation selected for in the presence of erythromycin (7 μg/ml for *ermC*) or chloramphenicol (Cm; 6, 12, or 20 μg/ml for cat_{LOW}, standard, or secondary cat_{GC} mutations, respectively). The conjugation of ptetM25.2 derivatives has been described (20). *E. coli* plasmid pES3 is plasmid pIP100 (4) with the *ermC* determinant inserted into the *Bgl* II site of the *iga* gene; pDF5 is plasmid pIP1 carrying the cat_{GC} gene in the *Bgl* II site of *iga* (4). pPH4 is plasmid pIP1 with *ermC* inserted into the *Hin*dIII/*Bgl* II site. pBA is a truncated version of vector pBR322 (23), and pRH60 is pBA carrying a 45-bp fragment flanked by *Bgl* II sites (AGATCTAAACAAAAAGCCGTCTGAAATG-GTTTCAGACGGCATCCTTTGTTCAGATCT) containing a gonococcal DNA uptake signal (11, 12). The ATG start codon of the *pilE* gene (23) was site-directedly mutagenized by PCR using pEMK40 ($pilE_{F3}$) as template and mutant oligonucleotides DF28 and DF29, following a previously published procedure (16). Two PCR reactions were carried out with primer pairs CG10/DF29 (upstream) and DF28/RH84 (downstream); the resulting PCR fragments were combined and further amplified using the distal primers DF4 and RH84. Cloning of the resulting fragment into the *Sal* I/*Hin*dIII sites of Hermes-11 generated pTR116, which was recombined with ptetM25.2 in strain N219 (20). The resulting plasmid pHTR93 was mobilized into N431, a nonpilated (P^-) Δ*pilE1,2* rifampicin-resistant (Rif^R) derivative of MS11 (20), to yield N516. Mutagenesis of the *pilE* translational start codon was confirmed by PCR sequencing (20) of the mutated *pilE* in

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Abbreviations: P^+, piliated; P^-, nonpiliated; Cm, chloramphenicol; Cm^R, Cm resistant; Rif, rifampicin; Rif^R, Rif resistant.

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pHTR93. Primers DF28 (AGGAGTAATTTTATCGATAC-CCTTC), DF29 (GAAGGGTATCGATAAAATTACTC-CT), DF4 and RH84 (20), and CG10 (13) have been described.

Other Techniques. Transformation frequencies were determined as described (8, 16) using 1 μg of chromosomal DNA prepared from N431 (Rif^R) or 1 μg of pDF5 plasmid DNA (Cm resistant; Cm^R) per 5×10^8 bacteria in 1 ml of medium; transformants were selected on GC agar plates containing Rif at 50 μg/ml or Cm at 10 μg/ml, respectively. Purified pili and $PilC_{His6}$ protein were obtained as described (19, 22). For immunoblotting, anti-PilE and anti-PilC sera were used and labeled with secondary antibodies (20).

RESULTS

Direct Function of PilC in Transformation. Deletion mutants of strain MS11 producing either PilC1 (N473), PilC2 (N472), or no PilC protein (N474, N477) were analyzed for transformation competence (Table 1). The $PilC^+$ strains N472 and N473 were both piliated (P^+), whereas the $PilC^-$ mutant varied at low frequencies ($\approx 10^{-6}$) between the P^- and P^+ phenotypes despite the synthesis of identical PilE proteins in both variants. Both phenotypes (expressed in N474 and N477, respectively) were separately assessed. In contrast to the $PilC^+$ *pilC* single-gene mutants and the wild-type strains, both P^+ and P^- *pilC1,2* double mutants were incompetent for transformation to Rif^R with chromosomal DNA from a Rif^R donor strain (Table 1). Since the competence defect of the $PilC^- P^+$ mutant N474 cannot be ascribed to a lack of pili formation, this indicates a direct role of PilC in gonococcal DNA transformation.

The transformation-defective mutants were complemented via conjugation in trans using the Hermes shuttle system based on the conjugative *Neisseria* plasmid ptetM25.2 (13, 20). Specifically, the nontransformable $PilC^- P^+$ mutant N474 was complemented with an expressed *pilC2* gene via the ptetM25.2 derivative pHTR93 (Fig. 1*B*). The resulting strain N475, which produced identical PilE, fully regained transformation competence (Table 1). Mobilization of the plain ptetM25.2 plasmid into N474 did not restore competence (data not shown). Complementation of the $PilC^- P^-$ strain N477 with pHTR93 not only restored transformation competence but also led to a P^+ phenotype as expected (strain N555; Table 1).

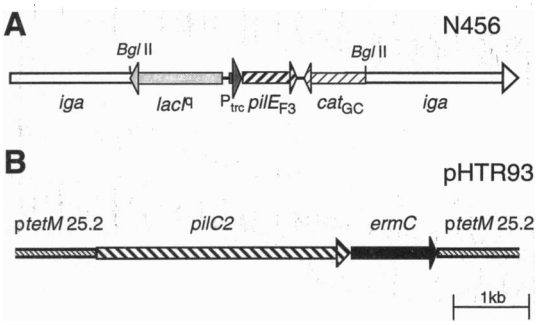

FIG. 1. Constructs used for the mutagenesis or genetic complementation of *N. gonorrhoeae* strains. (*A*) Structure of the inducible *pilE* cassette inserted into the *Bgl* II site of the chromosomal *iga* gene in strain N456. A similar inducible *pilE* cassette, but with the *ermC* marker, is contained in pHEMK40 described earlier (20). (*B*) For genetic complementation, the *pilC2* gene was inserted into the *Bam*HI–*Hin*dIII sites of the Hermes-6a shuttle vector to generate plasmid pHTR93 via gene replacement in ptetM25.2.

The single-gene mutants N472 (*pilC1*) and N473 (*pilC2*) differed with regard to the levels of transformation competence (Table 1). These mutants not only produced different PilC proteins but also structurally distinct PilE proteins, as judged from the migration in SDS gels (data not shown). Although the mutants were derived from the same parent, the changes in PilE probably occurred concomitantly with the introduction of the *pilC* mutations (21). It was, therefore, necessary to explore which of the two variable proteins was responsible for the difference between N472 and N473. For this purpose, spontaneous *pilE* deletion mutants of N472 and N473 were isolated and complemented in trans with the defined $pilE_{F3}$ gene carried on plasmid pHEMK40 (20). The resulting strains N454 and N452, which produced identical PilE

Table 1. Transformation competence of *N. gonorrhoeae* MS11 derivatives

Strain	Relevant genotype	Relevant phenotype* PilE	PilC	Piliation	Rif^R transformants/CFU	Source or ref(s).
N200	Variant (A)	$PilE_A$	PilC	P^+	5.3×10^{-4}	20
N238	Variant (F3)	$PilE_{F3}$	PilC	P^+	5.4×10^{-4}	9, 20
N263	Δ*pilE* (B2)	—	PilC	P^-	$<2 \times 10^{-8}$	20
N286	N431, pHEMK40	$PilE_{F3}{}^i$	PilC	P^i	†	This work
N336	Variant (E1)	$PilE_{E1}$	PilC	P^+	6.5×10^{-4}	9, 20
N452	N473, Δ*pilE*, pHEMK40	$PilE_{F3}{}^i$	PilC1	P^i	9.4×10^{-4}	This work
N454	N472, Δ*pilE*, pHEMK40	$PilE_{F3}{}^i$	PilC2	P^i	5.3×10^{-4}	This work
N456	Δ*pilE, iga*::$pilE_{F3}{}^i$	$PilE_{F3}{}^i$	PilC	P^i	†	This work
N472	*pilC1*::cat_{GC}	PilE	PilC2	P^+	1.3×10^{-3}	This work
N473	*pilC2*::cat_{GC}	PilE	PilC1	P^+	3.6×10^{-5}	This work
N474	*pilC1*::cat_{LOW}, *pilC2*::cat_{GC}	$PilE_{N474}$	—	P^+	$<2 \times 10^{-8}$	This work
N475	N474, pHTR93	$PilE_{N474}$	PilC2	P^+	9.0×10^{-4}	This work
N477	*pilC1*::cat_{LOW}, *pilC2*::cat_{GC}	PilE	—	P^-	$<2 \times 10^{-8}$	This work
N516	N431, pHTR116	—	PilC	P^-	$<2 \times 10^{-8}$	This work
N555	N477, pHTR93	PilE	PilC2	P^+	8.4×10^{-4}	This work

N336 and N238 are spontaneous variants of PilE derived by *in vitro* cultivation from strain MS11-A (i.e., N200); N263 is a PilE null mutant generated by spontaneous deletion of *pilE1,2*. The *pilC* single and double mutants (N472, N473, N474, N477) were constructed by allelic replacement of the central *pilC* gene regions by *cat* markers and, if necessary, subsequent selection for the P^+ phenotype. N456 was derived from N200 by insertion of the *iga*::$pilE_{F3}{}^i$ cassette (see Fig. 1*A*) and subsequently selected for the loss of the chromosomal *pilE* loci. The conjugative *N. gonorrhoeae* plasmids pHTR93 (PilC2), pHEMK40 ($PilE_{F3}$), and pHTR116 ($PilE_{F3.RNA}$) were constructed via the shuttle vector Hermes-6a (ref. 20; see Fig. 1*B*) and used for the complementation of transformation-incompetent mutants. Plasmid pHTR116 harbors an inducible $pilE_{F3}$ ($pilE_{F3}{}^i$) gene with a mutated ATG start codon; thus mRNA but no PilE protein is produced upon induction with isopropyl β-D-thiogalactoside. CFU, colony-forming units.

*P^i, Isopropyl β-D-thiogalactoside-inducible pili formation; —, respective protein not synthesized.

†See Fig. 3.

and different PilC proteins, showed similar transformation frequencies (Table 1), suggesting that it is not the two different PilC proteins that influence the transformation efficiency but rather the variant forms of PilE, perhaps in combination with PilC.

DNA Uptake Deficiency of PilE and PilC Mutants. A DNA uptake assay was developed using a pair of plasmids, only one of which (pRH60) carried an *N. gonorrhoeae* DNA uptake sequence (11, 12). This assay, which determined the conversion of plasmid DNA into a DNase-resistant form, was strictly dependent on this signal as evidenced by the lack of competence of the wild-type variants N336 and N200 for the control plasmid pBA versus pRH60 (Fig. 2). DNase-resistant pRH60 was not only recovered from the wild type but also from the *pilC1* and *pilC2* single-gene mutants N472 and N473, whereas the P^+ $PilC^-$ (*pilC1,2*) double-mutant N474 and the *pilE* deletion mutant N263 were absolutely DNA uptake-deficient (Fig. 2). The deficiencies could be restored by complementation with the *pilC2* and *pilE* genes, respectively (Figs. 2 and 3).

Requirement of PilE Rather than *pilE* Transcript. In contrast to the absolute defect of PilE synthesis based on the deletion of the *pilE* gene (Table 1), nonpiliated S- and L-pilin variants could still be efficiently transformed (8). To exclude a possible indirect effect of *pilE* gene expression (e.g., a regulatory effect of the *pilE* transcript on transformation competence), the *pilE* gene was mutated such that transcription was allowed but no PilE protein was produced. This was accomplished by site-specific mutagenesis of the translational start codon from ATG to ATC (see *Materials and Methods*). The resulting mutant N516, which still produces pilin mRNA but no PilE protein, exhibits an absolute transformation defect like the *pilE* deletion mutant (Table 1).

Small Amounts of PilE Suffice for Uptake and Competence. To assess the amount of PilE needed for DNA uptake and transformation, an inducible $pilE_{F3}$ gene was either incorporated into the chromosome of strain N456 (Fig. 1*A*) or inserted into the low-copy-number plasmid pHEMK40 (20). As compared to the wild-type level, PilE synthesis was slightly lower

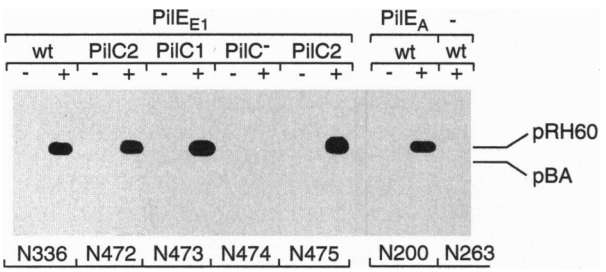

FIG. 2. DNA uptake by PilC and PilE mutants as determined by Southern blot analysis. The uptake of either pBA (−) or pRH60 containing the *N. gonorrhoeae* DNA uptake sequence (+) was analyzed; expected positions in the blot for the respective plasmids are indicated to the right. Relevant phenotypes of the strains tested (see Table 1) are shown for each lane. The DNA uptake assay was performed using the same amount of linearized pBA (*Sal* I) or pRH60 (*Sal* I), except that gonococci were treated with 100 μg of DNase I per ml 10 min after the addition of the DNA. After further incubation on ice for 15 min, 100 mM EDTA was added to the suspension followed by 5 min on ice. Cells were washed by centrifugation using 1 ml of proteose peptone medium containing 380 mM NaCl, resuspended in 500 μl of 50 mM glucose/25 mM Tris·HCl pH 8.6/10 mM EDTA, and lysed by subsequent treatments with lysozyme, sarcosyl, and proteinase K. The DNA was phenol extracted and prepared for Southern blot analysis using [^{32}P]-dCTP-labeled pBA as probe. Gonococci were incubated with the linearized form of either plasmid for 10 min and subsequently treated with DNase. DNA was then extracted and analyzed by Southern blotting using labeled pBA as a probe to determine whether the transforming DNA escaped DNase digestion. If DNase was omitted from the assay, both plasmids pBA and pRH60 gave strong signals (not shown). wt, wild type.

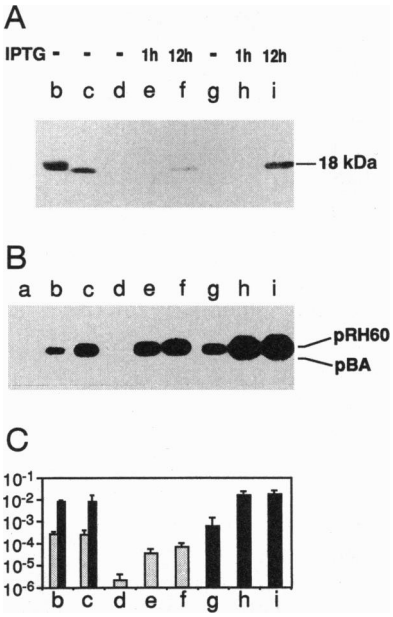

FIG. 3. DNA uptake and transformation competence in response to the production of PilE. (*A–C*) Three different analyses of the same experiment. (*A*) The relative amount of PilE produced by various strains was determined by immunoblotting. (*B*) A DNA uptake assay was performed with the same strains using linearized pBA (lane a, control) or pRH60 (lanes b–i). (*C*) Transformation competence was determined by the number of Rif^R (shaded bars) or Cm^R (solid bars) transformants per colony-forming unit. Use of two different markers was necessary due to the intrinsic Cm and Rif resistances of strains N456 and N286, respectively. Lanes a and b, strain N200 ($PilE_A$); lane c, N238 ($PilE_{F3}$); lanes d–f, N456 ($PilE_{F3}$); lanes g–i, N286 ($PilE_{F3}$). Gonococci were not induced (lanes a–d and g) or induced with 100 μM isopropyl β-D-thiogalactoside (IPTG) 1 h (lanes e and h) or 12 h (lanes f and i) prior to the analyses, as indicated.

or similar in the induced states for the chromosomal and the plasmid constructs, respectively; the difference was consistent with the copy number of the inducible *pilE* gene (Fig. 3*A*). In the repressed state or 1 h after induction of *pilE*, only very small quantities of PilE were produced by both strains (Fig. 3*A*). However, immediately after induction, a strong DNA uptake signal was observed similar to that of the wild-type strain (Fig. 3*B*). The transformation efficiencies in this experiment were determined with two different DNAs due to the intrinsic antibiotic resistances of the strains tested (Fig. 3*C*). The relative transformation efficiency was about 100-fold higher with plasmid pDF5 (Cm^R) compared to chromosomal Rif^R DNA. In general, transformation was very efficient despite the low level of PilE synthesis in some of the assays (Fig. 3*A*, lanes e, g, and h). Even in the repressed state, the transformation efficiency of N456 was still at 1% of the wild-type level. Therefore, small amounts of PilE are sufficient for transformation competence, consistent with our previous observation that S-pilin variants are fully competent (8); they produce only small amounts of intact PilE (21, 24). However, high amounts of PilE do not necessarily secure maximum transformation competence or DNA uptake, and DNA uptake does not strictly correlate with transformation competence (Fig. 3), suggesting a complex function of PilE in transformation competence. Furthermore it is evident that PilE variation influences transformation (ref. 8; Table 1) and to some extent also the DNA uptake efficiency (see Fig. 3, lanes b and c).

Biochemical Restoration of the *pilC* Defect. Recent studies suggest a physical association of PilC protein not only with the gonococcal pilus (19, 22) but also with the outer membrane where it is accessible by antibodies (unpublished data). We, therefore, addressed the question whether a PilC-dependent

transformation defect can be restored by supplementing intact gonococcal cells with purified PilC protein. A significant increase (10-fold) in transformation competence occurred after pretreatment of strain N556 (P^+, $PilC^-$) with purified PilC protein that was free of PilE (Table 2). This effect was enhanced with semipure PilC containing minor protein contaminants distinct from PilE. However, pretreatment of strain N556 with purified pili from P^+ $PilC^+$ gonococci (N200), but not with pili from a P^+ $PilC^-$ source (N556), led to a dramatic increase of competence of 3–4 orders of magnitude to near wild-type level (Table 2). Interestingly, competence could not be restored in the isogenic P^- $PilE^+$ $PilC^-$ variant N601 of strain N556 using the same pilus preparation (Table 2).

DISCUSSION

We demonstrate here an essential function of the phase-variable PilC proteins in the natural competence for transformation of *N. gonorrhoeae.* PilC proteins are produced in small quantities, copurify with gonococcal pili (19), are detectable by immunoelectron microscopy at the tip of the pili (22), and are also present in the outer membrane (unpublished data). The two variant PilC proteins produced by *N. gonorrhoeae* have been implicated in pilus biogenesis (19, 21); however, the assembly of morphologically intact pili is possible in the absence of PilC (20). In this case, another phase-variable pilus-associated factor appears to replace the assembly function of PilC (T.R., H.-J. Boxberger, and T.F.M., unpublished results). PilC-depleted pili are still capable of agglutinating human erythrocytes but have lost the ability to confer binding to epithelial cells (20). Here we show that transformation competence of PilC-deficient gonococci can be restored not only by genetic complementation with a functional *pilC* gene but also by treatment of PilC-deficient gonococci with PilC protein-enriched preparations (Fig. 4).

DNA uptake requires only small amounts of PilE, rather than long pilus fibers: both, gonococcal S variants, which produce a truncated soluble pilin, and are—if at all—poorly piliated, and L variants, which produce an over-length pilin and are devoid of any detectable pili, are still transformation competent (8). Furthermore, an assembly-incompetent, truncated pilin protein unable to form pili still supported transformation (18), whereas Δ*pilE* mutants were incompetent (8) or only very inefficiently transformed by comparison with wild-type gonococci (14). That the lack of a *pilE* transcript accounts for the defect could be ruled out.

Table 2. Complementation of transformation-deficient mutants with purified pili and PilC

Strain	Relevant phenotype	Addition of purified fractions	Transformation frequency
N200	P^+ $PilC^+$	—	4.9×10^{-4}
N600	P^+ $PilC_{His6}{}^+$	—	5.1×10^{-4}
N556	P^+ $PilC^-$	—	$<5.0 \times 10^{-9}$
N556	P^+ $PilC^-$	$Pili_{N200}$	1.1×10^{-5}
N556	P^+ $PilC^-$	$Pili_{N556}$	$<6.6 \times 10^{-9}$
N601	P^- $PilC^-$	$Pili_{N200}$	4.0×10^{-9}
N556	P^+ $PilC^-$	$PilC_{His6}$*	8.9×10^{-7}
N556	P^+ $PilC^-$	$PilC_{His6}$†	2.0×10^{-8}

N556 is an isogenic PilE variant of N474 (*pilC1,2*, P^+), N601 is a P^- $PilE^+$ variant of N556, which produces pilin of the same size as N556, but for an unknown reason does not assemble pili. Gonococci were suspended in 1 ml of proteose peptone medium and grown at 37°C for 1 h. Then either 10 mg of bovine serum albumin, 5 μg of purified pili, or $PilC_{H6}$ and 0.5 μg of plasmid pPH4 were added. The samples were grown for additional 4 h at 37°C before dilutions were plated on normal GC plates and antibiotic selection plates to determine the transformation frequency.

*Partially purified $PilC_{His6}$ protein containing additional proteins of 20, 36, and 40 kDa was added (22).

†Highly purified $PilC_{His6}$ protein was added (22).

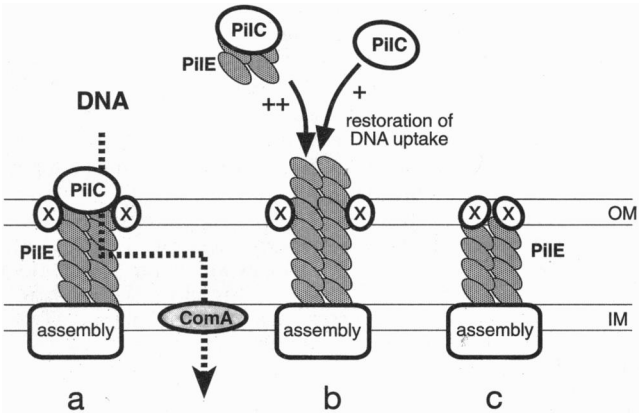

FIG. 4. Hypothetical model of DNA uptake in *N. gonorrhoeae.* Polymerization of the gonococcal type 4 pili is probably catalyzed by an inner membrane (IM)-associated assembly complex (25). DNA uptake requires PilC in combination with small amounts of PilE and possibly additional factors (X) associated with the outer membrane (diagram a). In the absence of PilC, gonococci vary between two phases, which either permit (diagram b) or prevent (diagram c) pilus formation (T.R., H.-J. Boxberger, and T.F.M., unpublished results). Only in P^+ variants (diagram b) can a PilC deficiency be functionally restored by supplementation of the bacteria with purified PilC (+), or better, PilC-containing pili (++). Translocation of DNA across the IM probably requires ComA, which does not affect pilus biogenesis (16). OM, outer membrane.

The deficiencies of PilC- and PilE-negative mutants in DNA uptake suggest that these proteins act on the bacterial cell surface. This notion is supported by the biochemical restoration of the PilC deficiency by the addition of PilC-containing protein fractions prior to gonococcal transformation. Uptake restoration is considerably more efficient if PilC-containing pili or partially purified PilC protein rather than pure PilC protein is provided. This indicates an interaction of PilC with PilE and other unknown factors enriched in crude PilC preparations. However, PilC preparations only restored the competence of a P^+ $PilC^-$ variant but not of an isogenic P^- $PilC^-$ variant. Therefore uptake restoration depends on the direct interaction of the exogenously added PilC protein complex with a surface-accessible pilus assembly complex (Fig. 4). It is conceivable that DNA uptake requires additional factors besides PilC and PilE, such as the gene product of *dud-1*, a gene recently identified by chemical mutagenesis (15), and factors involved in the pilus biogenesis.

Recent evidence provided by a variety of model systems suggests in more general terms the participation of type 4 pilus-related protein factors in the macromolecular transport across the membranes of both Gram-negative and Gram-positive bacteria (25). Besides the type 4 pili, these systems include the machineries for the secretion of various exoproteins, such as pullulanase of *Klebsiella oxytoxa* (26) and *Pseudomonas* exoproteins (27), and for the DNA uptake in *Bacillus subtilis* (28). Crucial factors for the *B. subtilis* competence are ComG Orf1–Orf7, some of which share homology with the neisserial pilin. An apparent difference, however, is that in *B. subtilis* the pilin-like ComG products probably serve for the uptake across the peptidoglycan layer and/or the cytoplasmic membrane of the Gram-positive organisms, whereas the neisserial pilin supposedly acts in the outer membrane. How this structural similarity can be fit into a common scheme explaining macromolecular transport across bacterial cell envelopes remains an open question.

Natural transformation competence in the genus *Neisseria* is considered to be a crucial mechanism for horizontal genetic exchange and the evolution of these microorganisms in the

absence of any known phage or chromosomal mobilization system (3, 6). However, additional roles for genetic competence have been hypothesized, such as a nutrition and a sensory function for the initiation of an SOS-like response (29). We anticipate that the future assessment of genetic competence will generate insights into the evolutionary and physiological principles associated with naturally competent microorganisms, many of which are pathogens.

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