Sequential action of factors involved in natural competence for transformation of *Neisseria gonorrhoeae*

Dirk Facius, Martin Fussenegger, Thomas F. Meyer *

Max-Planck-Institut für Biologie, Abteilung Infektionsbiologie, Spemannstr. 34, D-72076 Tübingen, Germany

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Abstract

We previously identified and genetically characterized several factors essential for the natural competence of transformation in *Neisseria gonorrhoeae*. Here we analyse the sequential action of these factors and dissect the overall transformation process into three distinct steps, (i) the sequence-specific uptake of transforming DNA into a DNase-resistant state, (ii) the transfer of DNA to the cytosol and (iii) the processing and recombination of the incoming with the resident DNA. While two pilus-associated factors, PilE and PilC, were previously implicated in the early DNA uptake event, we show here that three competence factors unrelated to pilus biogenesis, ComA, ComL and Tpc, are not essential for DNA uptake and rather act in a subsequent step. The respective mutants, however, lack the characteristic nucleolytic processing observed with the incoming DNA in both wild-type and non-transformable RecA-deficient *N. gonorrhoeae*, indicating that they are blocked in the processing and/or the delivery of DNA to the cytoplasm. A hypothetical model proposing a sequential action of the known gonococcal competence factors is presented.

Keywords: DNA uptake; Transformation competence; Gene transfer; Horizontal genetic exchange; Recombination; *Neisseria gonorrhoeae*

1. Introduction

*Neisseria gonorrhoeae* is a Gram-negative bacterial pathogen infecting mucosal surfaces of only human beings. Like many bacterial pathogens, *N. gonorrhoeae* is naturally competent for DNA transformation [1]. DNA transformation appears to be particularly relevant in the case of *N. gonorrhoeae* since it is not infected by any phage (including transducing phages) and is devoid of known genetic elements capable of mobilizing chromosomal determinants. Thus, natural transformation accounts for the rapid exchange and adaptation of virulence factors [2] and antibiotic resistances [3] by generating mosaic genes [3–5], for the exhaustive linkage equilibrium of genetic markers within the *N. gonorrhoeae* population [6], and contributes as a mechanism of pilin variation to the remarkable structural and antigenic diversity of pili [7–9].

The early description of transformation-deficient mutants by Biswas et al. [10] led to a dissection of the transformation process into two steps, a DNA uptake step whereby the transforming DNA is converted to a DNase-resistant state, and any subsequent events. While most of these potentially interesting mutants still await genetic characterization, one mutation affecting DNA uptake, *dud-1*, has recently
been characterized as an allele of the N. gonorrhoeae pilT gene [11] which encodes a cytoplasmic protein involved in type 4 pilus biogenesis, pilus retraction and twitching motility [12]. We recently reported on the role of two pilus-associated proteins in DNA uptake, i.e. the phase-variable PilC and PilE pilus subunit components [13]. In this study DNA uptake was strictly dependent on the presence of a DNA uptake signal in the transforming DNA [14].

We also characterized several different N. gonorrhoeae transformation determinants exhibiting no apparent function in pilus formation. The first determinant of this type, comA, is located adjacent to the iga locus and is common to almost all pathogenic and commensal Neisseria species [15]. Downstream of comA we detected another determinant required for full transformation competence, i.e. the comL gene which encodes an essential peptidoglycan-linked lipoprotein [16]. Furthermore, a second lipoprotein involved in transformation competence (Tpc) was identified by mini-transposon shuttle mutagenesis [17]. Tpc seems to be only loosely associated with the peptidoglycan and functions in the cell separation of dividing diplococci, and is also required for the expression of an invasive phenotype allowing N. gonorrhoeae to enter human epithelial cells. Finally, the RecA protein has been recognized as an essential transformation factor [18]. Any transforming DNA, including plasmids, is linearized during uptake [19] and thus requires a homologous counterpart in order to become either chromosomally integrated or re-circularized to a plasmid. Here we analyse the fate of transforming DNA in the various transformation-deficient mutants. The available data suggest a sequential order by which these factors act.

2. Materials and methods

2.1. Strains and plasmids

N. gonorrhoeae strain MS11 obtained from E.C. Gotschlich at the Rockefeller University, New York, is the progenitor of all variants and mutants used in this study: N200 is an authentic piliated variant (PilE₈) of strain MS11 [20]. N152 (P⁺) is a recA insertion mutant constructed by C.P. Gibbs in this laboratory; N263 is a spontaneous P⁻ pilE deletion mutant [21]; N474 is a piliated pilC1,2 double mutant [21]; N390 (P⁺) carries a TnMax1 insertion in the chromosomal comA gene [15]; N457 carries TnMax1 in comL [16]; N810 is a TnMax1 mutant in the tpc locus [17]. N. gonorrhoeae were grown on GC agar base with vitamin supplement (Becton Dickinson), at 37°C in 5% CO₂, and passaged daily. Maintenance of resistance marker by antibiotic selection (e.g. 7 µg/ml erythromycin for ermC or 10 µg/ml chloramphenicol for catgc) and of the proper colony morphologies was routinely monitored. E. coli plasmid pES3 is plasmid pIPlO [4] with an ermC determinant inserted into the BglII of the iga gene. Plasmid pBA is a truncated version of vector pBR322, and pRH60 is pBA carrying a 45 bp fragment flanked by BglII sites comprising a N. gonorrhoeae DNA uptake signal (AGATCTAACAAAAGCCGTCTGAAATGGTTTCAGACGGCATCTTTGTTCAGATCT) [14]. Plasmid pDF67 has been described [15].

2.2. DNA uptake and transformation assays

The DNA uptake assay was performed essentially as described [13] using 1 µg of linearized pBA (SalI), pRH60 (SalI), or pES3 (EcoRI). The bacteria were treated with 100 µg/ml DNase I 10 min after the addition of the DNA, unless otherwise indicated. 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 ml of 50 mM glucose, 25 mM Tris·HCl pH 8.6, 10 mM EDTA and lysed by subsequent treatments with 100 µg/ml lysozyme, 1% Sarcosyl and 100 µg/ml proteinase K. The DNA was phenol extracted and prepared for Southern blot analysis using [³²P]dCTP labelled pBA as probe.

3. Results

3.1. The comA, comL and tpc mutations do not affect DNA uptake

The recently cloned and characterized comA [18], comL [16] and tpc [17] genes were mutated by the
Fig. 1. DNA uptake by a transformation-competent variant of *N. gonorrhoeae* strain MS11 (N200) and recA (N154), comA (N390), comL (N457), tpc (N810), pilC (N474) and pilE (N263) mutants, as determined by Southern blot analysis. DNA uptake assays were performed using SalI-linearized pRH60 which contains an *N. gonorrhoeae* DNA uptake sequence. DNase-resistant DNA could be recovered from all mutants incubated for 10 or 20 min, except from the pilC and pilE mutants.

Mini-transposons of the TnMax series [22] and introduced in an isogenic background of strain MS11 variant N200. Representative mutants, i.e. N390 (comA), N457 (comL) and N810 (tpc), were piliated and also produced pilC and pilE, the only phase variable factors known to be essential for natural transformation competence [13]. The comA mutant contrasts with other transformation-deficient mutants in that it has no detectable pleiotropic phenotype [18]. Other incompetent mutants are additionally affected in pilus biogenesis [12,23–25], adhesion to epithelial cells [26], invasion and cell separation (tpc) [17], or exhibit an abnormal cell size (comL) [16]. The comA mutant as well as the tpc and comL mutants are, however, recombination-proficient as indicated by their ability to undergo intrinsic, transformation-independent pilE variation [18] (data not shown).

In contrast to the mutants deficient in the pilus-associated factors, PilE, PilC, and PilT (dud-l), where the transforming DNA is not taken up into the cells but rather remains attached to the cell surface accessible to the treatment with DNase [10,12,13], the comA, comL as well as the tpc mutant readily take up DNA into a DNase-resistant state. As shown in Fig. 1, DNA containing the *N. gonorrhoeae* uptake sequence, such as linearized pRH60, is internalised within minutes by the *N. gonorrhoeae* mutants N390, N457 and N810 and can be re-isolated from the cells after the degradation of the extracellular DNA by DNase I and detected by Southern hybridization.

Fig. 2. Time-dependent processing of pES3 DNA in wild-type and mutant *N. gonorrhoeae*. In this uptake assay EcoRI-linearized pES3 DNA was used and the process terminated at three different time points. The relevant genotypes of the strains tested are indicated. Lane a represents a control transformation with equal amounts of linearized pBA devoid of a *N. gonorrhoeae*-specific DNA uptake sequence.
3.2. Time-dependent processing of incoming DNA

In contrast to linearized pRH60 which remains stable inside all strains after uptake (Fig. 1), linearized plasmids which contain various inserts of N. gonorrhoeae DNA, e.g. pES3 (Fig. 2) or pDF67 (not shown), exhibit a partial, time-dependent degradation in the N. gonorrhoeae wild-type variant as well as in the recA mutant. The observed degradation is independent of the restriction site used for the linearization of the plasmid and affects only 50% of the transforming DNA while the remaining DNA was consistently found to be stable over a period of 2 h incubation. The molecular basis of this degradation is not understood but, since the phenomenon is not observed with pRH60, it may involve site-specific nucleases, such as restriction nucleases which are highly abundant in N. gonorrhoeae [27]. In contrast to other competent bacteria, the observed processing does not result in the accumulation of single-stranded DNA since treatment of the processed DNA with S1 nuclease did not lead to a significant change of the DNA pattern shown in Fig. 2. Interestingly, the time-dependent degradation is only found in the wild-type and the recA mutant but absent in the comA, comL and tpc mutants.

4. Discussion

The comA, comL and tpc mutants studied here represent a group of transformation-deficient mutants which are DNA uptake-proficient but blocked in subsequent steps of the transformation process preceding the processing and recombination of transforming DNA. The transformation process in N. gonorrhoeae can therefore be dissected into at least three distinct steps: (i) the initial sequence-specific binding and uptake of DNA into a DNase-resistant state, (ii) the transport into the cytosol and (iii) the nucleolytic processing and recombination with resident DNA. Fig. 3 illustrates a hypothetical model of the sequential action of known competence factors during the transformation process of N. gonorrhoeae. In the first step, the DNA is bound at the cell surface and subsequently transferred across the outer membrane in a double-stranded form whenever it contains the species-specific uptake signal [14]. In mutants which do not allow the transfer across the outer membrane the transforming DNA remains blocked at the cell surface where it can be completely digested by externally added DNase. Interestingly, all components involved in the DNA uptake are components of the pilus or the pilus biogenesis and assembly machinery. PilC and PilE were not only shown to represent essential DNA uptake factors but different combinations of these phase-variable proteins were implicated to influence the DNA uptake efficiency [13]. The N. gonorrhoeae PilT protein plays an essential role in pilus-associated phenotypes and was identified to be an allele of dud-I, the first uptake-deficient phenotype described [10,12]. PilE and PilT belong to a family of common components involved in the assembly of type 4 pili. DNA transfer systems, filamentous phage and protein-secretion apparatus [28]. Proteins involved in the transformation process of B. subtilis and H. influenzae also belong to this family [28]. We assume that other N. gonorrhoeae factors belonging to this family, including PilD and PilF [25], PilG [24], and Omc [28] which were shown to be deficient in pilus assembly and impaired in competence, function in DNA uptake as well.

The second step comprises the transfer across the periplasm, the murein sacculus and the inner membrane. A sequential order of the components involved in this second step, ComA, ComL and Tpc, cannot be dissected experimentally. However, the peptidoglycan-associated lipoprotein ComL and Tpc, which is implicated in the separation of the murein bilayer during cell division, may both mediate the passage of DNA through the periplasm. ComA, which is probably an integral inner membrane protein, may function in the transfer of the DNA across the inner membrane.

At a subsequent stage in transformation, a processing step takes place preceding the RecA-dependent recombination of the transforming DNA with the resident chromosome. Whether the observed processing is related to subsequent recombination events is unclear. A similar time-dependent degradation of the transforming DNA was observed by Biswas and colleagues [19] with strain FA759 using plasmid pFA10. The formation of single-stranded DNA may be a prerequisite for the recombination with resident DNA, however, the observed processing reaction did
not result in detectable amounts of single-stranded DNA. It remains to be determined where, in the periplasm or in the cytosol, the observed processing occurred and from which compartment the processed DNA form was derived.

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References


