Uptake-sequence-independent DNA transformation exists in *Neisseria gonorrhoeae*

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A DNA transformation dose-response curve of piliated (P⁺) gonococci with the use of cloned DNA containing a *pilE2-cat* fusion showed saturation at high and low levels of transforming DNA. At low DNA concentrations, transformation of the P⁺ strain MS11-A was effectively inhibited by a 1000-fold molar excess of the gonococcal transformation uptake sequence (GCUS). The same molar excess of the GCUS did not inhibit transformation of MS11-A at high DNA concentrations. In MS11-B2, a nonpiliated (P⁻), pilin-nonproducing, isogenic variant of MS11-A, the GCUS did not inhibit transformation at any level of transforming DNA. These data suggest that two mechanisms of transformation exist in P⁺ cells: one which utilizes the GCUS and one which does not. In MS11-B2 P⁻ cells, no evidence was found for the presence of the GCUS-dependent mechanism, suggesting that transformation in this background occurs solely by the GCUS-independent mechanism.

Keywords: dose-response, competition, pili

INTRODUCTION

Neisseria gonorrhoeae (the gonococcus) and *Neisseria meningitidis* are important obligate human pathogens that are naturally competent for DNA-mediated transformation (Catlin & Cunningham, 1961; Sparling, 1966). Transformation is probably the principal means of exchange of chromosomal markers between neisserial strains, since other means of chromosomal DNA transfer have not been observed (Cannon & Sparling, 1984). The mosaic nature of certain genes, most of which encode virulence determinants (Frosch & Meyer, 1992; Halter *et al.*, 1989; Spratt *et al.*, 1992), suggests that transformation occurs readily in nature and is likely to play a key role in obtaining genetic diversity. In addition, evidence has been provided to support the idea that gonococcal DNA transformation has a role in promoting the diversity of pili (Gibbs *et al.*, 1989; Norlander *et al.*, 1979; Seifert *et al.*, 1988), Opa proteins (Frosch & Meyer, 1992; Schwalbe & Cannon, 1986) and IgA₁ protease (Frosch & Meyer, 1992). These virulence factors are crucial in maintaining these organisms in the human population.

Gonococci are competent for DNA transformation during all phases of growth (Biswas *et al.*, 1977; Sparling, 1966). DNA transformation in the gonococcus involves

binding of DNA to cells (Dougherty *et al.*, 1979), followed by DNase-resistant uptake (Sparling, 1966) and homologous recombination (Kooimey & Falkow, 1987). Several genetic determinants involved in DNA uptake (*dud-1* or *pilT*; Biswas *et al.*, 1989; Kooimey *et al.*, 1994), DNA transport (*comA*; Facius & Meyer, 1993), processing (*ntr*; Biswas *et al.*, 1989) and recombination (*recA*; Kooimey & Falkow, 1987) have been identified. Outer-membrane proteins that bind gonococcal DNA have been described (Dorward & Garon, 1989), but a receptor for DNA remains undefined.

Gonococci are most efficiently transformed by genus-specific DNA (Dougherty *et al.*, 1979) and this process is presumed to involve a receptor that recognizes a specific 10 bp gonococcal transformation uptake sequence (GCUS) (Elkins *et al.*, 1991; Goodman & Scocca, 1988). This sequence occurs frequently on gonococcal DNA, often as a part of transcriptional terminators (Goodman & Scocca, 1988). However, evidence has also been obtained for lower efficiency gonococcal transformation by DNA that does not contain the GCUS (Stein, 1991), suggesting that the GCUS is not absolutely required for gonococcal DNA uptake. Moreover, gonococci can transport DNA molecules that do not contain this DNA sequence (Burnstein *et al.*, 1988).

An interesting feature of gonococcal transformation is the relationship between transformation efficiency and piliation phenotype or production of pilin, the major

Abbreviations: GCUS, gonococcal transformation uptake sequence; P⁺, piliated; P⁻, nonpiliated.

structural subunit of pili. Gonococci undergo phase variation, often reversibly, between pilated (P^+) and nonpilated (P^-) states (Kellogg *et al.*, 1963; reviewed by Seifert, 1992). Two general forms of P^- variants have been described: those that do not produce pilin and those that produce pilin monomers that are not assembled into pili (Swanson *et al.*, 1985). Whereas all P^+ gonococci can be efficiently transformed, nonreverting (pilin-non-producing) P^- variants are transformed less efficiently (Biswas *et al.*, 1977; Sparling, 1966; Swanson *et al.*, 1990). P^- variants which produce pilin monomers can be transformed at intermediate levels (Gibbs *et al.*, 1989; Zhang *et al.*, 1992), suggesting that the pilin protein facilitates the transformation process. The physical uptake of DNA in gonococci has been correlated with piliation, the production of the pilin monomer, and presence of the GCUS on transforming DNA; however, no direct relationship has been established between pilin production and recognition of the GCUS.

We have previously reported that cloned gonococcal DNA fragments carrying nonhomologous insertions transformed with greater efficiency when the size of the homologous fragment was increased relative to the size of the nonhomologous fragment (Boyle-Vavra & Seifert, 1993). Moreover, increased levels of input DNA also improved transformation efficiencies (Boyle-Vavra & Seifert, 1993). In this report, the dose-response of gonococcal transformation to various amounts of cloned DNA containing a heterologous *cat* insertion was studied. Since a saturation curve consisting of two portions was observed, we investigated the role of the GCUS and piliation phenotype in transformation at different DNA concentrations.

METHODS

Strains and media. Gonococci were grown on GC medium base (Difco) with added supplements (Kellogg *et al.*, 1963) (GCB) at 37 °C with 5% (v/v) CO₂. Chloramphenicol (Cm) was used at 10 µg ml⁻¹. MS11-A is a P^+ gonococcal strain which expresses the pilin gene from two pilin expression loci, *pilE1* and *pilE2* (Meyer *et al.*, 1984). MS11-B2 is a nonreverting P^- variant of MS11-A with a 1.4 kb deletion in both *pilE1* and *pilE2* (see Fig. 3) (Segal *et al.*, 1986). MS11Cm20 is a derivative of MS11-A which contains the mini-transposon mTn Cm (Seifert *et al.*, 1990) inserted in *pilE2*.

Plasmids. pNG1721CAT contains a 10.2 kb gonococcal insert from pNG1721 (Meyer *et al.*, 1984) containing a promoterless 780 bp Cm resistance marker (encoded by the *cat* gene) in the *MstII* site of *pilE2* (Taha *et al.*, 1988). At least one GCUS is present downstream of the *pilE* locus (data not shown). pNG1721CAT was prepared by either Triton lysis (Clewett & Helinski, 1969) or alkaline lysis (Birnboim & Doly, 1979) followed by CsCl/ethidium bromide purification (Sambrook *et al.*, 1989).

DNA transformation. Transformation was performed as described by Seifert & So (1991). Bacteria were grown on solid GCB medium for 18 h and swabbed into unsupplemented GC liquid medium containing 5 mM MgCl₂ (GCB-Mg) at 37 °C. The cell density was adjusted to about 10⁸ c.f.u. ml⁻¹ and the suspension diluted 1:10 into GCB-Mg at 37 °C containing the indicated concentrations of transforming DNA and GCUS

competitor. After 10 min at 37 °C, the cell/DNA suspension was diluted 1:10 into pre-warmed Kellogg-supplemented liquid GCB and incubated at 37 °C in 5% CO₂ for 4–5 h to allow for phenotypic expression of Cm resistance.

pNG1721CAT was prepared for transformation by digestion with *PstI*, extracted once with TE-equilibrated phenol and once with chloroform, and ethanol-precipitated (Sambrook *et al.*, 1989). Stock concentrations of DNA were determined by measuring the A_{260} . The double-stranded GCUS competitor DNA was prepared by boiling the oligonucleotide pair, GCU1 and GCU2 (Boyle-Vavra & Seifert, 1993), followed by slow cooling to promote annealing. In competition experiments, the GCUS oligomer was added to the transformation mix in 1000-fold molar excess over transforming DNA (pNG1721CAT), which was in excess of concentrations used previously to inhibit transformation (Goodman & Scocca, 1988). Transformants were plated in triplicate on GCB medium containing Cm and were counted at 24–27 h. C.f.u. were plated in triplicate on GCB medium and were scored after 18 h incubation.

Southern blotting. Gonococcal chromosomal DNA was isolated as described previously (Seifert *et al.*, 1990), digested with *BspDI* (an isoschizomer of *ClaI*) and separated on 0.8% agarose/TBE (Tris/borate/EDTA; Sambrook *et al.*, 1989) gels overnight at 50 V. Southern blotting was performed by capillary transfer as described by Sambrook *et al.* (1989). The pilin gene probe was the *HindIII*–*ClaI* fragment from pNG1100-1 (Meyer *et al.*, 1984) and the *cat* probe was the 780 bp *HindIII* fragment from pCM7 (Close & Rodriguez, 1982) radiolabelled with [α -³²P]dCTP (Amersham) by random primer labelling (Feinberg & Vogelstein, 1983). Blots were washed using high-stringency conditions (65 °C, 0.1% SDS, 0.1× SSC) (Sambrook *et al.*, 1989) and exposed to Kodak X-Omat film. Autoradiographs were scanned with a Bio-Rad Imaging Densitometer model GS-670 in conjunction with Molecular Analyst Image Analysis software (version 1.1). The final image in Fig. 3 was produced with Corel Draw software (version 3.0).

RESULTS

Dose-response of transformation of P^+ strain MS11-A

Our previous experiences with the transposon shuttle mutagenesis system had suggested that higher amounts of transforming DNA led to higher levels of gonococcal transformation (Boyle-Vavra & Seifert, 1993). To investigate this further, a dose-response curve for transformation with pNG1721CAT was generated. This plasmid contains 10.2 kb of cloned gonococcal DNA consisting of *pilE2* and its flanking sequences (Meyer *et al.*, 1984) and carries a promoterless *cat* gene downstream of the transcriptional start point of *pilE2* (Taha *et al.*, 1988). After binding and uptake of pNG1721CAT by gonococci, the *cat* gene is integrated into the *pilE2* region of the chromosome by homologous recombination. Since pNG1721CAT does not replicate in gonococci, Cm resistance in transformants is expressed from the chromosome.

Various amounts of plasmid DNA were used in transformations of the highly competent gonococcal strain MS11-A, which contains two pilin expression loci (Meyer *et al.*, 1984) and demonstrates stable piliation. Surprisingly, the dose-response curve produced with pNG1721CAT in MS11-A (Fig. 1) was different from

Fig. 1. Dose-response curve for gonococcal transformation with a *pil-cat* marker. The P^+ gonococcal strain MS11-A was transformed with varying amounts pNG1721CAT plasmid DNA (triangles) or chromosomal DNA (circles) isolated from MS11Cm20 and transformants were selected on $10 \mu\text{g Cm ml}^{-1}$. Each data point represents the mean of between three and seven experiments. Error bars indicate SEM.

dose-response curves previously described (Biswas *et al.*, 1977; Sparling, 1966). At low amounts of DNA, large increases in transformation frequency were seen in response to small changes in DNA concentration (Fig. 1). This was followed by an apparent saturation of transformation between 2 and $10 \mu\text{g}$ of DNA ml^{-1} . This first level of saturation was followed by a slight decrease in frequency at $20 \mu\text{g pNG1721CAT ml}^{-1}$ followed by a gradual increase in frequency with increasing DNA concentration (producing a gradual slope) reaching a final plateau between 40 and $70 \mu\text{g DNA ml}^{-1}$. At this concentration of DNA, as many as 39% of the cells exposed to the plasmid became Cm^R ; to our knowledge, this represents the highest level of transformation ever reported for the gonococcus.

The ability of pNG1721CAT to produce two levels of saturation for transformation was different from the single level of saturation previously reported when chromosomal DNA containing a streptomycin (Str) resistance marker (Biswas *et al.*, 1977; Sparling, 1966) was used. Such differences in results could be due to the selectable marker used to produce transformants or the physical form of the transforming DNA, i.e. chromosomal vs plasmid. To distinguish between these possibilities a dose-response curve of MS11-A transformation was produced using chromosomal DNA isolated from strain MS11Cm20, which contains a *cat* gene in *pilE2* (Seifert *et al.*, 1990). As seen in Fig. 1, the saturation curve produced by MS11Cm20 DNA resembled the plasmid dose-response curve at low DNA concentrations but did not show a significant rise in transformation at higher input DNA levels. Thus the dose-response curve produced by chromosomal DNA containing a Cm resistance marker more closely resembles the saturation curve previously reported for chromosomal DNA containing a Str resistance marker (Biswas *et al.*, 1977; Sparling, 1966) than the dose-response curve for plasmid DNA showing two

Fig. 2. Competition by the GCUS for transformation of a P^+ and nonreverting, pilin-nonproducing P^- strain. The P^+ strain was MS11-A (solid lines) and the P^- strain was MS11-B2 (dashed lines). Strains were transformed with pNG1721CAT DNA in the absence (triangles) or presence (circles) of a 1000-fold molar excess of a competing oligomer encoding the GCUS. Transformants were selected on $10 \mu\text{g Cm ml}^{-1}$. The data for MS11-A transformation without the GCUS also appear in the dose-response curve in Fig. 1. Transformations of MS11-A were repeated at least three times and transformations of MS11-B2 were repeated twice. The data for transformation of MS11-B2 at $1 \mu\text{g DNA ml}^{-1}$ represent transformants obtained in one of two experiments. All measurements of c.f.u. were performed in triplicate. Error bars indicate SEM.

levels of saturation (Fig. 1). The ability of plasmid and not chromosomal DNA to produce a second rise in transformation frequency is attributed to the homogeneous single gonococcal fragment in the plasmid DNA as opposed to heterogeneous chromosomal DNA, which has a low molar ratio of the transforming fragment relative to other competing chromosomal fragments.

Competition for transformation of P^+ cells by the GCUS

Since the GCUS has been shown to be utilized for efficient DNA uptake during transformation (Elkins *et al.*, 1991; Goodman & Scocca, 1988) we tested whether both portions of the transformation dose-response curve were dependent on uptake promoted by the GCUS. This was done by transforming MS11-A with pNG1721CAT at concentrations from different portions of the transformation dose-response curve in the presence of a 1000-fold molar excess (over transforming DNA) of double-stranded oligomer carrying two inverted copies of the GCUS. Using 1 and $10 \mu\text{g DNA ml}^{-1}$, the GCUS-oligomer was able to partially inhibit transformation of the P^+ strain, by 100-fold and 10-fold, respectively (Fig. 2). This is similar to results previously obtained in identifying and characterizing the GCUS (Elkins *et al.*, 1991; Goodman & Scocca, 1988). However, at $40 \mu\text{g pNG1721CAT ml}^{-1}$, the presence of excess GCUS produced transformation frequencies similar to those obtained without the GCUS (Fig. 2). Assuming that 1000-fold molar excess of the competitor was sufficient to

Fig. 3. Southern blot of MS11-B2 transformants of pNG1721CAT. Chromosomal DNA was isolated from MS11-A (M), MS11-B2 (B2), and four MS11-B2 Cm^R transformants (a–d) and digested with *Bsp*DI (a *Clal* isoschizomer). Blots were probed as described in Methods with either (a) a pilin gene probe, or (b) a *cat* gene probe. Hybridizing silent pilin copies (*pilS*) or pilin expression loci (*pilE*) are indicated on the left with their corresponding sizes in kb. The deleted *pilE1* (Δ *pilE1*) and deleted *pilE2* (*) bands appear in all the MS11-B2 variants at 2.6 and 2.8 kb, respectively, with deleted *pilE2* co-migrating with *pilS5*. *pilE2*-*cat* (#) comigrates with *pilS1*. *pilS7* is not shown but hybridizes equally well in all lanes. The band appearing at the top (>) of lanes with DNA from MS11-B2 variants is of unknown origin and variably appears in *Bsp*DI digests of gonococcal chromosomal DNA.

inhibit the GCUS-dependent process, the transformants obtained in the presence of the GCUS-containing oligomer were produced mainly by a GCUS-independent mechanism. These data suggest that two distinct processes were occurring in different portions of the transformation dose-response curve. One process, showing apparent saturation at approximately $2 \mu\text{g DNA ml}^{-1}$, was inhibited by the GCUS. The second process, showing saturation at higher levels of DNA, was not inhibited by the GCUS.

Association between piliation (or pilin production) and recognition of the GCUS

To test whether a relationship exists between recognition of the GCUS and production of pilin or pilus fibres in recipient bacteria, a pilin-nonproducing strain was transformed in the presence or absence of the GCUS oligomer. The P^- strain, MS11-B2, is nonreverting due to 1.4 kb deletions in both pilin expression loci (Segal *et al.*, 1986) and therefore is a stable pilin-nonproducing variant. DNA transformation of MS11-B2 with 1, 10 and $40 \mu\text{g pNG1721CAT ml}^{-1}$ in the absence of the GCUS competitor produced a dose-response with frequencies increasing from 10^{-9} to 10^{-7} Cm^R transformants per c.f.u. (Fig. 2) with increasing amounts of DNA. These low transformation frequencies are not attributed to poor recombination at the deleted chromosomal *pil* loci of strain B2 since this strain shows similar frequencies when transformed by DNA containing a point mutation that confers spectinomycin resistance (H. S. Seifert, unpublished). Also, these frequencies are similar to those previously reported for other nonreverting P^- strains (Biswas *et al.*, 1977; Sparling, 1966). In competition

experiments with the GCUS, a 1000-fold molar excess of the GCUS competitor did not inhibit transformation of MS11-B2 by pNG1721CAT at any DNA concentration tested. These data indicate that in the absence of pilin production or elaboration of pilin fibres transformation can occur by a GCUS-independent route.

Since some of the Cm^R transformants of MS11-B2 were obtained at a frequency that approached the spontaneous mutation rate (although Cm^R colonies were not observed at this level without added DNA), Southern analysis was performed with *pil* and *cat* gene probes to test whether the Cm^R colonies obtained were true transformants (Fig. 3). In blots probed with a *pil* gene probe, MS11-A contains the two expected bands (Meyer *et al.*, 1984) containing pilin expression at loci at 4.2 (*pilE2*) and 4.0 kb (*pilE1*) (Fig. 3a). The additional hybridizing bands in MS11-A represent partial, unexpressed, silent *pil* loci (*pilS*) (Haas & Meyer, 1986; Haas *et al.*, 1992). MS11-B2 contains deletions in both pilin expression loci and exhibits *pil*-hybridizing bands at 2.8 and 2.6 kb which correspond to deleted forms of *pilE2* and *pilE1*, respectively (Fig. 3a). The deleted form of *pilE2* in the MS11-B2 parent comigrates with *pilS5*, producing an apparently darker *pil* band at the 2.8 kb position. In contrast, the MS11-B2 transformants in lanes a, b, c and d do not exhibit the darker band at 2.8 kb, indicating that *cat* was inserted into the *pilE2* locus of the 2.8 kb band, which altered the band's mobility. Probing with the *cat* gene revealed that each transformant contains a *cat* gene in a band of a size consistent with a restored pilin expression locus migrating with or close to *pilS1* (Fig. 3b). The missing fragments corresponding to *pilS6* (Haas *et al.*, 1992) in lanes a, b and

d indicate that *pil* sequences in *pilS6* were involved in homologous pairing during the recombination reaction which resulted in the insertion of *cat* into *pilE2*. All of *pilS6* is upstream of *pilE2* in the gonococcal chromosome and a portion is carried on pNG1721CAT (Haas *et al.*, 1992; Meyer *et al.* 1984). The transformants in lanes a, b and d probably arose by homologous pairing between the silent sequences upstream of *pilE2* in pNG1721CAT and homologous sequences in the chromosomal *pilS6* locus resulting in crossing out of the intervening DNA between *pilS6* and the upstream silent copies of *pilE2*, including the *BspDI* site. We have previously noted similar pairing reactions between partial pilin copies of *pilS1* and *pilE1* (Seifert *et al.*, 1988). A simpler transformant is represented in Fig. 3(b), lane c, where the *cat* gene inserted into *pilE2* as a result of recombination between *pilE2* sequences in pNG1721CAT and the chromosome. These patterns are consistent with the proposed locations of homologous pairing and each transformant tested was therefore a true recombinant that had repaired the deletion of *pilE2* with input pNG1721CAT DNA. While the frequency of transformation of this P⁻ variant was lower than that of the P⁺ variant, the transformants are the result of homologous recombination representative of true transformation events.

DISCUSSION

We present several lines of evidence to indicate that two processes of transformation, which differ in their dependence on the GCUS, might function in gonococci. First, a dose-response study of plasmid DNA transformation of P⁺ gonococci showed saturation of transformation at low and high DNA concentrations, indicating two processes occurring in P⁺ cells. Second, as evidenced by the ability of the GCUS to reduce transformation frequency, a GCUS-dependent process was only evident in P⁺ cells and only at DNA concentrations from the first portion of the dose-response curve. Third, the GCUS-competition results indicated that transformation by a GCUS-independent process was present in both P⁺ and P⁻, pilin-nonproducing cells at all levels of transforming DNA.

Only the GCUS-independent mechanism was apparent in both P⁺ and P⁻ cells. In the P⁺ background, if the GCUS-independent transformation does not require a threshold level of DNA, the transformants obtained at lower DNA concentrations in the presence of the GCUS represent those obtained by the GCUS-independent mechanism (Fig. 2). Thus, at these low levels of DNA in the absence of competitor, both the GCUS-dependent and GCUS-independent mechanisms might function concurrently. The slight decrease in transformation frequency of the P⁺ strain observed at 20 µg pNG1721CAT ml⁻¹ (Fig. 1) indicates that transformation using the putative high-affinity receptor is inhibited at concentrations above the saturation point, at which time transformation by the second mechanism is apparent.

Since the GCUS did not decrease transformation frequencies of the P⁺ strain to levels as low as that of the P⁻ strain

at any level of pNG1721CAT, it can be assumed that the GCUS-independent mechanism is more efficient in the P⁺ background. Although the GCUS-independent mechanism appears less efficient in P⁻ cells than in P⁺ cells, it still results in very high transformation frequencies at high DNA concentrations in P⁺ cells. Since we have also observed that transformation of the P⁻ population in a reverting P⁻ gonococcal strain can be inhibited by the GCUS-containing oligomer (data not shown), it is likely that both pathways also operate in P⁻, pilin-producing backgrounds.

The GCUS-dependent mechanism was associated with a steep rise in the dose-response curve and apparent saturation at low amounts of DNA. The GCUS-independent mechanism in the P⁺ background was associated with a more gradual slope. Therefore, we hypothesize that the GCUS-dependent process could utilize a higher-affinity receptor. It seems likely that the GCUS-independent mechanism involves binding of DNA to a specific lower-affinity receptor rather than a non-specific process since this form of transformation is saturable in P⁺ cells. Also, it is difficult to envision that large DNA molecules could penetrate two membranes and the peptidoglycan layer simply by passive diffusion. We cannot determine from these data whether these two mechanisms differ from each other at the stage of transformation which involves binding of DNA to the cell or at a subsequent stage.

Consistent with our hypothesis for a GCUS-independent mechanism, in addition to the well-recognized GCUS-dependent mechanism of DNA uptake, is the residual level of DNA uptake seen in the presence of competing GCUS-containing plasmid DNA observed by Elkins *et al.* (1991). Also in support of two pathways of gonococcal DNA transformation are earlier observations of biphasic curves both for the rate of DNase-resistant uptake of DNA (Sparling, 1966) and for the DNA-concentration-dependent dose-response of gonococcal transformation (Sparling *et al.*, 1977). Our ability to obtain transformants at 70 µg DNA ml⁻¹ is also consistent with binding studies in gonococci which show saturation of binding of gonococcal DNA at this level (Dougherty *et al.*, 1979). In *Bacillus subtilis*, similar to results in Fig. 1, the presence of low- and high-affinity receptors for DNA was demonstrated for binding of DNA to membrane vesicles at three levels of saturation at 1, 10 and 70 µg DNA ml⁻¹ (Joenje *et al.*, 1975).

Swanson *et al.* (1990) previously characterized nonreverting P⁻ gonococcal strains as noncompetent. The transformation protocol used by Swanson's group did not include an incubation period for the transformed cells to express antibiotic resistance, which could explain the lower frequencies they also observed for their P⁺ strains (10⁻⁴ transformants per c.f.u.; Swanson *et al.*, 1990). The difference between this previous report and our findings could not be attributed to use of chromosomal DNA since we have observed similar transformation frequencies of a P⁻, nonreverting strain with chromosomal DNA (data not shown).

Since gonococci are highly autolytic (Hebeler & Young, 1975) and competent for DNA transformation (Sparling, 1966), it was suggested (Norlander *et al.*, 1979) and subsequently shown (Gibbs *et al.*, 1989; Seifert *et al.*, 1988) that uptake of DNA released by lysed cells could be the source of DNA used in nonreciprocal pilin gene recombination reactions responsible for phase and antigenic variation of pili. The putative GCUS-independent mechanism we propose provides a mechanism by which P^- gonococci could be transformed *in vivo* by chromosomal DNA released from neighbouring cells, resulting in repair of a deleted pilin expression locus by recombination with an intact pilin expression locus in the transforming DNA. The involvement of transformation in pilin recombination reactions is more likely to occur if local DNA concentrations are high and the resultant P^+ cells show a selective advantage over pilin-nonproducing, P^- cells. We can not know what the biologically relevant concentrations of DNA are in a human but presume that in a colony there could be local DNA concentrations that match these relatively high concentrations.

Although pilin production has been associated with transformation competence in gonococci, neither pilin monomers nor pili have been shown to bind DNA or inhibit DNA transformation (Mathis & Scocca, 1984; Sparling *et al.*, 1977). Thus, it has been suggested that other features of P^+ cells, besides pili, are involved in the transformation process, such as pilus biogenesis components or pilus-associated proteins. Supporting this notion is the finding that several components involved in pilus biogenesis in *Pseudomonas*, which produce a similar pilin type (type 4) as gonococci, are homologous to competence genes in *Bacillus subtilis* and *Haemophilus influenzae* (Hobbs & Mattick, 1993). The mutated gene in *dud1* mutants (Biswas *et al.*, 1989) responsible for decreased DNA uptake and transformation competence was recently identified as *pilT* (Koomey *et al.*, 1994), a gene which also affects twitching motility. Although *dud1* mutants were originally identified as P^+ (Biswas *et al.*, 1989), quantitative and qualitative changes were noted in pilus expression of *pilT* mutants (Koomey *et al.*, 1994). *pilC*, a pilus biogenesis component (Jonsson *et al.*, 1991) that can be found as a component of pili (Rudel *et al.*, 1995b), is essential for efficient gonococcal transformation, as shown by unusual *pilC* mutants that are capable of producing pili yet are not competent for transformation (Rudel *et al.*, 1995a). Taken together with these previous studies, our results suggest that only wild-type expression of the pilus fibre and its biogenesis components confers the high level of DNA transformation efficiency that corresponds to the first portion of the dose-response curve.

We propose two models to explain the relationship between GCUS-dependent (and possibly GCUS-independent) DNA transformation and pilin production or pilus biogenesis: (1) the pilus or components of the pilus transport apparatus are required to present the GCUS receptor on the cell surface, or (2) the pilus fibre or pilus transport machinery is used to transport DNA into the cell once DNA has specifically bound to the cell surface.

It is plausible that both models are correct and that full competence for transformation only occurs when both conditions are met. The identification of the GCUS receptor will help predict how the binding and transport of transforming DNA interacts with the pilus biogenesis apparatus.

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