Phase Variation of Gonococcal Protein II: Regulation of Gene Expression by Slipped-Strand Mispairing of a Repetitive DNA Sequence

George L. Murphy,*† Terry D. Connell,* Diana S. Barritt,* Michael Koomey,¹ and Janne G. Cannon*¹
* Department of Microbiology and Immunology
University of North Carolina
Chapel Hill, North Carolina 27599
†Present address: Burroughs-Wellcome Company, 3030 Cornwallis Road, Research Triangle Park, North Carolina 27709.

Summary

Expression of outer membrane protein II (PII) of Neisseria gonorrhoea is subject to reversible phase variation. Changes in the number of CTCTT units, leading to frame-shifting within the gene, are responsible for changes in PII expression. Phase variation mediated by the CTCTT repeat also occurred in E. coli, as assayed with a PII-alkaline phosphatase (phoA) gene fusion. Phase variation in both the gonococcus and E. coli was recA-independent, occurred at similar rates, and involved insertions or deletions of one or more repeat units. The characteristics of the phase variation process were consistent with a model in which expression of PII genes is regulated by slipped-strand mispairing of the DNA in the CTCTT repeat region.

Introduction

Many bacterial species that are obligate human pathogens have evolved novel genetic regulatory mechanisms for varying the array of surface components exposed to the defense systems of the infected host. Synthesis of such components may be subject to phase variation (reversible oscillation between different states of expression) and to antigenic variation (synthetic of antigenically different versions of a particular component). Neisseria gonorrhoeae, the causative agent of gonorrhoea, demonstrates both phase and antigenic variation of several different surface constituents, including a family of outer membrane proteins designated Proteins II (PII; also referred to as opacity proteins). The different PII proteins made by one strain can be distinguished by electrophoretic and antigenic differences among them, although all are heat-modifiable proteins of similar apparent molecular weight (24–30 kd) (Diaz and Heckels, 1982; Swanson, 1982; Swanson and Barrera, 1983). Each PII protein of a strain shows independent phase variation between on and off states of expression at a rate of $10^{-3}$–$10^{-4}$/cell/generation (Mayer, 1982; Black et al., 1984). It is possible to isolate gonococcal variants expressing from zero to at least three different PII proteins in different combinations on the surface of individual organisms (Swanson, 1982; Black et al., 1984; Robinson et al., 1988). PII phase variation occurs in vivo during gonococcal infection (Schwalbe et al., 1985) and is probably important in gonococcal pathogenesis (reviewed in Sparling et al., 1986; Blake and Gotschlich, 1987).

Analysis of different gonococcal strains indicates that there are at least nine complete PII structural genes in the gonococcal genome, although the actual number may be higher (Stern et al., 1984, 1986; Connell et al., 1988; van der Ley, 1988). Comparisons of DNA sequences of cloned PII genes show that the genes share a conserved framework, with differences among them confined primarily to two short hypervariable regions (Stern et al., 1986; Connell et al., 1988; van der Ley, 1988). The hypervariable regions encode variable antigenic determinants recognized by PII-specific monoclonal antibodies (Connell et al., 1988, and unpublished data). There is evidence that variability within the PII gene family is increased by recombination among PII genes that generates new assortments of hypervariable region sequences (Stern et al., 1986; Connell et al., 1988).

In all PII genes sequenced, the region of the gene encoding the hydrophobic core of the signal peptide is composed of identical repeats of the pentameric unit CTCTT (Stern et al., 1986; Connell et al., 1988; van der Ley, 1988). The number of repeats differs in different PII genes and can range from 7 to 27 repeat units (Stern et al., 1986). Stern et al. (1986) showed that PII genes are constitutively transcribed and that changes in the number of repeat units occur in PII transcripts as PII genes turn on or off. They proposed that the CTCTT repeats are involved in a translational control mechanism of phase variation. According to this model, changes in the number of CTCTT repeats place the PII gene in-frame with the ATG initiation codon (full-sized PII protein synthesized) or out-of-frame (truncated polypeptide synthesized).

There are several mechanisms that might be responsible for the high frequency changes in number of CTCTT repeats in PII mRNA. Some or all of the changes could result from slippage or stuttering of RNA polymerase as the genes are transcribed. Also, unequal crossing-over among the signal peptide coding regions of different PII genes in the chromosome could generate insertions or deletions of CTCTT repeat units. Such a recA-dependent recombinational mechanism is involved in antigenic variation of pilin protein in the gonococcus (Koomey et al., 1987).

An alternative model involves slipped-strand mispairing (SSM), which can occur during DNA replication or in non-replicating duplex DNA. Local denaturation of a DNA duplex can be followed by mispairing of bases in different copies of a short tandem repeat, leading to either insertions or deletions of the repetitive sequences (reviewed in Lovino and Gutman, 1987a). SSM has been suggested to be a major factor in the expansion of simple repetitive
DNA sequences in a wide variety of prokaryotes and eukaryotes. This mechanism has also been proposed to account for mutational hotspots for frameshifts at single-base runs (Streisinger et al., 1966; Streisinger and Owen, 1965; Pribrnow et al., 1981; Schaaper and Durin, 1987) and at tandem repeats of short sequence elements (Farabaugh et al., 1978; Albertini et al., 1982; Pribrnow et al., 1981), although it cannot account for all frameshift mutations (deBoer and Ripley, 1988). It has been suggested that transposon excision, and perhaps a number of other illegitimate recombination events, proceed by a similar mechanism (Albertini et al., 1982; Egner and Berg, 1981; Brunier et al., 1988). The SSM model makes a number of testable predictions: all size changes should involve addition or deletion of an integral number of discrete repeat units; the most common events should be those involving loss or gain of a single repeat unit, since these events will require minimal displacement of the two DNA strands; and the frequency of frameshift should increase with increasing length of the repeat region (Levinson and Gutman, 1987b). In this study, we have tested some of these predictions concerning the mechanism of PII phase variation.

Results and Discussion

Analysis of the CTCTT Repeats in the DNA of a Specific P.II Gene as It Changes Expression State

The CTCTT repeat region in PII genes is located in the signal peptide coding region, between the ATG initiation codon and the codons for mature PII protein. Depending on the number of CTCTT repeats, the codons for mature protein may be either in-frame or out-of-frame with the ATG codon. All of the PII genes that have been cloned to date are out-of-frame that were turned off in the gonococcal chromosome (Stern et al., 1966; Connell et al., 1986; van der Ley, 1988). We have been unable to clone in-frame PII genes, even on single-copy plasmid vectors, which has made it impossible to compare the DNA of on and off versions of a single PII gene. To confirm that phase variation of PII genes involves changes at the DNA level, we developed a means of identifying specific PII genes in a preparation of gonococcal chromosomal DNA and measuring the length of their signal peptide coding regions.

Gonococcal strain FA1090 produces six different identified PII proteins, designated PIIa through PIIf in order of increasing apparent molecular weight. We have produced a panel of monoclonal antibodies (MAbs) specific for unique surface exposed determinants on the PII proteins of this strain. Four MAbs bind individually to proteins PIIa, PIIc, PIIe, and PIIf. A fifth MAb binds to both PIIb and PIId (Black et al., 1984; Barratt et al., 1987). In previous studies, we used the MAbs to detect genetic transformation of PII genes into a different gonococcal strain and showed that the transformed population binds only the MAbs specific for the PII proteins that were expressed in the donor variant (Schwalbe and Cannon, 1986). Thus, a fragment of FA1090 DNA will yield MAb binding transformants only if it contains an intact gene expressing the PII protein recognized by the MAb.

To isolate a fraction of FA1090 DNA containing the PIIa and PIIe genes, we first determined that digestion of DNA from a PIIa- or PIIe-expressing variant with Clal did not inactivate PIIa or PIIe transforming activity. Clal-digested chromosomal DNA was subjected to agarose gel electrophoresis, and different size fractions were screened by the transformation assay to determine which contained the intact PIIa and PIIe genes. Both were contained in the 8–10 kb size fraction of Clal fragments, although not on the same fragment. No other intact PII genes were contained in this size fraction of DNA. We isolated a series of colony variants differing in expression of PIIa or PIIe (Figure 1), isolated the 8–10 kb size fraction of Clal fragments from each variant, and confirmed the expression state of the PIIa and PIIe genes in the isolated DNA fragments with the transformation assay. This size fraction was then digested with enzymes that cut in conserved sequences immediately flanking the CTCTT repeat region, electrophoresed on polyacrylamide gels, Southern blotted, and probed with an oligonucleotide homologous to the CTCTT repeats. This procedure allowed us to measure the precise lengths of the CTCTT regions of the PIIa and PIIe genes as they switched expression in different variants.

Two restriction fragments, containing the signal peptide coding DNAs of the PIIa and PIIe genes, hybridized to the probe (Figure 1). Changes in the length of the fragments occurred as the two genes switched on or off in different colony variants; changes in the large fragment correlated with switches in expression of PIIa, and changes in the smaller fragment correlated with switches in PIIe expression. All of the changes in length of the fragments involved addition or deletion of 5 bp or 10 bp, which corresponds to the size of one or two CTCTT repeats. In each case, the length of a fragment in a gene that was turned on was consistent with an in-frame gene, and its length in an off gene was consistent with an out-of-frame gene, based on comparisons to the DNA sequence of cloned FA1090 PII genes (Connell et al., 1988). This experiment demonstrated that changes in length of the CTCTT region, in increments corresponding to the size of the repeat unit, accompany phase variation of a specific PII gene. It is possible that transcriptional slippage also contributes to phase variation, but changes at the DNA level occurred in all of the phase switches we examined.

recA Independence of P.II Phase Variation

To determine if PII phase variation depends on gonococcal recA function, we compared PII switching in a recA derivative of strain FA1090 to that in the wild-type parent. FA1090recA produced colony variants capable of binding each of the five PII-specific MAbs. We isolated a PIIa-expressing variant of FA1090recA and three PII switches derived from it. The frequency with which PII variants appeared in the PIIa-expressing population was the same as for wild-type FA1090 (data not shown). Western blot analysis showed that the PIIa protein of FA1090recA had the same electrophoretic mobility as PIIa of FA1090
Figure 1. Southern Blot Analysis of the CTCTT-Containing Regions of a Plla Gene and a Pllb Gene as They Change Expression State

The diagrams on the left show the lineage of variants that were derived as single-step switchers in PII expression, and the photographs on the right show the Southern blot analysis of those variants. Variants were characterized as to PII expression with the panel of PII-specific MAbs. The designation PII- indicates that no PII proteins were expressed; the designation PIIa indicates that only PIIa was expressed in a variant. In (A), two independent PII- variants were derived from one PIIa variant. In (B), one of those PII- variants (PII-2) was used to derive four independent switchers in expression of either PIIa or PIIb. Chromosomal DNA of each variant was digested with Clal. The Clal fraction containing the intact Plla and Pllb genes was identified by the genetic transformation assay, then digested with HinfI and Hhal, which cut in conserved sequences immediately flanking the CTCTT repeats. The HinfI site is 22 bp upstream of the first CTCTT repeat; the Hhal site is 8 bp downstream of the last CTCTT repeat. Fragments were electrophoresed on polyacrylamide gels, Southern blotted, and probed with a CTCTT-specific oligonucleotide. To measure the changes in length of the fragments, they were compared with DNA sequencing reactions run on M13 clones of known DNA sequence and to size standards (100 bp and 115 bp) consisting of the same digests of cloned PII genes with known numbers of CTCTT repeats (lanes marked S). The fragment corresponding to the Plla gene is indicated with a closed circle; the fragment corresponding to the Pllb gene is indicated with an open circle. The faint bands visible in the Southern in (B) either represent partial digestion products, or are due to the unavoidable heterogeneity in PII expression that occurs within a colony variant.

(Figure 2). To examine the CTCTT repeat regions of PII genes in the recA strain, total chromosomal DNA of the variants was digested with the enzymes that cut on either side of the CTCTT repeat region, then electrophoresed, Southern blotted, and probed with the CTCTT-homologous oligonucleotide. Five major bands and several minor bands hybridized to the probe. A 90 bp band corresponding to 12 CTCTT repeats was present in the PIIa-expressing variant but absent in the three variants in which PIIa was turned off. In two of the PII- switchers, a new band of 95 bp corresponding to 13 CTCTT repeats was present. In the third PII- variant, no new band was
Figure 2. Analysis of PII Variants of FA1090 recA

(A) Western blot. Whole cells of different variants of FA1090 or FA1090recA were solubilized at 100°C, electrophoresed in a 4%-30% polyacrylamide gradient gel, transferred to nitrocellulose, and probed with MAb H.139 (PIIA-specific) and 125I-Protein A. The PIIa protein of FA1090 and FA1090recA migrates with an apparent molecular weight of 29K (solubilized at 100°C), determined by comparison to molecular weight standards. The three PII variants of FA1090recA were independent switchers derived from the PIIa variant of FA1090recA. Only the relevant portion of the gel is shown. (B) Southern blot. Chromosomal DNA of the four FA1090recA variants shown in (A) was digested with HinfI and Hhal, which cut in conserved sequences immediately flanking the CTCTT repeat regions of PII genes. The HinfI site is 22 bp upstream of the first CTCTT repeat; the Hhal site is 8 bp downstream of the last CTCTT repeat. The DNA fragments were electrophoresed, Southern blotted, and probed with the oligonucleotide specific for the CTCTT repeat region of the PIIa gene. The numbers at the right of the figure indicate the sizes of the fragments in bp (determined from the DNA sequencing reaction at the left side of the blot). The number of CTCTT repeats corresponding to the fragment lengths are indicated in parentheses.

Phase Variation of a PII-phoA Gene Fusion in E. coli

To determine if PII phase variation was dependent only on the region of the gene containing the CTCTT repeats, we constructed a gene fusion in which PII signal peptide coding DNA was fused to the structural gene for Escherichia coli alkaline phosphatase (phoA) with its own promoter and signal peptide coding region deleted, so that PhoA synthesis and export were under the control of the PII sequences (Hoffman and Wright, 1985). The gene fusion was constructed from a cloned out-of-frame PIIa gene, with the signal peptide coding DNA, the promoter, and approximately 350 bp of upstream sequence derived from the gonococcal clone (Figure 3). In the fusion construction, the codons for mature PhoA are in the same translational frame as those for mature PII protein, so that production of PhoA protein requires a frame-shift placing the phoA coding sequence in the same translational frame as the PII ATG initiation codon. If the ATG codon remains out-of-frame with the rest of the gene, translation of PhoA terminates prematurely. The activity of PhoA, which can be detected with the chromogenic substrate Xp, is dependent on secretion of the protein (Hoffman and Wright, 1985). Therefore, detection of PhoA production from an in-frame gene by blue colony color also requires appropriate secretion of the protein across the inner membrane.

E. coli HB101 containing the gene fusion cloned in pBR322 formed blue colonies of varying color intensity on media containing Xp, but no white colonies. However, when the fusion was cloned into plasmid pOU71 (Larsen et al., 1984), which replicates as a single copy per chromosome at 30°C, there was reversible phase variation of blue to white and white to blue colonies (Figure 4). The inability to detect blue-white phase variation when the gene fusion was present on a multicopy vector may have been due to independent phase variation of the gene copies in a cell, resulting in production of some PhoA by cells in each colony. All subsequent experiments with the PII-phoA gene fusion were done with pFLOB901 (the derivative of single-copy vector pOU71) in cultures maintained at 30°C.

The rate of phase variation of the PII-phoA fusion in E. coli HB101 (recA) containing pFLOB901 was 1.2 ± 0.2 x 10^-3/cell/generation for blue to white transitions and 2.6 ± 0.3 x 10^-4/cell/generation for white to blue transitions (mean ± sem; n = 10 independent colonies for each). In E. coli RR1, which is isogenic with HB101 except recA+, the rate for blue to white switching was 2.2 ± 0.4 x 10^-3/cell/generation (n = 6). Thus, the rate of blue-white phase variation in these strains of E. coli was similar to the rate of PII phase variation in the gonococcus, and was recA-independent.

We also cloned the gene fusion into pLES2, a gonococcal-E. coli shuttle vector, and mobilized it into the gono-
Figure 3. Diagram of the PII-phoA Gene Fusion (pFLOB8000)

The gene fusion was constructed by ligating a fragment containing the phoA gene (lacking its promoter and signal peptide coding region) into a unique site in an out-of-frame FA1090 Plla gene cloned in pBR322. The solid line indicates vector sequence, and the box indicates the fusion gene construction. The open area represents the inserted phoA-containing fragment; the solid black and dotted areas represent the Pllα coding sequence and flanking sequence, respectively, from the gonococcal clone. The upstream flanking sequence derived from the gonococcal clone (350 bp) does not contain any open reading frames with appropriate signals for initiation of transcription and translation (Connell et al., 1999). The DNA sequence at the fusion junction is shown, starting with the ATG initiation codon for the first amino acid of the PII signal peptide. The codons for phoA, starting at Glnt4, are in the same frame as the codons for mature PII protein. The Sall-Xhol fragment from pFLOB900 was cloned into the single copy vector pOU71, resulting in plasmid pFLOB901.

Mechanism of Phase Variation of the Gene Fusion

If phase variation of the gene fusion in E. coli involves SSM of the repeated CTCTT element, the process should show the following features: all changes will involve integral numbers of discrete repeat units; there will be a bias towards loss or gain of single units; and the rate of frameshifting will increase as the length of the repetitive region increases (Levinson and Gutman, 1987a, 1987b). To characterize the events accompanying blue–white phase variation of the fusion construction, we sequenced the signal peptide coding regions of 17 variants of HB101(pFLOB901) that had undergone PhoA phase transitions. In each case, phase transitions between blue and white colony types were accompanied by changes in the number of CTCTT repeats. Sixteen of the switches involved deletions (six) or insertions (10) of a single repeat; one switch was accompanied by insertion of four CTCTT units (data not shown). All of the blue colonies contained plasmid with a number of CTCTT repeats placing the ATG initiation codon and the codons for mature PhoA in the same frame; the fusion gene in all of the white colonies was out-of-frame. No other DNA sequence alterations occurred in the signal peptide coding region of these variants.

Once the sequence analysis indicated that the mechanism of phase variation did involve changes in CTCTT number, we analyzed a larger set of phase variants using the Southern blotting method described earlier (Figure 1) to measure the length of the CTCTT region in each variant. Fifty-one variants of HB101(pFLOB901) were analyzed. Every switch in PhoA expression was accompanied by a change in the length of the CTCTT repeat region, always by 5 bp or a multiple of 5 bp (data not shown). Forty-five were accompanied by insertion or deletion of 5 bp, 4 by changes of 10 bp, 1 by insertion of 20 bp, and 1 by a deletion of 35 bp. We also analyzed six PhoA phase transitions in strain RR1 (recA+) containing pFLOB901; all involved changes of 5 bp in the length of the CTCTT repeat region (one deletion and five insertions).

In each of three sequential cycles of isolating phase variants, we chose the ones with the longest and shortest CTCTT regions to use as the source for another cycle of variants (Figure 5). The longest signal peptide coding regions we obtained contained 17 CTCTT repeats in a PhoA+ colony and 25 repeats in a PhoA- colony. The shortest were 12 (PhoA+) and 11 (PhoA-) repeats. We do not know if these numbers represent the actual limits of length of the CTCTT repeats, or if greater extremes would be found by screening additional variants. The phase variation rate (white to blue) for the variant with 25 repeats (PhoA-25) was 4.6 ± 1.2 x 10^-4/cell/generation; the rate for the variant with 11 repeats (PhoA-11) was 1.9 ± 0.3 x 10^-4/cell/generation (five separate determinations for each). Southern blot analysis of plasmid from several of
the PhoA+ switchers derived from PhoA-25 showed that they generally lost large numbers of repeats (>6), whereas the PhoA+ derivatives of PhoA-11 gained single CTCTT repeats. It is possible that the mechanism for loss of CTCTTs from a longer repeating stretch might be different than from a shorter one. However, because every colony will actually contain a mixture of cells with different numbers of CTCTT repeats, it is impossible to conclude with certainty that those PhoA+ progeny arose in single steps from PhoA-25.

Because this gene fusion will not detect all possible frameshifts, it cannot be used to compare relative frequencies of deletion and insertion. For example, starting with a blue (PhoA+) colony, loss or gain of one CTCTT would result in a white (PhoA-) phenotype. Starting with a white colony with the gene in the -1 frame, addition of one CTCTT would cause a detectable phase shift to blue, but loss of one CTCTT would not change the phenotype of the colony. Since loss or gain of single CTCTT units was the most common event, the rate of blue to white phase variation should be greater than that of white to blue. Consistent with this prediction, the blue to white rate in HBl01 (pFLOB901) was 4-fold higher than the white to blue rate.

Detection of phase variation by blue colony formation also indicated that the unusual signal peptide encoded by the repeating CTCTT element functioned appropriately to direct secretion of the fusion protein in E. coli. The repeating sequence Leu–Phe–Ser–Ser–Leu encoded by the CTCTT repeats forms the hydrophobic core of the PII signal peptide. In a gene with 12 CTCTT, the hydrophobic core of the encoded signal peptide would be 20 amino acids long; that of a gene with 24 repeats would be 40 amino acids long. All are longer than the hydrophobic
cores of most prokaryotic signal peptides that have been studied (von Heijne, 1985; Pollitt and Inouye, 1987). Production of active alkaline phosphatase is dependent on secretion, but may not require processing of the signal peptide, so we do not know if the unusually long signal peptides were processed. The range of variation in CTCTT number will undoubtedly be constrained by the requirement for a translated signal peptide that will function in the secretion and localization of PII.

In these experiments, we analyzed a total of 74 phase transitions in E. coli containing the PII-phoA gene fusion. All of the transitions involved loss or gain of CTCTT units, as shown directly in variants that were sequenced and indirectly in variants analyzed by Southern blotting. There was no evidence for changes in other sequences in the signal peptide coding region or in partial CTCTT units. The majority of the transitions (67 of 74) involved loss or gain of single CTCTT units. The phase transition rate (white to blue) increased 2.5-fold with an increase from 11 to 25 CTCTT T repeats. Although this magnitude of increase in rate with increasing repetitive region length is not as dramatic as that observed for single-base runs (Streisinger and Owen, 1985; Pribnow et al., 1981; Kunkel, 1986), it is in rate with increasing repetitive region length is not as dramatic as that observed for single-base runs (Streisinger and Owen, 1985; Pribnow et al., 1981; Kunkel, 1986), it is similar to that obtained for a repeating 2 bp element (Levinson and Gutman, 1907b).

**Regulation of Gene Expression by SSM**

The process of SSM has been implicated in evolutionary expansion of repeated sequences and in mutational hotspots for frameshifts but has not heretofore been shown to be a mechanism for regulation of gene expression. The features of CTCTT-mediated frameshifting in the gonococcus and in E. coli were consistent with the model in which PII phase variation involves SSM. Phase variation was an inherent property of the repeated CTCTT sequence elements and did not depend on specific gonococcal factors or on mature PII coding sequences. Phase variation of the PII-phoA gene fusion in E. coli involved the same events (loss and gain of CTCTT) and occurred at the same high rate (10−3−10−5/cell/generation) as PII phase variation in the gonococcus. The process was recA-independent in both the gonococcus and E. coli, indicating that homologous recombination is probably not involved in these events. Although recA mutations do not abolish all recombinational events (Matfield et al., 1985), the high rate of frameshifting of the single cellular copy of the PII-phoA gene in the recA E. coli strain argues against the likelihood of a recombination-based mechanism. Even variation of the gene fusion in E. coli showed the predicted characteristics of loss or gain of integral numbers of CTCTT units, bias toward single unit changes, and increased frameshifting rate with increased length of the repetitive region.

SSM requires transient formation of single-stranded DNA, prior to the misalignment of copies of a repeated sequence. It is interesting to note that polypurimidine/polypyrimidine stretches of DNA, such as the CTCTT repeat studied here, are susceptible to local denaturation and the formation of unusual DNA structures (Johnston, 1988; Hun and Dahlberg, 1988; Wells, 1988). The data presented here support the hypothesis that phase variation of gonococcal PII proteins is dependent only on the special properties of the CTCTT repeat sequence in its interaction with the replication apparatus and is regulated by a novel mechanism involving SSM and frameshifting within the signal peptide coding regions of PII genes.

**Experimental Procedures**

**Bacterial Strains, Plasmids, and Plasmid Constructions**

N. gonorrhoeae strain FA1090 has been previously described (Black et al., 1984; Connell et al., 1986). FA1090 colony variants were grown on GC Base agar (Difco) with the supplements of Kellogg et al. (1963) at 37°C in a 5% CO2 atmosphere. All FA1090 variants were non-plasmid. E. coli strains HB101 (Maniatis et al., 1982), RRI (Maniatis et al., 1982), and JM83 (Veira and Messing, 1982) were grown on LB agar (Maniatis et al., 1982) or MacConkey agar (Difco). When appropriate, ampicillin (50 μg/ml) or X5 (5-bromo-4-chloro-3-indolyl phosphate, 40 μg/ml; Sigma) were added. Although all E. coli strains used possessed a chromosomal phoA locus, the gene is not expressed except under low phosphate conditions (Torriani, 1990). Colonies were white on plates of LB plus XP E. coli strains were made competent with CaCl2 and transformed as described (Maniatis et al., 1982).

Plasmid pFLOB907 contains the out-of-frame PII gene of FA1090 on a 1.6 kb Sau3A1 fragment cloned into the BamHl site of pBR322 (Connell et al., 1986). To construct the PII-phoA gene fusion (designated pFLOB900), pFLOB709 was linearized at a unique NotI site located 15 bp into the coding region of mature PIIa protein. The phoA gene, beginning with the codon for Glu1, was isolated on a 3 kb PstI fragment from plasmid pCH2 (Hoffman and Wright, 1985). Both DNA fragments were blunt ended with mung bean nuclease and ligated, resulting in plasmid pFLOB901. The 3 kb SalI-XhoI fragment was cloned into the unique SalI site of the E. coli--N. gonorrhoeae shuttle vector pLE52 (Stein et al., 1983), resulting in plasmid pFLOB902.

**Immunological Techniques**

The five MAbs specific for the six identified PII proteins of FA1090 have been previously described (Black et al., 1984; Barritt et al., 1987). Procedures for their use in ELISA, colony blot RIA, SDS-PAGE, and Western blotting have been described (Black et al., 1984).
Southern Hybridizations
Gonococcal chromosomal DNA was isolated by the method of Stern et al. (1984). Plasmid DNA was isolated by the alkaline lysis method (Maniatis et al., 1982). DNA was digested with restriction enzymes (BRL, New England Biolabs) according to the manufacturer’s specifications.

For analysis of chromosomal fragments, Clal-digested FA1090 DNA was electrophoresed through 0.7% agarose gels; DNA in the 8–10 kb size range was electroeluted and further digested with Hinfi and HhaI, which cut on either side of the CTCCT repeat region in the signal peptide coding region of PII genes. The Hinfi site is 22 bp upstream of the first CTCCT repeat; the HhaI site is 8 bp downstream of the last CTCCT repeat. The sites for these enzymes are conserved in all published PII gene sequences (Stern et al., 1986; Connell et al., 1988; van der Ley, 1988). The enzymes Hinfi and Ddel were used to isolate the corresponding region of the PII-phoAgene fusion from pFLOB901; the Ddel site is 5’ to downstream of the last CTCCT repeat in the gene fusion construction. Small chromosomal and plasmid DNA fragments were electrophoresed through 6% polyacrylamide–8 M urea gels and electroblotted to GeneScreen (New England Nuclear) or Zetabind (AMF)

basal's solution, and 20 mM NaPPi at 42°C (Maniatis et al., 1982). Oligonucleotides were synthesized and purified as previously described (Connell et al., 1988). Hybridizations were done in 0.5x SSPE (1x SSPE: 150 mM NaCl, 10 mM NaH2PO4, 1 mM EDTA [pH 7.4], 0.5% SDS, 5x Denhardt’s solution, and 20 mM NaPPi) at 42°C (Maniatis et al., 1982). Filters were washed twice in 1x SSPE, 1x SDS, 5 mM NaPPi at 42°C–45°C for 45 min under high stringency conditions. Size standards were sequencing reactions done on M13 clones of known DNA sequence by the method of Sanger et al. (1977).

Measurement of Phase Transition Rates
Rates of phase transition were determined as described by Eisenstein (1981), using the formula (MN)/g. Where M/N is the ratio of the minority population (PhoA+ or PhoA–) to the total population of colony-forming units, and g is the number of generations of growth from a single cell to the total number of cells within a colony. Control phase transition rates (PhoA+ to PhoA–) for E. coli HB101(pOU71) and RR1(pOU71) were less than 1 x 10–9/Cell/generation.

DNA Sequencing
Plasmids used for DNA sequencing were prepared as described above. An 820 bp Sph1-SalI fragment of pFLOB91 from PhoA expression switchers was cloned into M13mp18 (New England Biolabs) of the PII gene (5’-CCGCTTTGAAGCATCAGTG-3’). DNA from at least four independent M13 clones was sequenced for each of the PhoA variants analyzed, to exclude the possibility that changes in the length of the CTCCT region were occurring during the M13 transfection (Levinson and Gutman, 1980).

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