

NOTES

Pathogenic Neisseriae Can Use Hemoglobin, Transferrin, and Lactoferrin Independently of the *tonB* Locus

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Redundant TonB systems which function in iron transport from TonB-dependent ligands have recently been identified in several gram-negative bacteria. We demonstrate here that in addition to the previously described *tonB* locus, an alternative system exists for the utilization of iron from hemoglobin, transferrin, or lactoferrin in *Neisseria meningitidis* and *Neisseria gonorrhoeae*. Following incubation on media containing hemoglobin, *N. meningitidis* IR3436 (*tonB exbB exbD* deletion mutant) and *N. gonorrhoeae* PD3401 (*tonB* insertional mutant) give rise to colonies which can grow with hemoglobin. Transfer of Hb⁺ variants (PD3437 or PD3402) to media containing hemoglobin, transferrin, and/or lactoferrin as sole iron sources resulted in growth comparable to that observed for the wild-type strains. Transformation of *N. meningitidis* IR3436 or *N. gonorrhoeae* PD3401 with chromosomal DNA from the Hb⁺ variants yielded transformants capable of growth with hemoglobin. When we inactivated the TonB-dependent outer membrane hemoglobin receptors (HmbR or HpuB) in the *Neisseria* Hb⁺ variants, these strains could not grow with hemoglobin; however, growth was observed with transferrin and/or lactoferrin. These results demonstrate that accumulation of iron from hemoglobin, transferrin, and lactoferrin in the pathogenic neisseriae can occur via a system that is independent of the previously described *tonB* locus.

Acquisition of iron from transferrin, lactoferrin, heme, and heme-containing compounds in the pathogenic *Neisseria* spp. involves a family of distinct iron-regulated outer membrane receptors (1, 3, 5, 7–11, 14–16, 20, 21). The iron-repressible hemoglobin receptor HmbR of *Neisseria meningitidis* (20, 21) and HpuB of *N. meningitidis* and *N. gonorrhoeae* (7, 15, 16) are required for binding and utilization of hemoglobin or hemoglobin bound to haptoglobin, respectively. A second gene (*hpuA*), predicted to encode a lipoprotein, is found upstream of *hpuB*, and recent studies have demonstrated that both HpuA and HpuB are required for the utilization of hemoglobin in the pathogenic *Neisseria* spp. (7, 16). An *N. gonorrhoeae* *hmbR* homolog has been identified; however, the presence of a stop codon in the coding sequence in the gonococcal *hmbR* gene indicates that *N. gonorrhoeae* does not produce a functional HmbR (7, 21).

Energy for the transport of iron from host iron-binding proteins across the outer membrane into the periplasmic space in a number of gram-negative organisms is provided by TonB, in association with the ExbB and ExbD proteins (6, 12). The TonB system uses the proton motive force of the cytoplasmic membrane for the passage of ligands into the periplasm. Receptors which require energy supplied via the TonB system are termed TonB dependent and have amino acid homology in several regions termed TonB boxes. The TonB box represents the domain of the bacterial receptor which physically interacts with the energy-transducing protein TonB. The *Neisseria* HmbR and HpuB hemoglobin receptors have homology to TonB-dependent outer membrane receptors (7, 20), and

N. meningitidis and *N. gonorrhoeae* *tonB*, *exbB*, and *exbD* homologs have been identified (2, 22). *N. meningitidis* and *N. gonorrhoeae* *tonB*, *exbB*, and *exbD* insertional mutants were observed to grow with hemin; however, these mutants were reported to be deficient in the ability to utilize hemoglobin, transferrin, and/or lactoferrin (2, 22).

It was recently reported that in *Vibrio cholerae*, a second TonB ExbB ExbD system is operative, and this system was demonstrated to functionally replace the TonB1 system for the acquisition of TonB-dependent ligands associated with the accumulation of iron (17). Likewise, in *V. parahaemolyticus*, *V. alginolyticus*, *Serratia marcescens*, *Yersinia pestis*, and *Pseudomonas aeruginosa*, two *tonB* genes have been identified (13, 18, 24). Based on these recent reports, we have examined a newly constructed *N. meningitidis* *tonB exbB exbD* deletion mutant and an *N. gonorrhoeae* *tonB* insertional mutant for the ability to grow with heme, hemoglobin, transferrin, and/or lactoferrin as sole iron sources. We have demonstrated that in addition to the previously described *tonB* locus, an alternative system is operative in *N. meningitidis* and *N. gonorrhoeae* for the utilization of iron from hemoglobin, transferrin, and/or lactoferrin. We have clearly demonstrated that this system can function with the TonB-dependent receptors HmbR in *N. meningitidis* and HpuB in *N. gonorrhoeae* for growth with hemoglobin.

Bacterial strains and growth conditions. *N. meningitidis* strain IR1072 is a serogroup C clinical isolate which is *hmbR hpuB*. Attempts to PCR amplify the *hpuB* gene in IR1072 failed; however, we were able to amplify the *hpuB* gene from IR1075 using the same primers (data not shown). In addition, strain IR1072 is unable to grow with hemoglobin complexed to haptoglobin (20, 21). *N. meningitidis* IR3436 is a *tonB exbB exbD* deletion mutant constructed following transformation of strain IR1072 with a plasmid construct in which the *tonB exbB exbD* locus was deleted and replaced with the spectinomycin

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TABLE 1. Bacterial strains used in this study

Strain	Genotypic and phenotypic description	Reference or source
<i>N. meningitidis</i>		
IR1072	Clinical isolate, serogroup C (<i>hmbR</i> ⁺ <i>hpuB</i>)	20
IR3436	IR1072 but with <i>tonB</i> <i>exbB</i> <i>exbD</i> deletion	Stojiljkovic, unpublished
PD3437	IR3436 variant which grows with hemoglobin, transferrin, and lactoferrin	This study
PD3438	PD3437 but with <i>hmbR</i> ::Km ^r	This study
PD3439	IR3436 transformed with genomic DNA from PD3437	This study
IR1075	Clinical isolate, serogroup C (<i>hmbR</i> ⁺ <i>hpuB</i> ⁺)	21
IR2200	IR1075 but with <i>tonB</i> ::Km ^r	22
IR1098	IR1072 but with <i>hmbR</i> ::Km ^r	22
MC58	Clinical isolate, serogroup B (<i>hmbR</i> ⁺ <i>hpuB</i> ⁺)	Lab collection
MC5801	MC58 but with <i>tonB</i> <i>exbB</i> <i>exbD</i> deletion	This study
<i>N. gonorrhoeae</i>		
340	Wild type (<i>hmbR</i> ⁺ <i>hpuB</i> ⁺), but HmbR phenotypically not functional	Lab collection
PD3401	340 but with <i>tonB</i> ::Km ^r	This study
PD3402	PD3401 variant which grows with hemoglobin and transferrin	This study
PD3403	PD3402 but with <i>hpuB</i> ::Erm ^r	This study
PD340E	340 but with <i>hpuB</i> ::Erm ^r	Lab collection

gene (I. Stojiljkovic, unpublished data). Genomic DNA isolated from IR3436 was used to transform *N. meningitidis* MC58, the serogroup B clinical isolate which has been fully sequenced, generating strain MC5801, an additional *tonB* *exbB* *exbD* deletion mutant. The *N. gonorrhoeae* *tonB* insertional mutant was constructed by transformation (4) of *N. gonorrhoeae* 340 with *N. meningitidis* IR2200 chromosomal DNA (Table 1). *N. gonorrhoeae* strain 340 does not utilize lactoferrin as an iron source. *N. meningitidis* or *N. gonorrhoeae* strains

were typically maintained on gonococcal base (GCB) medium containing 1% Kellogg supplement and incubated aerobically (5% CO₂) at 37°C for 16 to 18 h (9). When necessary, kanamycin (50 µg/ml), erythromycin (1.5 µg/ml), or spectinomycin (100 µg/ml) was used.

Characterization of *Neisseria tonB* mutants. The *tonB* mutation in *N. meningitidis* and *N. gonorrhoeae* strains was confirmed by either colony or genomic PCR analysis. The deletion of the *tonB* *exbB* *exbD* genes in *N. meningitidis* strains IR3436,

TABLE 2. Oligonucleotide primers for PCR confirmation of *N. meningitidis* and *N. gonorrhoeae* *tonB*, *hmbR*, and *hpuB* mutations

Primer	Sequence	Wild-type product	Mutant product
del-A	5' GCGATCCGGTCTCAGTGGGTATAGC 3'	<i>tonB</i> , <i>exbB</i> , <i>exbD</i> genes and 400 nt ^a of flanking DNA upstream of the <i>tonB</i> gene and downstream of the <i>exbD</i> gene (2.8-kb fragment)	3.0-kb fragment corresponding to the omega cassette (2.2 kb) with 400 nt of flanking DNA
del-D	5' GGCTCGAGCGATATGGGGCAGGGGGTTCA 3'		
tonB-F2	5' TGCTATTATTATGCCCGAATT 3'	In <i>N. meningitidis</i> , F2 binds nt 101 upstream and R1 anneals to nt 724 downstream of the putative +1 initiation start codon (ATG) of the <i>tonB</i> gene, producing a 825-bp fragment	<i>tonB</i> gene with a kanamycin cassette insertion, producing a fragment of 2.03 kb
tonB-R1	5' TTTTCACGATTTTAACGGAC 3'		
		In <i>N. gonorrhoeae</i> , F2 anneals to nt 21 upstream and R2 anneals to nt 739 downstream of the putative +1 initiation start codon (ATG) of the <i>tonB</i> gene, producing a 760-bp fragment	<i>tonB</i> gene with a kanamycin cassette insertion, producing a fragment of 1.96 kb
hmbR-F1	5' AACAAAGAAATGATACGCGAC 3'	F1 anneals to nt 184 and R1 anneals to nt 928 downstream of the putative +1 initiation start codon (ATG) of the <i>hmbR</i> gene, producing a 744-bp fragment	<i>hmbR</i> gene with a kanamycin cassette insertion, producing a fragment of 1.94 kb
hmbR-R1	5' AGAGGTTGGTGTACGCCGT 3'		
hpuB-F3	5' TCAAACCCGTATTGGCTG 3'	F3 anneals to nt 11 upstream of the putative +1 initiation start codon (ATG). R2 anneals to nt 2 upstream of the putative stop codon (TAA) of the <i>hpuB</i> gene, producing a 2.39-kb fragment	<i>hpuB</i> gene with a erythromycin cassette insertion, producing a fragment of 3.69 kb
hpuB-R2	5' GCACATCAGGAGCTTAGA 3'		

^a nt, nucleotides.

PD3437, and PD3439, as well as in strain MC5801, was confirmed using primers del-A and del-D, designed to amplify the entire *tonB* *exbB* *exbD* locus and 400 bp of flanking DNA upstream of *tonB* gene and downstream of the *exbD* gene (Table 2). To confirm the disruption of the *tonB* gene in the *N. gonorrhoeae* insertional mutant, primers tonB-F2 and tonB-R1 were designed to amplify homologous regions between the 5' end of the gene, upstream of the ATG codon, and the 3' end of the gene, upstream of the stop codon. Briefly, a single colony was suspended in 100 μ l of distilled H₂O and incubated at 80 to 100°C for 5 min. A 10- μ l volume of this crude preparation was then used for colony PCR analysis. Growth of *N. meningitidis* and *N. gonorrhoeae* strains was examined in a plate assay or in broth as described in the figure legends.

Construction of *N. meningitidis* *hmbR* and *N. gonorrhoeae* *hpuB* mutants. *N. meningitidis* *hmbR* and *N. gonorrhoeae* *hpuB* insertional mutants were constructed in *N. meningitidis* strain PD3437 and *N. gonorrhoeae* strain PD3402, respectively, by transformation as previously described (4). To disrupt the *hpuB* gene, *N. gonorrhoeae* strain PD3402 was transformed using chromosomal DNA (1.0 μ g) from *N. gonorrhoeae* strain PD340E (Table 1) and transformants were selected on GCB plates containing erythromycin (1.5 μ g/ml) and kanamycin (2 μ g/ml). To disrupt the *hmbR* gene, *N. meningitidis* strain PD3437 was transformed using chromosomal DNA (1.0 μ g) from *N. meningitidis* strain IR1098 (Table 1) and transformants were selected on GCB plates containing kanamycin (25 μ g/ml) and spectinomycin (50 μ g/ml). The inactivation of *hmbR* and *hpuB* genes in strains PD3437 and PD3402 was confirmed by PCR (data not shown).

Utilization of hemoglobin, transferrin, and lactoferrin is not absolutely dependent on the *tonB* locus in the pathogenic *Neisseria* spp. To determine if an alternate system independent of the previously described *tonB* locus could function in hemoglobin utilization in *N. meningitidis*, we examined the ability of an *N. meningitidis* *tonB* *exbB* *exbD* deletion (IR3436) mutant to grow with different iron sources. Both the wild-type and *tonB* *N. meningitidis* strains were capable of growth with Fe or heme as sole iron sources (data not shown). Discrete colonies around hemoglobin disks were observed with *N. meningitidis* strains IR3436 (*tonB* *exbB* *exbD* deletion) (Fig. 1B) and MC5801 (*tonB* *exbB* *exbD* deletion) (data not shown). Furthermore, when a single colony of *N. meningitidis* IR3436 isolated from around a hemoglobin disk was transferred to fresh GCB plates supplemented with desferal, spectinomycin, and hemoglobin and incubated for 18 h, confluent growth was observed (data not shown). We designated this hemoglobin-utilizing variant strain PD3437. When PD3437 was examined for the ability to use other iron sources in a plate assay, growth comparable to that of the wild-type strain IR1072 around disks containing hemoglobin, transferrin, or lactoferrin was observed (Fig. 1B). Furthermore, we also examined the growth of *N. meningitidis* strain PD3437 in liquid culture with hemoglobin or heme as a sole iron source. For these studies, hemoglobin was supplemented with human serum albumin. In agreement with the results of the plate assays, we found that with hemoglobin as the sole iron source, *N. meningitidis* strain PD3437 grew at levels comparable to the original wild-type strain IR1072 (data not shown).

The *tonB* *exbB* *exbD* deletion in *N. meningitidis* strains IR3436 and PD3437 was confirmed by PCR analysis. As expected, we amplified a 2.8-kb fragment in the wild-type strain IR1072 and a 3.0-kb fragment in the *tonB* *exbB* *exbD* deletion mutants, strains IR3436 and PD3437 (Fig. 1A). Taken together, these results indicate that a stable change allowing the acquisition of iron from hemoglobin, transferrin, and lactofer-

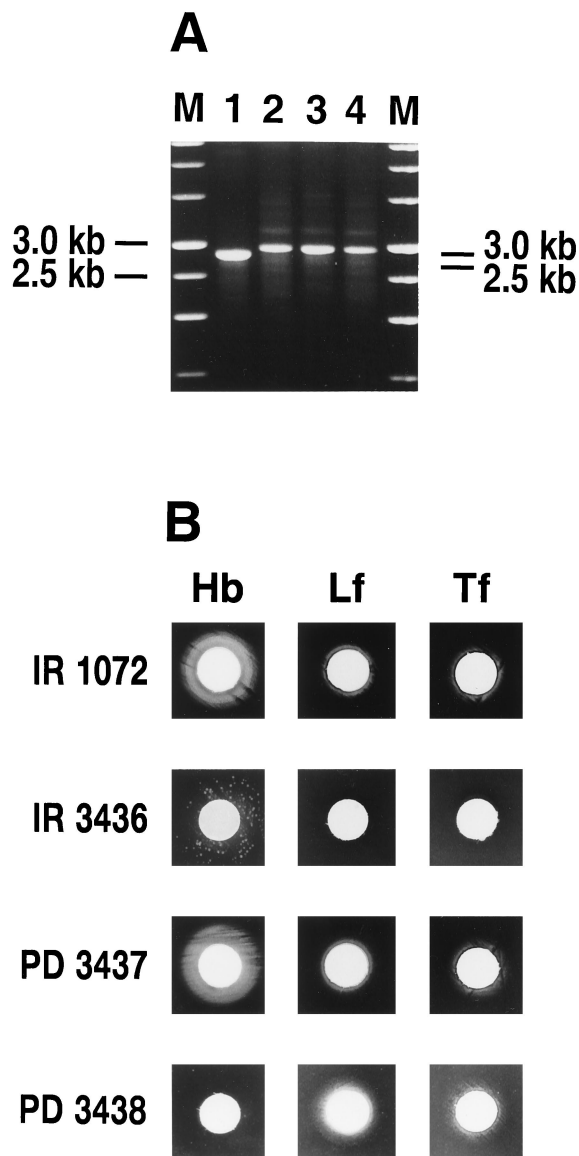


FIG. 1. (A) The *tonB* *exbB* *exbD* deletion in *N. meningitidis* strains IR3436, PD3437, and PD3439 was confirmed by PCR. The *tonB* gene was amplified by colony PCR using *N. meningitidis* primers specific for the *tonB* gene (Table 2). Lanes: M, molecular size standard; 1, IR1072; 2, IR3436; 3, PD3437; 4, PD3438. Molecular sizes are indicated on the left; those on the right correspond to the approximate sizes of the expected amplified products. (B) Growth of *N. meningitidis* IR3436, PD3437, and PD3438 with various iron sources. A 10- μ l volume of human hemoglobin (Hb) (10 mg/ml) or an iron-saturated human transferrin (Tf) (50 mg/ml) or lactoferrin (Lf) (25 mg/ml) was used. These solutions were applied to paper disks and placed onto the GCB-desferal (60 μ M) plates seeded with bacteria (10^8 CFU). Strain IR1072 is the wild-type strain from which IR3436 and PD3437 were derived. Growth was recorded after 18 to 36 h; results are from one experiment and are representative of three separate experiments.

rin had occurred and suggest that a locus independent of *tonB* *exbB* *exbD* can be utilized for growth with TonB-dependent ligands. Such a phenotype would be expected if a silent homolog of the *tonB* locus was present in *N. meningitidis*.

Using genomic DNA from the *N. meningitidis* *tonB* mutant (IR2200), we constructed a gonococcal *tonB* mutant in strain 340 and demonstrated that this strain (PD3401) grew with heme and ferric nitrate as sole iron sources (data not shown).

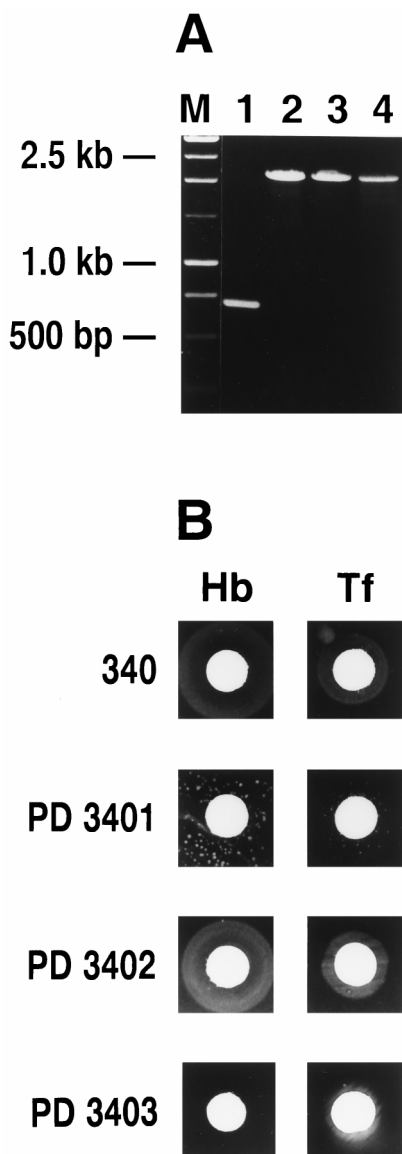


FIG. 2. (A) Insertional inactivation of the *tonB* gene in *N. gonorrhoeae* strains PD3401, PD3402, and PD3404, was confirmed by PCR as described in the legend to Fig. 1. Lanes: M, molecular size standard; 1, 340; 2, PD3401; 3, PD3402; 4, PD3403. Molecular sizes are indicated on the left. (B) Growth of *N. gonorrhoeae* PD3401, PD3402, and PD3403 with various iron sources. Strains were examined for their ability to utilize various iron sources in a plate assay as described in the legend to Fig. 1. *N. gonorrhoeae* strain 340 is the wild-type strain from which strains PD3401 and PD3402 were derived. Growth was recorded after 18 to 36 h; results are from one experiment and are representative of three separate experiments. Hb, hemoglobin; Tf, transferrin.

As observed with the *N. meningitidis tonB* deletion mutant, we also observed colonies around disks containing hemoglobin and transferrin with strain PD3401 (Fig. 2B). We further characterized a hemoglobin-utilizing variant of strain PD3401 and designated this variant PD3402. When PD3402 colonies were transferred to GCB plates supplemented with desferal, kanamycin, and hemoglobin, confluent growth was observed (Fig. 2B). Furthermore, when PD3402 was transferred to media containing disks with various iron sources, growth was observed around the transferrin and hemoglobin disks, and this was comparable to the growth observed for the wild-type strain (strain 340) (Fig. 2B). To confirm that the hemoglobin utiliza-

tion phenotype was not due to a reversion of the *tonB* mutation, we amplified the *tonB* gene from strains 340, PD3401, and PD3402 by PCR using primers specific for the *tonB* gene. As expected, the wild-type *tonB* gene was amplified as a ~760-bp fragment from strain 340 whereas a ~2.0-kb fragment was amplified in strains PD3401 and PD3402 (Fig. 2A). This was also confirmed by Southern blot hybridization analysis using the *tonB* gene as a probe; one intact copy of the *tonB* gene was observed in strain 340, and one copy of the disrupted *tonB* gene was observed in PD3401 and PD3402 (data not shown).

Utilization of hemoglobin independently of the *tonB* locus is dependent on the *N. meningitidis* HmbR and *N. gonorrhoeae* HpuB hemoglobin receptors. We next inactivated the hemoglobin receptors HmbR in *N. meningitidis* PD3437 and HpuB in *N. gonorrhoeae* PD3402. *N. meningitidis* strain IR3436, from which strain PD3437 was derived, is naturally *hpuB*, and thus inactivation of *hmbR* in this strain should result in a strain unable to grow with hemoglobin as a sole iron source. Likewise, since the *hmbR* locus is not expressed in *N. gonorrhoeae* (7, 21), the inactivation of the *hpuB* gene in *N. gonorrhoeae* strain PD3402 should result in a strain unable to grow with hemoglobin as the sole iron source. If the ability to utilize hemoglobin independently of the *tonB* locus was due to the removal of heme independent of HmbR or HpuB and subsequent transport into the cell via a heme acquisition pathway, we would expect that *hmbR hpuB* strains should still utilize heme from hemoglobin for growth, since heme transport in *N. meningitidis* and *N. gonorrhoeae* occurs via an HmbR- and HpuB-independent mechanism (16, 20). The inactivation of *hmbR* or *hpuB* in these strains was confirmed by PCR. As expected, we observed a ~744-bp PCR fragment using primers specific to an internal fragment of the *hmbR* gene in *N. meningitidis* strain PD3437 (data not shown). A PCR fragment of ~1.9 kb was observed in the *N. meningitidis hmbR* insertional mutant strain PD3438, corresponding to the insertion of the kanamycin cassette. Likewise, using primers specific for the *N. gonorrhoeae hpuB* gene, we observed a ~2.4-kb fragment in *N. gonorrhoeae* strain PD3402 (data not shown). A PCR fragment of ~3.7 kb was observed in *N. gonorrhoeae* strain PD3403, corresponding to the insertion of the erythromycin cassette into this strain (data not shown).

N. meningitidis PD3438 and *N. gonorrhoeae* PD3403 exhibited normal growth with heme (data not shown), transferrin, and/or lactoferrin compared to the wild-type strains IR1072 and 340 (Fig. 1B and 2B). However, we did not observe any growth of strain PD3438 or PD3403 with hemoglobin as the sole iron source (Fig. 1B and 2B). These results clearly indicate that the ability to utilize hemoglobin independently of the above-described *tonB* locus in *N. meningitidis* and *N. gonorrhoeae* does not result from the removal of heme and subsequent transport into the cell via a heme acquisition pathway. These results also indicate that in *N. meningitidis* and *N. gonorrhoeae* a second TonB system must function with HmbR or HpuB for the transport of iron from hemoglobin. Furthermore, our observations suggest that the putative second *tonB* locus may be silent, and expression can be detected only when the first *tonB* locus is inactive.

The ability to utilize hemoglobin independently of the *tonB* locus can be genetically transformed into *N. meningitidis* IR3436 or *N. gonorrhoeae* PD3401. The frequency of the appearance of *N. meningitidis* hemoglobin-utilizing (Hb⁺) variants for strain IR3436 was determined to be 1.37×10^{-7} . Likewise, the frequency of the appearance of *N. gonorrhoeae* Hb⁺ variants for strain PD3401 was similar to that observed for *N. meningitidis* (2.9×10^{-7}). To determine if the ability to utilize hemoglobin independently of the *tonB* locus was encoded by a gene which

was now functional in the Hb⁺ variant (strain PD3437), we transformed *N. meningitidis* IR3436 with chromosomal DNA isolated from strain PD3437. Transformation of *N. meningitidis* strain IR3436 with chromosomal DNA from strain PD3437 yielded transformants capable of growth with hemoglobin at a frequency of 1.07×10^{-4} . We randomly picked Hb⁺ transformants and transferred these colonies to media containing disks with various iron sources; for all Hb⁺ transformants tested, growth was observed around hemoglobin-, transferrin-, and lactoferrin-containing disks and was comparable to that observed for strain PD3437 (data not shown). The deletion of the *tonB* *exbB* *exbD* genes in *N. meningitidis* strain PD3439 was confirmed by PCR analysis (Fig. 1A).

Likewise, we obtained similar results when we transformed *N. gonorrhoeae* strain PD3401 with chromosomal DNA isolated from the Hb⁺ variant (strain PD3402). Transformation of *N. gonorrhoeae* strain PD3401 with chromosomal DNA isolated from strain PD3402 yielded transformants capable of growth with hemoglobin at a frequency of 4.9×10^{-3} . We also randomly picked *N. gonorrhoeae* Hb⁺ transformants and transferred these colonies to media containing disks with various iron sources. As observed for *N. meningitidis* PD3439, we observed proficient growth of strain PD3404 around transferrin- and hemoglobin-containing disks. We also confirmed the *tonB* mutation in strain PD3404 by PCR analysis (Fig. 2A). These results indicate that the ability to utilize hemoglobin independently of the previously described *tonB* locus is encoded by a gene which is functional and stable in *N. meningitidis* strain PD3437 and *N. gonorrhoeae* strain PD3402.

Concluding remarks. The results obtained in this study indicate that the previously described *tonB* locus is not solely responsible for the utilization of hemoglobin, transferrin, or lactoferrin in the pathogenic *Neisseria* spp. Based on these results, we postulate that *N. meningitidis* and *N. gonorrhoeae* possess a second, alternative TonB system for the utilization of iron from TonB-dependent ligands. Our results also indicate that in *N. meningitidis* and *N. gonorrhoeae* the second TonB system clearly functions with HmbR or HpuB for the acquisition of iron from hemoglobin. We found that the frequency of colonies which could grow with hemoglobin and transferrin was relatively low. Indeed, this may explain why, in previous studies (2, 22), these colonies either were not observed or appeared at a relatively low frequency (depending on the duration of incubation) and therefore were not reported. In addition, differences in the growth assays, the media used for plate assays (complex versus defined), the concentration of the various iron sources, or the concentration of organisms used may explain this discrepancy. In our studies we utilized plate assays with 100 µg of hemoglobin per disk (1.5 µM final concentration), whereas Stojiljkovic and Srinivasan (22) used plate assays with 50 µg of hemoglobin per disk (0.75 µM final concentration). In studies reported by Biswas et al. (2), the ability of gonococcal *tonB* *exbB* *exbD* mutants to utilize various iron sources was examined by streaking colonies on CDM plates containing various iron sources with a final hemoglobin concentration of 1 µM. Our observations suggest that the putative second *tonB* locus may be silent and that expression can be detected only when the first *tonB* locus is inactive. Furthermore, the ability to obtain stable Hb⁺ transformants following transformation with chromosomal DNA from the *N. meningitidis* or *N. gonorrhoeae* Hb⁺ variants (PD3437 or PD3402 respectively) indicates that, once this silent second *tonB* locus is expressed, its expression is stable.

In summary, we propose that the pathogenic *Neisseria* spp. possess a second TonB system which functions to transduce energy to TonB-dependent outer membrane receptors. Exam-

ination of the gonococcal and recently completed meningococcal database for *Neisseria tonB* homologs did not reveal any open reading frames with significant homology to any previously described *tonB* locus. This is not an unexpected finding, given that *V. cholerae tonB2* has only 23% homology with *Neisseria tonB*. Similarly, *P. aeruginosa tonB2* demonstrates 28% homology to *P. aeruginosa tonB*. Attempts to find conserved regions in both *tonB* proteins among these species were unsuccessful. We have constructed a genomic library from the hemoglobin-utilizing variant *N. meningitidis* PD3437, and studies are currently in progress to identify the second *Neisseria tonB* locus by complementation analysis.

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