Identification of an EF-Tu protein that is periplasm-associated and processed in Neisseria gonorrhoeae

Stephen F. Porcella, R. J. Belland and R. C. Judd

INTRODUCTION

Elongation factor-Tu (EF-Tu) is a ubiquitous protein involved in protein synthesis and may compose 10% of the total cellular protein of a rapidly dividing Escherichia coli bacterium (Van der Meide et al., 1982). EF-Tu has also been described as the dominant protein released in periplasmic extracts of E. coli (Jacobson et al., 1976). In Gram-negative bacteria, EF-Tu is typically encoded by two almost identical genes, tufA and tufB. Therefore, EF-Tu is a generic term which may be applicable to either EF-TuA, EF-TuB, or both. These tuf genes have been identified and well characterized in E. coli and they differ in only one amino acid (Arai et al., 1980; Jones et al., 1980). During conditions of nutrient limitation, EF-Tu appears to be methylated and cytoplasmically membrane bound (Young & Bernlohr, 1991; Young et al., 1990). Southern analysis has demonstrated that the gonococcus (Neisseria gonorrhoeae) has two tuf genes (Goldstein et al., 1989); a situation similar to that in E. coli (Jaskunas et al., 1975) and Salmonella typhimurium (Hughes, 1990).

The 41–45 kDa proteins of N. gonorrhoeae have had numerous characteristics described for them (Chen et al., 1984; Hill & Judd, 1988, 1989; Judd et al., 1991; Shafer, 1988; Shafer & Judd, 1991; Shafer & Morse, 1987; Swanson, 1981; Zak et al., 1984). Two closely migrating 44 kDa proteins have been found in periplasm and non-periplasm fractions of N. gonorrhoeae (Judd & Porcella, 1993; Porcella & Judd, 1993). In an effort to determine which 44 kDa proteins are responsible for the observed characteristics, we purified a 44 kDa protein from the periplasm of N. gonorrhoeae. This protein was cleaved with cyanogen bromide (CNBr) and a peptide fragment was sequenced and found to be highly homologous to EF-Tu (Judd & Porcella, 1993).

In this report we describe the further purification of this periplasm-associated 44 kDa protein and the production of polyclonal antibodies. This antiserum, designated 44 kDa periplasmic antiserum, was preabsorbed with E. coli lysates and immunoblotted against whole-cell lysates of N. gonorrhoeae. Reactivity of the antisera to a 44 kDa

A 44 kDa protein is a dominant component of periplasmic extracts of Neisseria gonorrhoeae. Peptide sequence generated from a cyanogen-bromide-cleaved fragment of this protein indicated sequence homology with elongation factor-Tu (EF-Tu). Polyclonal antiserum was made against the 44 kDa protein purified from periplasm extracts of N. gonorrhoeae. The preabsorbed antiserum was immunoblotted against whole-cell lysates on two-dimensional gels. A 44 kDa protein and a smaller 37 kDa protein were recognized by this antiserum. A N. gonorrhoeae λ phage DNA library was screened and a clone expressing a 44 kDa protein was identified. The DNA insert in this clone contained several genes homologous to genes contained in the str operon of Escherichia coli. One ORF product with a calculated molecular mass of 43 kDa was highly homologous to the EF-TuA of E. coli. A synthetic peptide antiserum specific for a portion of the C terminus of EF-Tu confirmed that the 37 kDa protein in whole-cell lysates of N. gonorrhoeae was a processed form of EF-Tu. Deletion of the tufA gene homologue in N. gonorrhoeae was attempted but was unsuccessful.

Keywords: Neisseria gonorrhoeae, tufA, EF-Tu, processing

The GenBank accession number for the sequence reported in this paper is L36380.
protein and a smaller 37 kDa protein implied that the antiserum was sufficiently specific. A gonococcal library was immunoblotted with the polyclonal antiserum and a clone was identified which constitutively produced only one 44 kDa protein. Sequence analysis of the N. gonorrhoeae DNA within this clone indicated that it contained several str operon-like genes, including a single ORF with a predicted product of molecular mass 44 kDa that was highly homologous to EF-TuA.

EF-Tu has recently been shown to be a primary target in a phage exclusion mechanism where the majority of the molecule is cleaved at a highly conserved site producing a 37 kDa protein (Yu & Snyder, 1994). We used a synthetic, EF-Tu-specific, peptide antiserum previously described (Judd & Porcella, 1993), to probe N. gonorrhoeae whole-cell lysates in two-dimensional gels. We found that both the 44 kDa protein and the smaller 37 kDa protein reacted with the antiserum. These results indicate that the 37 kDa protein in N. gonorrhoeae is a processed form of EF-Tu.

Analysis of the function of EF-TuA and B in E. coli has recently been shown to be a primary target in the transposon-inactivated gene in an unknown genomic location.

METHODS

Bacteria. These included N. gonorrhoeae strains MS11 (courtesy of John Swanson, Rocky Mountain Laboratory, Hamilton, MT) and FA19 (courtesy of William Shafer, Dept of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA). E. coli XL1 was obtained from Stratagene.

Transparent, non-piliated gonococci were grown at 37 °C on clear typing medium as previously described (Swanson, 1982). Cells were passaged daily and large transparent colonies were selected. Cells were harvested at 18 h and washed twice in Dulbecco's PBS (DPBS).

Protein purification. Protocols for whole-cell lysates have been previously described by Judd (1982). Periplasmic fractions of N. gonorrhoeae were isolated as previously described (Judd & Porcella, 1993). Five hundred microlitres of solubilized FA19 periplasmic extract was layered onto 9% (v/v) SDS-PAGE preparative gels and electrophoresed. Gels were stained and the periplasmic extract was layered onto discontinuous buffer system of Laemmli (1970). High-resolution two-dimensional gel electrophoresis was performed using the Bio-Rad's Mini-Protean II 2-D kit.

Preparation of antisera. Monospecific, polyclonal rabbit antisera was generated against a N. gonorrhoeae strain FA19 periplasm-associated 44-kDa protein following standardized immunological protocols (Judd, 1991). Preabsorption of the antiserum was performed by first growing E. coli strain XL1-Blue (Stratagene) overnight in 100 ml Luria-Bertani (LB) medium. Cells were harvested by centrifugation at 5000 g for 10 min at 4 °C and resuspended in 3 ml 50 mM Tris/Cl (pH 8.0), 10 mM EDTA (pH 8.0). The suspension was frozen and thawed three times and sonicated at full power for six periods of 20 s at 4 °C. DNAse (Sigma) was added to the homogenate to a final concentration of 1 unit ml⁻¹ and centrifuged at 12000 g for 10 min at 4 °C. The supernatant was absorbed onto nitrocellulose paper, dried, and subsequently incubated at 10 min intervals in the 44 kDa periplasmic antiserum. The filters were developed according to standard chromogenic Western blot procedures (Maniatis et al., 1982). All E. coli cross-reactive antibodies were presumed to be removed from the antiserum when nitrocellulose filters no longer showed any reactivity during Western blot development.

Polyclonal antibodies were prepared against a multiple antigenic peptide containing the sequence FRKLLDEGQAA as previously described (Judd & Porcella, 1993)

Cloning and DNA sequence analysis. A N. gonorrhoeae library, generated by partial digestion of MS11 genomic DNA with the restriction enzyme EcoRI and subsequent ligation into the EcoRI site in the Lambda Zap II vector (Stratagene), was screened with the 44 kDa periplasmic antiserum following the manufacturer's protocol (Stratagene). Positive clones were plaque purified and rescued as described in the Stratagene protocol. Plasmid DNAs were isolated and purified for sequencing using the Qiagen Midi prep kit (Qiagen). Sequencing of the cloned DNA inserts was performed using forward and reverse primers (Stratagene) and synthetic oligonucleotides made using an ABI oligonucleotide synthesizer at the Murdoch Molecular Biology Facility at the University of Montana and at Rocky Mountain Laboratories in Hamilton. Sequencing reactions were performed using the dideoxy-nucleotide chain-termination method (Sanger et al., 1977). Nucleotide sequencing also was performed using an Applied Biosystems model 373A automated DNA sequencer and PRISM ready reaction DyeDeoxy Terminator cycle sequencing kits according to manufacturer's instructions (Applied Biosystems). Nucleotide and deduced amino acid sequences were analysed and manipulated using the University of Wisconsin Computer Genetics Group version 7.3 (GenBank database release 82.0) (Devereux et al., 1984) and MacVector version 4.1.1 [International Biotechnologies-Kodak] software packages. Potential promoter sites within DNA sequences were searched and assigned similarity scores based upon known E. coli promoter sequences using the MACTARGSEARCH algorithm (Mullican et al., 1984).

Gel electrophoresis. SDS-PAGE was performed using the discontinuous buffer system of Laemmli (1970). High-resolution two-dimensional gel electrophoresis was performed as described by O'Farrell (1975) using Bio-Rad's Mini-Protean II 2-D kit.

Shuttle mutagenesis. The shuttle mutagenesis protocol previously described by Seifert et al. was used (Seifert & So, 1991; Seifert et al., 1986, 1990) to insertionally inactivate the cloned $\text{tufA}$ gene. The N. gonorrhoeae DNA insert in clone 961 was first digested with ClaI and EcoRI and a 1.7 kb fragment was gel purified. This 1.7 kb fragment, which contained the entire $\text{tufA}$ gene as well as rpsJ, was cloned into the shuttle mutagenesis vector pHS6 via ClaI and EcoRI sites and mutagenized following the guidelines previously described (Seifert et al., 1986, 1990; Seifert & So, 1991). pHS6 constructs that contained mutagenized $\text{tufA}$ genes were PCR mapped using primers specific to the DNA 5' of the $\text{tufA}$ gene (5' G'T'GGAAGTGGAAATCCGG 3', designated 5'Tuf) and 3' (5' AAG-CTTTTCAGGGCGTAC 3', designated 3'Tuf) of the $\text{tufA}$ gene. Orientation of the chloramphenicol marker respective to the $\text{tufA}$ gene was determined using the 5'TufA or 3'TufA primers and a primer specific to the minus strand of the chloramphenicol gene (5' TACCAAGCGTACGCCGTTTC 3', designated Cat). A mutagenized $\text{tufA}$ gene was identified which
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The fact that so many proteins can be observed to migrate
whole-cell lysates of N. gonorrhoeae
RESULTS AND DISCUSSION

PCR and Southern analysis of mutants. PCR was performed
using a Perkin-Elmer Cetus Thermocycler and Taq polymerase,
under reaction conditions previously described (Sambrook et al., 1989). PCR products were gel purified for ligation or
Southern blot hybridization procedures using the Qiaex DNA
purification kit (Qiagen). PCR mapping of the genomic DNA of
the chloramphenicol-resistant mutants, wild-type MS11
genomic DNA (negative control), and the transforming DNA
(positive control) was performed using the oligonucleotides
S\(^{\text{Tuf}}\), 3\(^{\text{Tuf}}\) and Cat described above. Total genomic DNA was
isolated from N. gonorrhoeae transformation mutants using a
DNA extraction kit (Stratagene) following the procedures
recommended by the manufacturer.

Southern blotting and hybridization experiments were per-
formed under high-stringency conditions following the pro-
tocol previously described (Cohen, 1990). Specific probes were
intronitically labelled using the Boehringer Mannheim random
labelling primer kit.

RESULTS AND DISCUSSION

Two-dimensional isoelectric-focusing SDS-PAGE: gels of
whole-cell lysates of N. gonorrhoeae strain MS11 showed
that many proteins (about 11) that differ in pl migrate at
a molecular mass of 44 kDa (Fig. 1). This same result has
been seen for N. gonorrhoeae strain FA19 (data not shown).
The fact that so many proteins can be observed to migrate

at this molecular mass may explain why so many diverse
functions have been associated with this protein. Immunob-
lotting of MS11 whole-cell lysates separated by two-
dimensional gel electrophoresis with the preabsorbed
44 kDa periplasmic antisera showed that two proteins,
with calculated molecular masses of 44 kDa and 37 kDa,
were recognized by the antisera in Fig. 1. This
experiment has been repeated with N. gonorrhoeae strain
FA19 whole-cell lysates with identical results (data not shown).

The 44 kDa periplasmic antisera was used to probe a N.
gonorrhoeae lambda Zap II genomic library. Four clones
were isolated which reacted with the antisera. Of the four reactive clones, one slow-growing E. coli recom-
binant clone, designated 961, was selected for further analysis.
Of the four clones originally isolated, the 961 clone produced the least amount of 44 kDa protein by
immunoblot comparison of the \(i\) plaques, yet it was the
only clone which did not die after the second passage
following rescue. Fig. 2 (a, b) shows the results of two-
dimensional gel analysis and immunoblotting of 961 and
host cell XL1 with the 44 kDa periplasmic antisera.
There was minimal cross reactivity with E. coli XL1 proteins by the preabsorbed antisera while a protein
from clone 961 with a molecular mass of 44 kDa was
observed to react. Interestingly, in Fig. 2(a), a smaller
molecular mass protein of approximately 37 kDa was not
seen to react with the 44 kDa periplasmic antisera in
cut 961 blots. Based upon the specificity and reactivity
of the antisera, we believe we have cloned the periplasm-
associated 44 kDa protein of N. gonorrhoeae (Fig. 1).

Induction of the 961 clone with IPTG did not appreciably
alter expression of the 44 kDa protein on one-dimensional
SDS-PAGE gels (data not shown). This result suggests

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**Fig. 1.** Two-dimensional gel electrophoretic analysis of N. gonorrhoeae strain MS11 probed with 44 kDa periplasmic
antisera. Whole-cell lysates of N. gonorrhoeae strain MS11 were stained with Coomassie brilliant blue and probed with
44 kDa periplasmic antisera (Anti-44 kDa periplasmic). The first dimension isoelectric focusing (IEF) direction is shown.
Samples were run in the second dimension on a 10% SDS-PAGE gel and either blotted for antibody probing or stained
with Coomassie brilliant blue.

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that the protein is expressed from an endogenous promoter element located in the cloned fragment and that expression was not solely the result of the lac promoter in the pSK vector.

Of note, there are striking differences between the protein profiles for 961 and XL1 (Fig. 2a, b). This disparity in the protein profile may be evidence of a weakly growing E. coli recombinant. It is possible that the 44 kDa protein is toxic to E. coli and that the 961 clone may contain a truncated insert or a cloning artifact which lowers the expression and/or toxicity of this gene.

**Sequence analysis of the 961 clone**

The gonococcal DNA fragment contained within the recombinant clone was sequenced in its entirety and this sequence is shown in Fig. 3. The length of the sequenced fragment is 2439 bases. A partial ORF at the 5' end of the DNA insert was found to encode a protein of calculated molecular mass 24.9 kDa. This protein was searched against the databases and found to be most homologous to E. coli EF-G (Blast score 282, probability $4.1 \times 10^{-86}$). A Bestfit analysis showed this partial ORF to be 76.7% similar and 65.0% identical to E. coli EF-G. Therefore, based upon this high percentage of identity it is most likely that this partially cloned gene is a N. gonorrhoeae fusA homologue. Immediately downstream of the fusA homologue was a potential ribosome-binding site followed by a second ORF which codes for a protein of calculated molecular mass 43 kDa. This protein was searched against the databases and found to be highly homologous to E. coli EF-TuA (Blast score 1675, probability $4.7 \times 10^{-228}$). A Bestfit analysis of this protein compared to E. coli EF-TuA showed 90.6% similarity and 82.9% identity between the two proteins. A Bestfit analysis with E. coli EF-TuB showed 90.3% similarity and 82.9% identity. Although it is not possible to differentiate whether or not the N. gonorrhoeae EF-Tu protein is an EF-TuA or EF-TuB homologue based upon these percentage identities or similarity scores, we believe that the presence of a fusA gene immediately upstream and an rpsJ gene (discussed below) immediately down-

![Fig. 2. Two-dimensional gel electrophoretic analysis of E. coli host strain XL1 and the 961 clone probed with 44 kDa periplasmic antiserum. (a) 961 clone stained with Coomassie brilliant blue (CBB) and probed with 44 kDa periplasmic antiserum (Anti-44 kDa periplasmic). (b) E. coli host strain XL1 stained with Coomassie brilliant blue and probed with 44 kDa periplasmic antiserum (Anti-44 kDa periplasmic). The first dimension isoelectric focusing (IEF) direction is shown. Samples were run in the second dimension on a 9% SDS-PAGE gel and either blotted for antibody probing, or stained with Coomassie brilliant blue.](image-url)
Neisseria gonorrhoeae EF-Tu is processed

During the double-stranded sequencing of this DNA insert, it was discovered that the putative start codon for the tufA gene was an ATA codon. Oligonucleotide primers were made 5' (5'TTGGAAAGTGAAACTCCG 3') of the start codon and 3' (5'TTACCATGGTCA-ACGTG 3') of the start codon and a 424 bp product was amplified by PCR from the chromosome of N. gonorrhoeae strain FA19 and MS11. These two PCR products were sequenced with the same PCR primers and it was determined, as shown in Fig. 3, that the start codon for tufA was actually ATG for these two strains. It is tempting to speculate that the mutation in the 961 clone may have been selected by enhanced E. coli survival, thus producing the ATO codon artifact.

To confirm the genetic linkage of fusG, tufA and rpsJ genes, genomic DNA was isolated from N. gonorrhoeae strain MS11 and FA19 and PCR reactions were performed on these templates with primers situated within the 3' end of the fusG gene (5' GTTGAGTGGAAAACCTCGG 3') and a primer located downstream of the rpsJ gene (5' CAGCGCATCGTAGTTIT 3'). A PCR product of 1.9 kb, a size consistent with that predicted, was produced (data not shown). A linkage map of the fusG, tufA and rpsJ genes based upon this PCR data and the sequence data is shown in Fig. 3(b).

The str operon in E. coli is composed of rpsL, rpsG, fusG, tufA, and tufA (Jinks-Robertson & Nomura, 1987). Downstream of the TAA stop codon for the rpsJ gene in the N. gonorrhoeae cloned fragment are sequences suggestive of a transcriptional stop signal (Platt, 1986). This stop signal is composed of exact inverted repeats of 14 bp flanking 5 bases of non-homology as shown in Fig. 3. This structure suggests that this is the end of the str operon for N. gonorrhoeae. The location of the rpsJ gene in the gonococcal str operon is in contrast to the str operon in E. coli; the significance of this finding is unclear.

Expression of the str operon in E. coli is controlled by a promoter in the rpsL gene which resides immediately upstream of rpsG and fusA. In E. coli, expression of tufA is higher than that of the other genes in the operon. This has been in part explained by the presence of several potential promoter elements in the terminal portion of fusA (Zengel et al., 1984). Promoter searches of the terminal region of the gonococcal fusG gene and the intergenic region between fusG and tufA were performed using an algorithm based upon E. coli consensus promoter sequences (Mulligan et al., 1984). Only a single potential promoter was identified, upstream of the gonococcal tufA gene (Fig. 3), which has a sequence similarity score of 48% denoting a relatively weak promoter.

**Processing of the N. gonorrhoeae EF-Tu protein**

To determine whether the 37 kDa protein which reacted with the 44 kDa periplasmic antiserum was in fact a truncated or a processed form of EF-Tu, antibody similarity and 68.9% identity between the two proteins. This high percentage of identity indicates that this ORF is most likely a gonococcal rpsJ homologue.

stream of the EF-Tu ORF indicates that these genes constitute a portion of the str operon of the gonococcus. Therefore, based upon this str operon homology, the EF-Tu ORF is most likely encoded by tufA. Immediately downstream of the tufA homologue was a potential ribosome-binding site followed by a third ORF. This third ORF coded for a protein of calculated molecular mass 11.8 kDa. This protein was searched against the databases and found to be most homologous to E. coli S10 (Blast score 365, probability 1.0 × 10^-45). A Bestfit analysis of this ORF compared with E. coli S10 showed 82.5%
Fig. 4. One- and two-dimensional gel electrophoretic and immunoblot analysis of *N. gonorrhoeae* strain FA19 probed with an EF-Tu-specific antiserum. One- and two-dimensional gel electrophoretic analysis of *N. gonorrhoeae* strain FA19 immunoblotted with the peptide-specific EF-Tu antiserum (FRKLLDEGQA). The blot was subsequently stained with Indian ink. The 44 kDa and 37 kDa proteins reactive with the anti-FRKLLDEGQA antisera and visualized with the India Ink are shown with asterisks.

previously generated against a portion of the gonococcal EF-Tu protein sequence (FRKLLDEGQA) known to be highly conserved amongst many EF-TuA and EF-TuB proteins (Judd & Porcella, 1993) was used in immunoblots of one- and two-dimensional gels of *N. gonorrhoeae* strain FA19 whole-cell lysates. This antiserum reacted with both the 44 kDa and 37 kDa proteins as shown in Fig. 4. The antiserum was immunoblotted against the 961 recombinant clone and only the 44 kDa protein was detected (data not shown). These results suggest that the gonococcal EF-Tu protein is processed or cleaved to a smaller molecular mass form (37 kDa) in *N. gonorrhoeae* but not in the *E. coli* recombinant. Of note, previous use of this antiserum in one-dimensional immunoblots of *N. gonorrhoeae* strain FA19 lysates detected the 37 kDa protein in residue extracts while it failed to detect the 37 kDa protein in whole-cell lysates (Judd & Porcella, 1993). Where a less dilute mixture of the antiserum was used in previous studies a 37 kDa protein was readily detected in whole cell lysates as shown in Fig. 4.

Evidence for a truncated or processed EF-Tu protein was recently described in a phage exclusion system in *E. coli* (Yu & Snyder, 1994). The exclusion mechanism was due to a prophage encoded *Lit* protease specific for EF-Tu. Cleavage at a very specific and highly conserved sequence produced a 37 kDa EF-Tu fragment which halted protein synthesis as a mechanism of immunity in the infected cell. The conserved site where endogenous cleavage has been demonstrated in *E. coli* (Yu & Snyder, 1994) and predicted for *N. gonorrhoeae* is at position 60 in the EF-TuA protein as shown in Fig. 3. The appearance of a lower molecular mass EF-Tu protein in the gonococcus is the first example of EF-Tu cleavage in a bacterium other than *E. coli* K-12 strains and has interesting implications with regard to possible gonococcal phage exclusion mechanisms, gonococcal prophage elements, or an as yet undefined endogenous self-EF-Tu cleavage system. It is also important to note that no phage capable of infecting the gonococcus has ever been described.

EF-Tu appears to be influenced by nutrient availability and growth rate conditions, being methylated and bound to the cytoplasmic membrane during nutrient-limiting conditions (Young & Bernlohr, 1991; Young et al., 1990). The putative methylation site described for *E. coli* and proposed for *N. gonorrhoeae* is at Lys-57 in the EF-TuA protein sequence as shown in Fig. 3. EF-Tu concentrations, in terms of freely soluble ternary complexes, have been shown to directly affect growth and translation elongation rates (Tubulekas & Hughes, 1993). A computer simulation of the *E. coli* translation system suggests that the concentration of EF-Tu should have a direct cause-and-effect relationship upon the dynamics and accuracy of protein synthesis (Pingoud et al., 1990). Therefore, self-cleavage of EF-Tu, with concomitant modulation of the rate of protein synthesis and/or the possible removal of the methylated portion of EF-Tu may have relevance to gonococcal survival and growth in diverse, nutrient-fluctuating environments.

**Attempted deletion of tufA**

A fragment of DNA containing the *tufA* gene was cut from the cloned insert in 961 using *ClaI* and *EcoRI* enzymes. This fragment was ligated into the pHSS6...
primer (5' TTGGTGTGAGG AGTGA
lanes marked MS11-Tuf-2 and MS11-Tuf-3 a further
constructed by PCR using primers internal to the hvf4
transformed into pili-positive EMS1 1 hJ.
HindIII-restricted lanes is in fact the
hybridizing fragment(s) is present while the wild-type
mutagenesis vector and mutagenized. The fragment
containing the insertionally inactivated tufA gene was
categorized by PCR using the oligos 3' TufA, 5' TufA
and Cat. The defective chloramphenicol-containing trans-
poson was located within the tufA gene in a 5'-to-3'
orientation. The transposon resided approximately 0.6 kb
from the 5' end of the ClaI/EcoRI fragment and 1.1 kb
from the 3' end of the fragment. The mutagenized DNA
was obtained from the vector by digesting with NotI.
After gel purification, the linear DNA fragment was
transformed into pili-positive MS11 N. gonorrhoeae and
mutants resistant to chloramphenicol were selected.
Twenty-four slow-growing, chloramphenicol-resistant
mutants were identified. The plates were rechecked for
mutants growing extremely slowly by longer incubation
times, but no colonies were detected. From the 24
mutants, two were randomly selected (designated MS11-
Tuf-2 and MS11-Tuf-3) and characterized by Southern
analysis results show that the probe hybridized
PCR analysis using the 3' TufA, 5' TufA and Cat primers
was performed on the wild-type MS11 DNA produced a 1-5 kb fragment;
consistent with the expected size for this portion of the strep.
PCR analysis of the wild-type DNA and the
transforming DNA with the 5' TufA and Cat primer
produced no 1 kb fragment in the wild-type and a
fragment of 2.0 kb (consistent with the location of the transposon) for the transforming DNA. Genomic DNA
was isolated from the two mutants and PCR, using the
5' TufA and 3' TufA primers, produced a 1-5 kb fragment
for each mutant, thereby indicating that an intact copy of the
transposon still existed. PCR analysis with the 5' TufA
and Cat primer produced a fragment of 2 kb in the two
mutants indicating that the transforming DNA was
located in the chromosome in an unknown location. This
PCR analysis confirmed the Southern blot data in that
presumably functional copies of the tufA gene were still
intact while the transforming DNA was present but
located in an unknown region of the chromosome.

It is generally believed that the 1% nucleotide difference
that exists between the tufA and tufB genes in E. coli and
S. typhimurium is sufficient to prevent high levels of homologous recombination between the genes. Given
this premise and the fact that roughly 1.7 kb of strep
operon-specific DNA flanked the transposon we believed that the odds of recombination occurring at the tufB locus were
minimal. Given that our primary interest was in the
deletion of the tufA gene, we decided to characterize the

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**Fig. 5.** Southern blot analysis of N. gonorrhoeae tufA mutants. Southern blot analysis was performed with MS11 genomic DNA (MS11), MS11 mutant transformant MS11-Tuf-2 genomic DNA and MS11-Tuf-3 genomic DNA digested with HindIII (H), HindIII + ClaI (HC), or ClaI (C).
remaining 22 mutants by PCR analysis alone using the 3’TufA, 5’TufA and Cat primers. Unfortunately, all of the remaining 22 mutants produced PCR profiles identical to those previously described for MS1-Tuf-2 and MS11-Tuf-3. Therefore, we were unable to functionally delete the tufA gene of N. gonorrhoeae.

The tufB gene of E. coli has been inactivated by insertion of a Mu phage genome (van de Klundert et al., 1978; Young & Furano, 1981) while the tufA gene has been shown to be essential (Vijgenboom & Bosch, 1987). It is possible that in our mutagenesis procedures a potential polar effect upon the rpsJ gene may have occurred. Given the unusual location of the rpsJ gene in the N. gonorrhoeae str operon (as compared to the location of this gene in E. coli) we do not know whether this gene is essential in this context.

The presence of heterodiploids arising during the mutagenesis of a potentially essential gene such as tufA is similar to the phenomenon observed with the pilA gene, a gene thought to be essential in N. gonorrhoeae (Taha et al., 1988). These results are consistent with the notion that the tufA gene of N. gonorrhoeae is essential.

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