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Characterization of the *rho* Genes of *Neisseria gonorrhoeae* and *Salmonella typhimurium*

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We have cloned and sequenced the genomic regions encompassing the *rho* genes of *Neisseria gonorrhoeae* and *Salmonella typhimurium*. Rho factor of *S. typhimurium* has only three amino acid differences with respect to the *Escherichia coli* homolog. Northern (RNA) blots and primer extension experiments were used to characterize the *N. gonorrhoeae rho* transcript and to identify the transcription initiation and termination elements of this cistron. The function of the Rho factor of *N. gonorrhoeae* was investigated by complementation assays of *rho* mutants of *E. coli* and *S. typhimurium* and by in vivo transcription assays in polar mutants of *S. typhimurium*.

Transcription termination in prokaryotes specifies the 3′ ends of the transcripts and plays a fundamental role in the control of gene expression. Transcription termination is accomplished by two mechanisms. In the first one, the only requirement is the presence of *cis* elements (46). In the second (Rho-dependent) one, both *cis* elements and *trans*-acting factors are required; of these, the best-known factor is the Rho protein of *Escherichia coli* (36). Rho is composed of identical subunits of 419 amino acids that form hexamers in solution; the protein has helicase and RNA-dependent ATPase activities (36). Rho is essential for cell growth (9), and its expression is autogenously regulated at the transcriptional level by a Rho-dependent attenuation mechanism (32). Rho-dependent termination is responsible for the phenomenon of polarity, for transcriptional attenuation, and for preventing the synthesis of unused transcripts during conditions of physiological stress (1, 36). Together, these data indicate a more general role for Rho-dependent transcription termination in the cell metabolism and suggest that Rho factor might be present and functional in many bacterial genera.

Evidence for the presence of a Rho-like activity in gram-negative species relies upon enzymatic assays and Western blot (immunoblot) experiments using an antibody directed against Rho of *E. coli* (4). Only in *Salmonella typhimurium* has Rho factor been partially characterized at the genetic and biochemical levels (15, 24, 25). The existence of a Rho-like activity in gram-positive bacteria has not been convincingly demonstrated (4, 26). Recently a putative *Bacillus subtilis rho* gene has been identified by homology comparison. Contrary to *E. coli rho*, this gene is not essential since its disruption causes only protonophore resistance and sensitivity to low growth temperature (37).

In the present work, we have isolated from genomic libraries and sequenced the *rho* genes of *S. typhimurium* and *Neisseria gonorrhoeae*. We have analyzed the transcripts and the transcription initiation and termination sites of the *rho* cistron in *N. gonorrhoeae*. Rho function was investigated by complementation tests and by studying the effects of Rho on known transcription terminators in vivo.

Cloning and sequencing of *rho* genes. Bacterial strains used in this study are listed in Table 1. Genomic DNA of pathogenic *N. gonorrhoeae* T2 and *S. typhimurium* TM218 prepared as previously described (6), digested with *Eco*RI, and hybridized to an *E. coli* DNA fragment spanning the *rho* gene (Fig. 1A, probe A) showed hybridization to single bands of 5,500 and 3,500 bp, respectively (data not shown). Genomic DNA libraries from these two strains were constructed in the λgt10 vector system (Stratagene) by using *Eco*RI total digest. Phage plaques were screened (40) with probe A at 55°C, and clones containing *Eco*RI fragments of the expected length were isolated and further characterized. The presence of an *Eco*RI site within the coding region of *rho* gene from both species required further screening. A clone containing the contiguous distal *Eco*RI fragment of 2,800 bp was isolated from the same library of *S. typhimurium* screened with probe B (Fig. 1A). A new library of *N. gonorrhoeae* T2 was constructed in the λgt10 vector by using *Pvu*II total digest and screened with the homologous probe C (Fig. 1A) at 68°C to isolate a clone containing the overlapping *Pvu*II fragment of 6,000 bp. We then established detailed restriction maps of the cloned inserts by subcloning different fragments in plasmid vectors (Fig. 1A). Plasmid pEG25 carrying the wild-type *rho* gene of *E. coli* has been previously described (21). Plasmid pNG100 was obtained by subcloning a 5,500-bp *Eco*RI fragment of *N. gonorrhoeae* in pBR322. Plasmid pNG101 is a pUC18 derivative carrying a 1,500-bp *Pvu*II-*Eco*RI fragment from pNG100. Plasmids pNG102 and pNG103, containing the entire *rho* gene of *N. gonorrhoeae*, were obtained by subcloning a 3,300-bp *Eco*RI fragment in the dephosphorylated *Eco*RI site of pNG101, verifying the relative orientation by restriction analysis, and subcloning a 1,500-bp *Ssp*I fragment from pNG102 in the dephosphorylated *Sma*I site of pSP72. Plasmid pST100 is a pUC19 derivative carrying a 3,500-bp *Eco*RI fragment of *S. typhimurium*.

The nucleotide sequences of a 2,558-bp region of *S. typhimurium* (Fig. 2) and of a 1,849-bp region of *N. gonorrhoeae* (Fig. 3) were determined by the dideoxy-chain termination procedure (41). We have identified two open reading frames (ORFs) of 330 and 1,260 nucleotides in *S. typhimurium* (Fig. 2). These regions are highly homologous (91%) to *E. coli trx*A, encoding thioredoxin, and *rho* genes. The homology extends to the 5′ and 3′ regions of the two ORFs, including sequences

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TABLE 1. Bacterial strains

Strain	Genotype	Reference or source
E. coli		
DH5α	F⁻ *endA1 hsdR17* (r_K^- m_K^+) *supE44 thi-1* λ⁻ *recA1 gyrA96 relA1* Δ*lac*U169 φ80d*lacZ*Δ15M	22
AD1600	F⁻ His⁻ Str^r *relA1 galOP3*::IS2 *rho15*::IS*1*	9
S. typhimurium		
SC684	*hisO1242 hisG2148 rho111*	8
SC685	*hisO1242 hisG2148*	8
TM218	*hisO1242 hisD2352 his-3962*	38
RM926	*hsdLT*(r⁻ m⁺) *hsdSA*(r⁻ m⁺) *hsdSD*(r⁻ m⁺) *metA22 metE551 trpD2 leu val rpsL120 galE*	C. Grubmeyer
N. gonorrhoeae T2		5

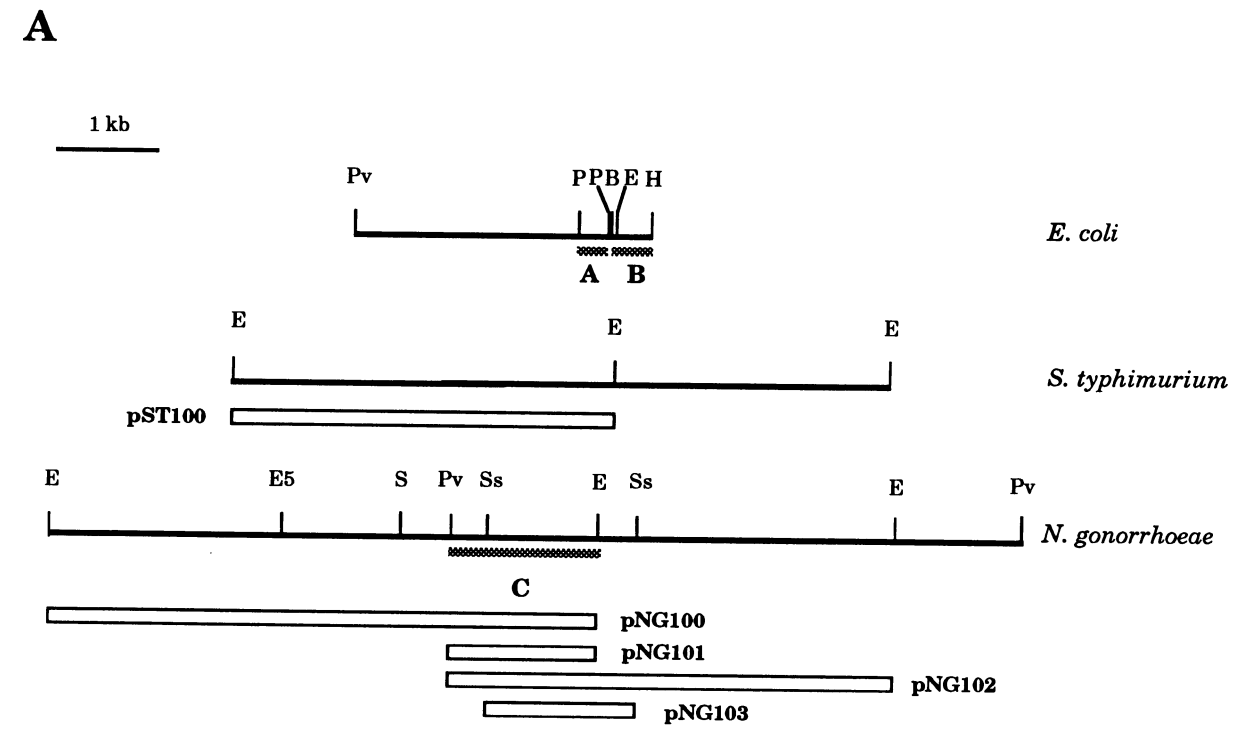
deputed to the initiation and termination of transcription (Fig. 2) (32, 35). Two ORFs of 264 and 1,260 nucleotides were identified in *N. gonorrhoeae* (Fig. 3). The first truncated ORF is homologous (70%) to the distal portion of the *ppsA* gene of *E. coli*, encoding phosphoenolpyruvate synthase (33). Downstream of the *ppsA* stop codon (Fig. 3), as in *E. coli* (33), there is a palindromic structure with features of a Rho-independent terminator (46). The second ORF is homologous (67%) to the *E. coli rho* gene, and the homology is confined to the coding region. Codon preferences in these cistrons are in agreement with general trends observed in other *S. typhimurium* (27) and *N. gonorrhoeae* (45) genes.

Homology comparison of gene products. The amino acid sequence of the *trxA* gene product of *S. typhimurium* is identical to that of the *E. coli* protein (Fig. 2 and data not shown). The proteins encoded by the *rho* genes of *S. typhimurium* and *E. coli* differ for only three amino acids (Asn-20/Ser-20; Leu-55/Val-55; Phe-121/Tyr-121) (Fig. 4), two of which are conservative replacements (10).

The amino acid sequence of the carboxyl-terminal portion of the *ppsA* gene product of *N. gonorrhoeae* has 74% identity and 85% similarity to the *E. coli* homolog (Fig. 3 and data not shown). The protein encoded by the *rho* gene of *N. gonorrhoeae* when aligned with the *E. coli* homolog shows an overall identity of 71% (Fig. 4); taking into account conservative amino acids substitutions, the similarity is much higher (87%). The similarity is not distributed evenly in the three structural domains in which Rho protein is subdivided (11, 36): 60% identity and 77% similarity in the 130-amino-acid-long RNA binding domain, 73% identity and 87% similarity in the 152-amino-acid-long ATP binding domain, and 74% identity and 91% similarity in the 137-amino-acid-long third domain (Fig. 4). Studies in *E. coli* have identified several consensus sequences in Rho with homology to functionally related proteins (36).

Analysis of *N. gonorrhoeae* Rho factor shows that residue Cys-202, previously believed to participate in RNA binding (31) and subsequently shown to be dispensable (36), is substituted by a valine (Fig. 4). In the RNA binding domain, the sequence between residues 15 and 20 (ITLGEN) has been tentatively identified (11) as the homolog of the cytidine-binding part (ITIGLN) of the regulatory subunit of the aspartate transcarbamylase (28). This sequence is modified to LELAEE in *N. gonorrhoeae*, and in *S. typhimurium*, one of the three amino acid changes occurs in this region (ITLGES). The sequence between residues 20 and 98 has homology with several RNA-binding proteins and includes the GFGF consensus (36), which is also present in *N. gonorrhoeae*. In the ATP binding domain, two consensus sequences ($GXGXXGX_nK$) and (GX_nGKT) for nucleotide-binding proteins (3, 44) are present in *E. coli* (residues 169 to 181 and residues 178 to 185) and are conserved in *N. gonorrhoeae*. In addition to Lys-181 and Lys-184, also Asp-265, believed to be important for the catalytic activity of Rho (12), is conserved. A prediction of the secondary structure of the Rho protein of *N. gonorrhoeae* shows conservation of the five β strands believed to form a hydrophobic pocket required for the interaction with ATP (13, 17).

Transcriptional analysis of the *N. gonorrhoeae rho* gene. There are very few studies on the identification and function of sequences involved in the initiation and termination of transcription in *N. gonorrhoeae*. Among them are the *pilA* (42) and P29 (30) genes, for which 5′ ends of transcripts were identified by S1 mapping and sizes of the transcripts were determined by Northern (RNA) blotting. The *rho* gene of *N. gonorrhoeae* was subjected to a transcriptional analysis in the heterologous *E. coli* host transformed with the recombinant plasmid pNG100. Total bacterial RNA was extracted as previously described (20). The transcription initiation site was mapped by primer extension (16) at a C residue 85 bases upstream of the translation initiation codon (Fig. 5A, lane 1). Upstream of the mapped RNA start site there are sequences that conform to consensus elements of a promoter region (Fig. 3). An analysis of the sequence for potential transcription terminators showed that a region capable of assuming a secondary structure with the typical features of a Rho-independent terminator (46) is located 22 nucleotides downstream of the stop codon (Fig. 3). *N. gonorrhoeae* strains were grown in GC agar plus supplement G (Diagnostic Pasteur) in a candle extinction jar or in GC broth (34) plus 1% Vitox (Oxoid). Northern blot analysis (43) of total RNA from *S. typhimurium* SC684 harboring plasmid pNG103M (Fig. 5B, lane 2) or from *N. gonorrhoeae* T2 (Fig. 5B, lanes 3 and 4) revealed a single RNA species of about 1,400 nucleotides. The estimated length of the transcript from the 5′ end to the run of U residues following the palindrome would be 1,410 nucleotides, a figure in agreement with the Northern blot data, indicating that transcription terminates at the canonical Rho-independent terminator immediately downstream of the ORF (Fig. 1B and 3). The identification of putative Rho-independent transcription terminators (14) at the end of *N. gonorrhoeae* genes has been done on the basis of structural data, i.e., the presence of palindromic structures which often include as an inverted repeat the 10-nucleotide (GCCGTCTGAA) DNA uptake sequence (DUS) which plays a fundamental role in the process of transformation (14, 18). DUS, which is rather frequent in the genomic DNA of *N. gonorrhoeae* (19), is present as an inverted repeat in the *rho* terminator and in single copy on the opposite strand at nucleotides 323 and 434 between the *ppsA* and *rho* genes (Fig. 3). It has been suggested that DUS originated as a regulatory signal and that its role in transformation has evolved to take advantage of its frequency and distribution in the genome (18,


A
1 kb
Pv
P P B E H
A B
E. coli
E
E
E
S. typhimurium
pST100
E
E5
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Ss
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Ss
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C
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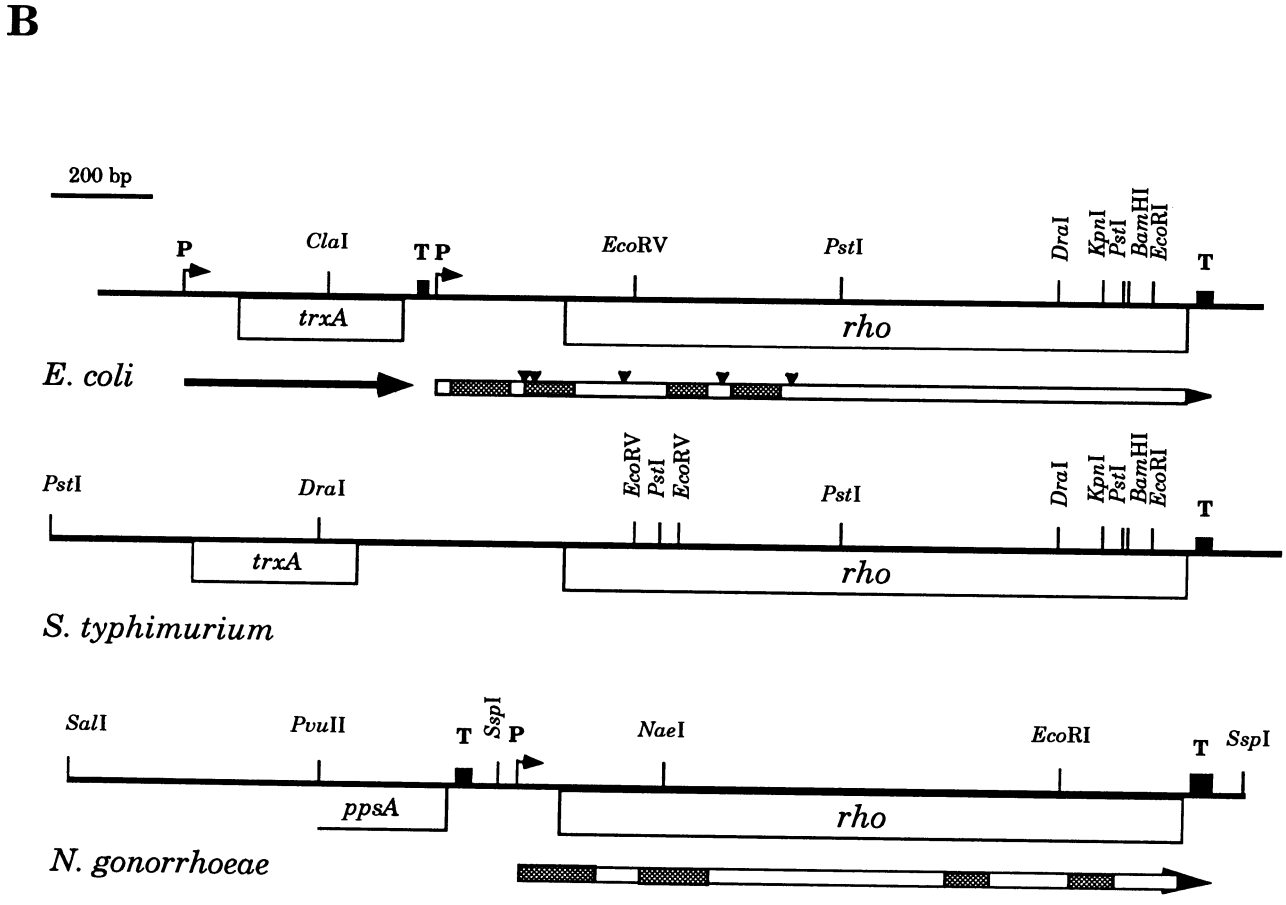
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200 bp
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ClaI
T P
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E. coli
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S. typhimurium
SalI
PvuII
T
SspI
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NaeI
EcoRI
T
SspI
ppsA
rho
N. gonorrhoeae

FIG. 1. (A) Physical map of the genomic regions spanning the *rho* gene. Shown are the *E. coli* region (top) cloned in plasmid pEG25 (21), the two contiguous *Eco*RI fragments of *S. typhimurium* (middle), and the overlapping *Eco*RI and *Pvu*II fragments of *N. gonorrhoeae* (bottom) isolated from the genomic libraries. Shaded boxes indicate the fragments used as probes (A, 566-bp *Pst*I fragment; B, 710-bp *Bam*HI-*Hin*dIII fragment; C, 1,500-bp *Pvu*II-*Eco*RI fragment). Restriction sites: B, *Bam*HI; E, *Eco*RI; E5, *Eco*RV; H, *Hin*dIII; P, *Pst*I; Pv, *Pvu*II; S, *Sal*I; Ss, *Ssp*I. The regions cloned in the different plasmids are indicated by open boxes. (B) Structural and functional characteristics of the genomic regions spanning the *rho* gene. A partial restriction map of the genomic regions aligned with respect to the *rho* gene of *E. coli* is shown. Coding sequences are indicated by rectangles and gene symbols. Promoters (P) and terminators (T) are indicated. Direction of transcription and length of the transcripts are indicated by arrows. Rho-dependent transcription termination elements (2) are indicated by stippled rectangles within the transcripts. Black triangles identify 3′ ends of attenuated transcripts previously described in *E. coli* (32).

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CTGCAGCTCTTCCTGAGTGGTGAGCAGCTCTTCTTTACGGGTACCGGTTTGCGCCTGCCCTGCAACGTCACGACCCGCCAGCGTTACGGAAGGGCCAGTG   100

CTGAATGGGCGTACAATTATAAACCCTTTTTTTTTCAAGGGCTTCAACCACCTGTGGTGCAGGGCGAAGTCGGAAAACTTCTGTTCTGTTAAATGTGTTT   200

TGCTCATAGTGTGGTAGAATATCAGCTTACTATTGCTTTACGAAAGCGTATCCGGTGAAATAAAGTCAACCTTTGTTGGCGAAGTTAACATCAGCACCTC   300

GTTGGTTAATGCTACACCAACACGCCAGGCTTATTCCTGTGGAGTTATATATGAGCGATAAAATTATTCACCTGACTGACGACAGTTTTGACACGGATGT   400
                                                  MetSerAspLysIleIleHisLeuThrAspAspSerPheAspThrAspVa

ACTCAAAGCGGACGGGGCTATCCTCGTTGATTTCTGGGCAGAGTGGTGCGGGCCGTGTAAAATGATCGCTCCGATTCTGGATGAAATCGCTGACGAATAT   500
lLeuLysAlaAspGlyAlaIleLeuValAspPheTrpAlaGluTrpCysGlyProCysLysMetIleAlaProIleLeuAspGluIleAlaAspGluTyr

CAGGGCAAATTGACCGTTGCCAAACTGAACATTGACCAGAACCCAGGTACTGCGCCTAAATATGGCATCCGCGGTATTCCGACTCTGCTGCTGTTTAAAA   600
GlnGlyLysLeuThrValAlaLysLeuAsnIleAspGlnAsnProGlyThrAlaProLysTyrGlyIleArgGlyIleProThrLeuLeuLeuPheLysA

ACGGCGAAGTGGCGGCAACCAAAGTAGGCGCACTGTCTAAAGGTCAGTTGAAAGAGTTTCTCGACGCCAATCTGGCGTAATACCTTTTATACGTCGAATG   700
snGlyGluValAlaAlaThrLysValGlyAlaLeuSerLysGlyGlnLeuLysGluPheLeuAspAlaAsnLeuAla...

ACAAAGGCGCTGTATGGTCAGGCGTCTGTCATTCGACGAATGATTAAGGTATCAGGCAGTTTCGTGGCGTCTGTTGTCCTTACGTTATTCTTAAATTGTC   800

AGGATCTCTGGACGCCCGGTCTGAGTCGTGCTAAGTTAGTATTGACTTCGAATTAAACATACCTTATTAAGTTTGAATCTGGTTTTATCCGTCACTTCCC   900

GTTTTTTCTCGCACGAGAAGTGGAAAGATTCCTGGCTCTTCGCTCATTCCGTCTTGTCGTTTCAGTTCTGCGTACTTTCCTGTGACCAGACAGCGAACAG  1000

ACATGAGTTGATAGCCGTAAACAGGCATGGATGACCCTGCCATACCATTCACAACATTAAGTTCGAGATTTACCCCAAGTTTAAGAACTCACACCATTAT  1100
                                                                                                      Me

GAATCTTACCGAATTAAAGAATACGCCGGTTTCTGAGCTGATCACTCTCGGCGAAAGTATGGGGCTGGAAAACCTGGCCCGTATGCGCAAGCAGGACATT  1200
tAsnLeuThrGluLeuLysAsnThrProValSerGluLeuIleThrLeuGlyGluSerMetGlyLeuGluAsnLeuAlaArgMetArgLysGlnAspIle

ATTTTTGCCATCCTGAAGCAGCACGCAAAGAGTGGCGAAGATATCTTTGGCGACGGTGTGGTGGAGATATTGCAGGATGGATTTGGTTTCCTCCGTTCTG  1300
IlePheAlaIleLeuLysGlnHisAlaLysSerGlyGluAspIlePheGlyAspGlyValValGluIleLeuGlnAspGlyPheGlyPheLeuArgSerA

CAGACAGCTCCTACCTCGCCGGTCCTGATGATATCTACGTTTCCCCCAGCCAAATCCGCCGTTTCAACCTCCGCACTGGTGATACCATTTCTGGTAAGAT  1400
laAspSerSerTyrLeuAlaGlyProAspAspIleTyrValSerProSerGlnIleArgArgPheAsnLeuArgThrGlyAspThrIleSerGlyLysIl

TCGCCCGCCGAAAGAAGGTGAACGCTATTTTGCGCTGTTGAAAGTTAACGAAGTTAACTACGACAAACCGGAAAACGCCCGTAACAAAATCCTCTTTGAG  1500
eArgProProLysGluGlyGluArgTyrPheAlaLeuLeuLysValAsnGluValAsnTyrAspLysProGluAsnAlaArgAsnLysIleLeuPheGlu

AACTTAACCCCGCTGCACGCAAACTCTCGTCTGCGTATGGAGCGTGGTAACGGTTCTACCGAAGACTTAACGGCGCGCGTTCTGGATCTGGCTTCGCCGA  1600
AsnLeuThrProLeuHisAlaAsnSerArgLeuArgMetGluArgGlyAsnGlySerThrGluAspLeuThrAlaArgValLeuAspLeuAlaSerProI

TCGGTCGCGGCCAGCGCGGTCTGATTGTCGCGCCGCCGAAAGCGGGTAAAACCATGCTGCTGCAGAACATCGCGCAGAGCATCGCGTATAACCACCCGGA  1700
leGlyArgGlyGlnArgGlyLeuIleValAlaProProLysAlaGlyLysThrMetLeuLeuGlnAsnIleAlaGlnSerIleAlaTyrAsnHisProAs

CTGCGTGCTGATGGTGCTGCTGATTGACGAACGTCCGGAAGAAGTGACCGAGATGCAGCGTCTGGTAAAGGGCGAAGTGGTTGCTTCTACCTTTGACGAA  1800
pCysValLeuMetValLeuLeuIleAspGluArgProGluGluValThrGluMetGlnArgLeuValLysGlyGluValValAlaSerThrPheAspGlu

CCGGCATCCCGCCACGTTCAGGTTGCCGAAATGGTTATCGAGAAGGCGAAGCGTCTGGTTGAACACAAGAAAGACGTTATCATCCTGCTCGACTCCATCA  1900
ProAlaSerArgHisValGlnValAlaGluMetValIleGluLysAlaLysArgLeuValGluHisLysLysAspValIleIleLeuLeuAspSerIleT

CCCGTCTGGCGCGCGCCTACAACACCGTGGTGCCGGCTTCCGGTAAAGTATTGACCGGTGGTGTGGACGCTAACGCCCTGCATCGTCCGAAGCGTTTCTT  2000
hrArgLeuAlaArgAlaTyrAsnThrValValProAlaSerGlyLysValLeuThrGlyGlyValAspAlaAsnAlaLeuHisArgProLysArgPhePh

CGGCGCGGCGCGTAACGTGGAAGAGGGCGGTAGCCTGACTATCATCGCGACGGCGCTAATCGATACCGGTTCCAAGATGGACGAAGTTATCTACGAAGAG  2100
eGlyAlaAlaArgAsnValGluGluGlyGlySerLeuThrIleIleAlaThrAlaLeuIleAspThrGlySerLysMetAspGluValIleTyrGluGlu

TTTAAAGGCACCGGTAACATGGAGCTGCATCTCTCGCGTAAGATCGCTGAAAAACGTGTCTTCCCGGCTATCGACTACAACCGTTCCGGTACCCGTAAAG  2200
PheLysGlyThrGlyAsnMetGluLeuHisLeuSerArgLysIleAlaGluLysArgValPheProAlaIleAspTyrAsnArgSerGlyThrArgLysG

AAGAGCTGCTCACCACTCAGGAAGAGCTGCAGAAAATGTGGATCCTGCGTAAAATCATCCATCCGATGGGTGAAATTGACGCGATGGAATTCCTCATTAA  2300
luGluLeuLeuThrThrGlnGluGluLeuGlnLysMetTrpIleLeuArgLysIleIleHisProMetGlyGluIleAspAlaMetGluPheLeuIleAs

CAAACTGGCGATGACCAAAACTAATGACGACTTTTTCGAGATGATGAAGCGCTCATAACCTGGTCTTTACCTGAAAACGCCACGTTTTTACGTGGCTTTT  2400
nLysLeuAlaMetThrLysThrAsnAspAspPhePheGluMetMetLysArgSer...

TGTTTTGCGATTCATCCTGGCGTCATGTCATATAAACTGGTCATCGGGATATGCGCTACAGAGTAACACCATGCACAATACACATTGTTTGCGGTAAAAG  2500

TGTAATGTTCTAACAGGTCTCTTCTAGGAGCTGGCGTAACGCATTATACTTAAGGATA                                            2558
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FIG. 2. Nucleotide sequences of the *trxA* and *rho* genes from *S. typhimurium*. The *trxA* −35 (189 to 194) and −10 (215 to 220) sites and Shine-Dalgarno box (341 to 344) are underlined, and the catabolite gene activator protein-binding sequence (192 to 211) is overlined. The *rho* −35 (809 to 814) and −10 (832 to 837) sites and Shine-Dalgarno box (1083 to 1087) are underlined, and the transcription terminator (2374 to 2400) is indicated by convergent arrows. The amino acid sequences of *trxA* (351 to 677) and *rho* (1099 to 2355) are indicated below the sequence.

FIG. 3. Nucleotide sequence of the *rho* region from *N. gonorrhoeae*. Putative −35 (365 to 370) and −10 (388 to 393) consensus sequences are underlined. The black square below nucleotide 400 indicates the transcription initiation site. The arrow below nucleotides 486 to 502 indicates the synthetic oligonucleotide used in primer extension experiments. The putative transcription terminators (274 to 304 and 1767 to 1809) are indicated by convergent arrows. The DUSs are indicated by thick black lines. The amino acid sequences of *ppsA* (1 to 261) and *rho* (485 to 1741) are indicated below the sequence.

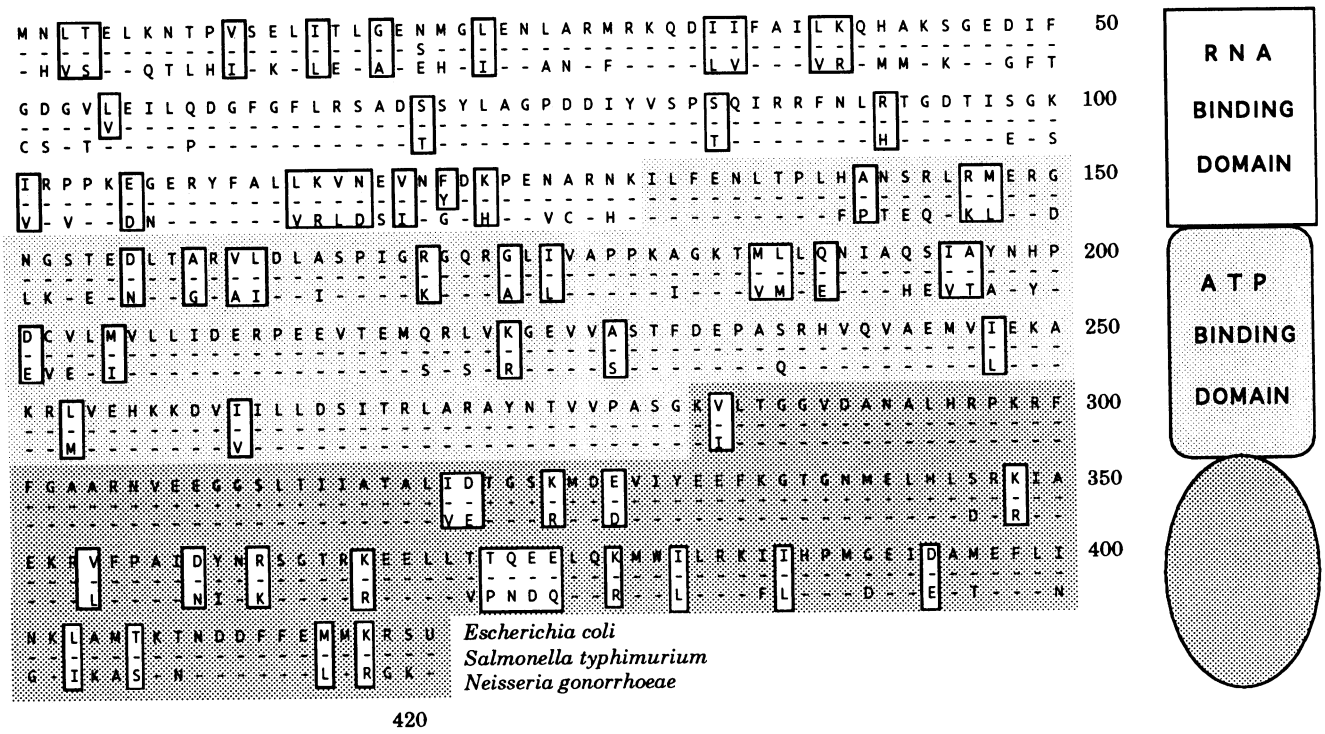

FIG. 4. Alignment of the amino acid sequences of Rho from *E. coli*, *S. typhimurium*, and *N. gonorrhoeae*. Residues are indicated by the single-letter code. Identical amino acids are indicated by dashes with respect to the *E. coli* sequence; conservative replacements are boxed. The three domains into which Rho protein is subdivided are shown by stippled areas of different intensity as indicated on the right.

19). The potential role of DUS in the termination of transcription remains to be established.

Rho factor synthesis in *E. coli* is autogenously regulated at the transcriptional level by a self-dependent attenuation mechanism (32). Transcription termination elements (TTE) recognized by Rho factor in the RNA have features that allow their identification (2, 39). TTE are present in the *rho* gene of *E. coli* upstream of the 3′ ends of the attenuated transcripts (Fig. 1B). TTE are also found in the *rho* gene of *N. gonorrhoeae* at the 5′ end and in the coding region (Fig. 1B), suggesting that Rho expression might be autogenously regulated also in this species.

Translational analysis and functional assays of Rho protein of *N. gonorrhoeae*. The 419-amino-acid-long putative Rho protein of *N. gonorrhoeae* has an estimated M_r of 47,366, very similar to the *E. coli* value of 47,010. To obtain independent evidence that the DNA sequence was correct and that the protein was actually encoded and expressed by the recombinant plasmids, we performed in vitro transcription-translation experiments. In vitro expression of plasmid-encoded proteins was obtained in an S30 extract prepared from *E. coli*, making use of a translation kit (Amersham) and following the manufacturer's instructions to obtain [^{35}S]methionine-labelled gene products. Analysis of the labelled proteins in sodium dodecyl sulfate (SDS)-polyacrylamide gels (29) showed that a protein comigrating with the one produced from plasmid pEG25 (21) that harbors the *rho* gene of *E. coli* (Fig. 6A, lane 3) was efficiently synthesized from plasmids pNG102 (data not shown) and pNG103 (Fig. 6A, lane 2).

Strains carrying mutations in the *rho* gene are conditionally lethal and exhibit a temperature-sensitive phenotype (9, 24). *E. coli* AD1600 and *S. typhimurium* SC684 (Table 1) were grown in LB broth at 32°C to optical densities at 550 nm of 0.4 and 0.7, respectively. Strains were transformed with different plasmids (Table 2), and transformants were isolated on LB-ampicillin plates at 32°C. Individual clones were tested for growth at 32 and 42°C. Transformations were performed as described by Hershfield et al. (23). Transformation of *S. typhimurium* strains required plasmid modification through an intermediate passage in $r^- m^+$ strain RM926. Modified plasmids are denoted by an M. The *rho* gene of *N. gonorrhoeae* was able to restore the temperature-resistant phenotype in *E. coli* and *S. typhimurium* mutants (Table 2).

The function of *N. gonorrhoeae* Rho protein in transcription termination was investigated in vivo. In polar mutants, cryptic Rho-dependent transcription terminators within cistrons are unmasked and induce the appearance of short transcripts with different 3′ ends (2). In particular, five major transcripts are detected by S1 nuclease (Sigma) mapping performed as previously described (7) in *S. typhimurium* SC685 harboring a polar

TABLE 2. Complementation of *rho* mutants of *E. coli* and *S. typhimurium*

Strain	Plasmid	Growth at:	
		32°C	42°C
AD1600 (*rho15*::IS*1*)		+	−
AD1600 (*rho15*::IS*1*)	pUC18	+	−
AD1600 (*rho15*::IS*1*)	pNG102	+	+
AD1600 (*rho15*::IS*1*)	pEG25	+	+
SC684 (*rho111*)		+	−
SC684 (*rho111*)	pSP72M	+	−
SC684 (*rho111*)	pNG103M	+	+
SC684 (*rho111*)	pEG25M	+	+

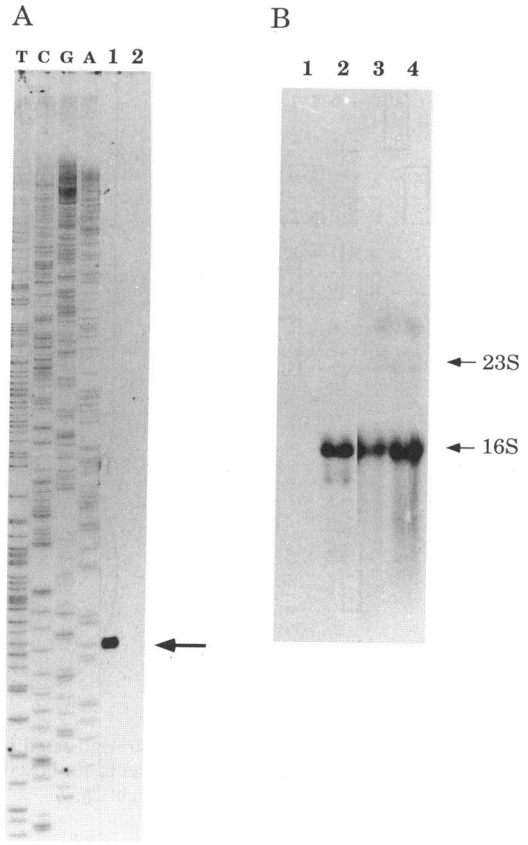

FIG. 5. Primer extension and Northern blot analyses of *rho* RNA. (A) Five nanograms of the 5′-end-labelled synthetic 17-base oligonucleotide complementary to nucleotides 486 to 502 (Fig. 3) was annealed to 5 μg of RNA from *E. coli* DH5α transformed with plasmid pNG100 (lane 1) or pBR322 (lane 2) and extended. Samples were electrophoresed on a 6% polyacrylamide denaturing gel alongside with sequencing ladders used as molecular size markers. The arrow indicates the primer extension product. (B) Total RNA from *S. typhimurium* SC684 (10 μg; lane 1), SC684 transformed with plasmid pNG103M (10 μg; lane 2), and *N. gonorrhoeae* T2 (10 μg [lane 3] or 20 μg [lane 4]) was electrophoresed on 1% agarose–formaldehyde gels, transferred to Hybond-N (Amersham) membranes, and hybridized to a ^{32}P-labelled 399-bp *Eco*RI-*Sal*I fragment from the *N. gonorrhoeae* *rho* gene. Positions of the rRNAs (23S and 16S) are indicated. Exposure times were 4 (lanes 1 and 2) and 18 (lanes 3 and 4) h.

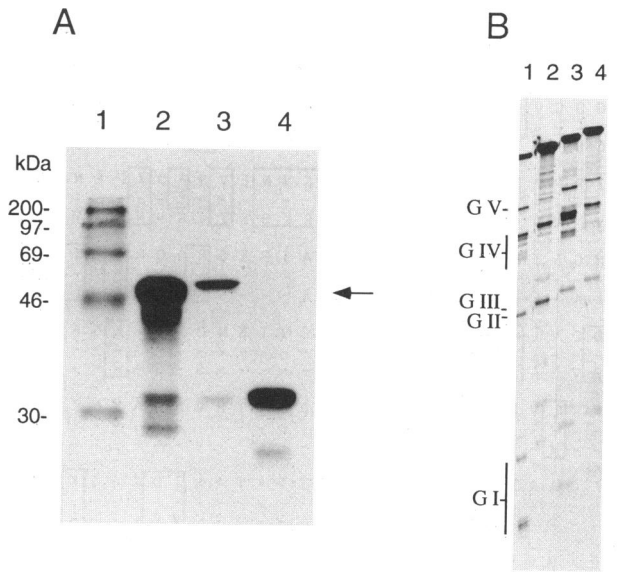

FIG. 6. (A) Analysis of in vitro translation products of *N. gonorrhoeae* Rho. Aliquots of the in vitro translation assays were electrophoresed on SDS–12% polyacrylamide gels and fluorographed. Lane 2, plasmid pNG103; lane 3, plasmid pEG25; lane 4, vector plasmid pSP72. The molecular sizes of ^{14}C-labelled markers (Amersham) (lane 1) are indicated on the left. The arrow indicates the Rho protein. (B) Mapping of the 3′ ends of *his*-specific transcripts produced in vivo. Total RNA (20 μg) extracted from *S. typhimurium* SC685 Rho$^+$ (lane 1), SC684 Rho$^-$ (lane 2), SC684 harboring plasmid pEG25M (lane 3), and pNG103M (lane 4) were hybridized to a 3′-end-labelled (40) 359-bp *Sau*3AI fragment spanning the 3′ end points of the prematurely terminated transcripts and digested with S1 nuclease. Protected bands corresponding to the different RNA 3′ ends are indicated in roman numerals at the left.

mutation (*hisG2148*) in the *hisG* cistron (Fig. 6B, lane 1) (8). In a *rho* genetic background (*rho111*), there is a variable decrease of the short *his* transcripts, the first and fifth being most affected, as well as a restoration of readthrough transcription (Fig. 6B, lane 2) (8). Transformation of strain SC684 (*rho111 hisG2148*) with plasmids harboring the *E. coli* (Fig. 6B, lane 3) or *N. gonorrhoeae* (Fig. 6B, lane 4) *rho* gene restored, at least partially, the transcriptional pattern of the polar mutant SC685.

The data presented in this report show that Rho factor is highly conserved among different gram-negative bacteria. The overall genomic organization and relevant regulatory elements of *rho* are the same in *E. coli* (32, 35) and *S. typhimurium* (Fig. 1B and 2). These observations and previous studies (15, 24, 25) suggest that *rho* gene function, expression, and regulation are similar in the enterobacteria. In *N. gonorrhoeae*, *rho* has a different genomic organization. It is not preceded as in *E. coli* and *S. typhimurium* by *trxA* but by *ppsA* (Fig. 1B and 3), a gene that in *E. coli* maps very distant from *rho* (37 versus 85 min) (33). Nevertheless *rho* of *N. gonorrhoeae* has the same functions of its enterobacteria homologs, as shown by complementation tests and in vivo transcription assays. The fact that Rho factor of *N. gonorrhoeae* is able to interact with *E. coli* RNA polymerase indicates that it recognizes similar sequence signals and is functionally compatible with an evolutionary diverged RNA polymerase.

Nucleotide sequence accession numbers. The sequences reported here have been deposited in the EMBL/GenBank/DDBJ Nucleotide Sequence Data Libraries under accession numbers Z21789 and Z21790.

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