Function of the Novel Subdomain in the RNA Binding Domain of Transcription Termination Factor Rho from *Micrococcus luteus*

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Transcription termination factor Rho from *Micrococcus luteus*, a high G + C Gram-positive bacterium, contains an unusual extra sequence within its RNA binding domain that is rich in Arg, Glu, and Asp residues and deficient in hydrophobic residues. To determine the role of this extra sequence, we compared the biochemical properties of a variant lacking nearly all the extra sequence, des(60–300) Rho, to that of wild-type *M. luteus* Rho. The two forms had very similar properties except for binding ATP (3, 4). A comparison of the RNA binding properties of the two forms of this Rho with normal and inosine-substituted RNAs were found to correlate fully with their termination properties. These results indicate that the arginine-rich extra sequence is directly involved in the selection of the termination site and support the hypothesis that the sequence is present in *M. luteus* Rho to facilitate its binding to *M. luteus* transcripts, which are likely to have a high degree of base-paired secondary structure because of their high proportion of G residues.

Transcription termination factor Rho is a strongly conserved component in bacteria (1). To cause termination, it first binds to the nascent RNA and then uses the energy derived from ATP hydrolysis to mediate the dissociation of the RNA (2). The Rho polypeptide has two distinct domains, one for binding RNA, the other for binding ATP (3, 4). A comparison of the rho genes isolated from several different phylogenetic groups has revealed that the ATP binding domain is more highly conserved than the RNA binding domain (1).

The most radical divergence that has been discovered so far is the presence of a 150–250-residue extra sequence in the RNA binding domain of the Rhos in organisms from the high G + C Gram-positive group (5, 6). This group includes *Micrococcus luteus*, the *Streptomyces*, and the *Mycobacteria*. Four Rho sequences from this group are known and they all have similar extra sequences. In *M. luteus*, the main extra sequence is between the phylogenetically conserved residues Ile48 and Gly312, and it is unusual in its high proportion of Arg, Glu, and Asp residues and the very low abundance of hydrophobic residues. We refer to it as the arginine-rich extra sequence. Although the sequences of these extra residues in the four known examples are not highly conserved, their compositions and properties are very similar (6), suggesting that they have a similar function and that the extra sequences arose through adaptive evolution. Since the RNA transcripts in these organisms would have a high proportion of G residues, they are likely to have high levels of base-paired secondary structures. One possible function for the insert is to give Rho the ability to bind to and cause termination of transcripts that would have a high degree of secondary structure (5).

Rho factor from *M. luteus* was found to cause termination of transcription in a heterologous system with *Escherichia coli* RNA polymerase and a λ DNA template (5). The *M. luteus* Rho differed from the *E. coli* Rho in being able to terminate transcription at far upstream sites on that template, at sites that were not normally used by *E. coli* Rho. Since *E. coli* Rho binds preferentially to RNA that is largely single-stranded and rich in unpaired cytosine residues (2) and since the λ *cro* transcript that was available for interaction with Rho when RNA polymerase was at the upstream sites of the *cro* is predicted to have a substantial degree of base-paired secondary structure (7), these results suggest that *M. luteus* Rho was using these sites because it was capable of binding to highly structured transcripts.

In this report we test the role of the arginine-rich extra sequence in the RNA binding domain of *M. luteus* Rho on the specificity of the termination by comparing the properties of wild-type *M. luteus* Rho with those of a variant, called des(60–300) Rho, that lacks most of the extra sequence. We also test the role of the RNA secondary structure on the process by using ITP to replace GTP for the synthesis of RNA, which would have less stable base pairing. The results indicate that the extra sequence does have a major role in allowing the *M. luteus* Rho to bind to RNA with a relatively high degree of base-paired secondary structures.

**EXPERIMENTAL PROCEDURES**

**Materials**—*E. coli* Rho protein was provided by Lislott Richardson (Indiana University). *M. luteus* Rho was purified as described elsewhere (8). NusG was a gift from Barbara Stitt (Temple University). Bicyclomycin was obtained from Fujisawa Pharmaceutical Co. Ltd. (Osaka, Japan). *E. coli* RNA polymerase was purchased from Epicentre Technologies. Enzymes used in DNA manipulations were purchased from New England Biolabs. Radioactive nucleotides were from ICN Radiochemicals. Ribonucleotides, deoxynucleotides, and deoxyribonucleotides were purchased from Pharmacia Biotech Inc. Polynucleotides were from Miles Laboratories.

Construction of the Overexpression Vector pBN21—Ligation of the 2021 bp *BamHI/Sphi* fragment (113 to +2076) from pMLRHOSK + (1, 9) into the approximately 4-kilobase *BamHI/Sphi* of pBN10 (5).
provided the entire M. luteus rho gene on plasmid pTB1 (1200 to +2076). pBN15, a plasmid containing a deletion of bp 1150 to +2076 of the 5’ rho’ sequence in pMLRHSOK+ was prepared by ligation of the large EcoRI/BglII fragment of pMLRHSOK+ that had been blunt ended by treatment with Klenow DNA polymerase. pBN15 was then digested with BamHI and ligated with the 268-bp fragment in pBT1, creating this framing deletion. This fragment was cloned into the 2994-bp BamHI/EcoRI fragment of pMLRHSOK+ creating the in-frame deletion of Arg20 to Glu200 (rho bp 180 to +900) in plasmid pBN16.

The Arg20 to Glu200 region (rho bp 180 to +900) in plasmid pBN16 was flanked by two unique restriction sites, SpaI and NcoI. Digestion of pBN16 with these restriction endonucleases released a 118-bp fragment, which was swapped for the 906-bp NheI/SphI fragment in pBT1, introducing the desired deletion into the M. luteus rho gene (pTB3). The approximately 2550-bp BamHI/EcoRI fragment from pBT1 was ligated in the overexpression vector pTSEs (Invitrogen) to create pBN18.

Primers WN7 (5’-CATAGGGCTATGCTGACATCCTTCCAGCAGGAGGCAGCGGAGG-3’) and WN3 (5’-GGCGGCTCTCCCGCGCGCGGG-3’) were used to amplify with a polymerase chain reaction a 247-bp DNA fragment in M. luteus rho (12 to +235). Primer WN7 includes the addition of NdeI and Nhel sites at the 5’ end of the M. luteus rho gene. Additionally, the M. luteus translation initiation codon at +1, GTG, was changed to ATG. Digestion of the polymerase chain reaction product with Nhel and SphI yielded a 118-bp fragment, which was ligated into the 4130-bp NheI/SphI fragment of pTB18 to create pBN19. This resulted in the in-frame placement of M. luteus des(60–300) rho into plasmid pTSEs and added the primary amino acid sequence MRGRSHHHHHHHHGMASGM to the N terminus. Plasmid pBN21 was constructed by ligation of the 1398-bp Nhel/EcoRI fragment from pBN19 into the 5330-bp NheI/EcoRI fragment of vector pET28a (Novagen) which added the primary amino acid sequence MGSSHHHHHHSSGLVPRGSHMASTM to the N terminus. Verification of the sequence changes were confirmed by DNA sequencing (10).

Purification of M. luteus des(60–300) Rho—Plasmid pBN21 containing the M. luteus rho gene with amino acid positions Arg20 to Glu200 (numbering included Met1) was transformed into the protease-deficient (ompT-) E. coli strain BL21DE3[pLysS]. A colony of BL21DE3[pBN21, pLysS] was used to inoculate 150 ml of 2 x YT containing chloramphenicol (25 µg/ml) and kanamycin (50 µg/ml). The culture was grown to 0.4. Overexpression was induced by the addition of IPTG to 1 mM. The culture was aerated an additional 3 h at 37 °C with moderate shaking. Cells were pelleted by centrifugation (10 min, 3000 x g), washed once in STE (100 mM NaCl, 10 mM Tris-HCl, pH 7.8, 1.0 mM EDTA), pelleted as above and stored at −20°C for up to 2 months.

The cell pellet (1 g) was resuspended in 5 ml of grinding buffer (50 mM Tris-HCl, pH 7.8, 0.1 M NaCl, 5% glycerol, 2 mM EDTA, and 0.1 mM dithiothreitol) and blended at low speed with a Virtis homogenizer for 10 s. The dialyzed pool was applied to a 1-ml (0.5 cm x 3 cm) Ni-nitrilotriacetic acid resin (Qiagen) column and washed with 10 column volumes of washing buffer. The His-tagged protein was eluted with a 150-mM NaCl gradient (0.1 M to 1.5 M) in buffer TGED. Fractions containing poly(C) dependent ATPase activity were pooled, concentrated with a Centricon-100 (Amicon), and dialyzed overnight against 1 liter of buffer F (50 mM Tris-HCl, pH 7.8, 0.15 M NaCl, 0.1 mM EDTA, 0.1 mM dithiothreitol, and 50% glycerol). The protein was stored at −20 °C.

In Vitro Synthesis of RNA—In vitro transcription reactions were synthesized in vitro with T7 RNA polymerase (11). Plasmid pIF2, which contains the rho gene with a modified promoter recognized by T7 RNA polymerase, was digested with either Taq1 or HaeIII restriction endonuclease to use as a template to synthesize runoff transcripts of 378 or 115 nucleotides, respectively. 3 pmol of template were added to a 100-µl reaction containing 10 mM MgCl2, 40 µM each of UTP, CTP, GTP, and 0.125 mM [α-³²P]ATP (350 nCi/µmol) and 40 units of RNasin (Promega). To synthesize RNA molecules in which the guanosine residues were replaced with inosine residues, 2 µM ITTP was substituted for 2 µM GTP in the polymerization reaction. The addition of 5 µM GMP was found to enhance the product yield. Synthesis was initiated by the addition of 200 units of T7 RNA polymerase, and the reaction was incubated for 2 h at 37 °C. RNA of the desired length was gel-purified on a 6% polyacrylamide gel containing 7 M urea after electrophoresis at 30 watts for 2 h. The RNA was electroeluted from the gel slice, precipitated with 0.1 vol of ethanol, and stored at −80 °C. Typical yields were 20 pmol for guanosine-incorporated RNA or 100 fmol for inosine-substituted RNA.

RNA Filter-binding Assays—³²P-Labeled RNA obtained by in vitro synthesis was diluted into 50 µl of transcription reaction mix (150 mM potassium glutamate, pH 7.8, 40 mM Tris acetate, pH 7.8, 4 mM Mg(OAc)2, 1 mM dithiothreitol, 0.02% Nonidet P-40, 0.002% acetylated bovine serum albumin, 1% glycerol) to a final concentration of 0.2 mM. This mixture was added to various concentrations of Rho protein (final concentration from 0.05 to 100 nM) and incubated at 25 °C for 10 min. Reactions were filtered under vacuum on Biorace NT filters (Gelman) which had been prewetted in binding buffer. The filters were washed with 6 volumes of wash buffer (binding buffer without the acetylated bovine serum albumin), dried, and assayed for radioactivity by scintillation counting. Sample values were adjusted for the fraction of actively binding protein which was determined by the method of Witherell and Uhlenbeck (12). Data were analyzed with the GraFit program v. 3.0 (Erithacus Software Ltd).

Transcription Termination Assays—Reactions were performed with E. coli RNA polymerase-initiated cro DNA complexes as described previously (5). When inosine-substituted RNA was characterized, ITTP was substituted for GTP in the reaction mixtures with the following modifications. ITTP was used in the formation of the A²⁴ complex at 40 µM rather than 4 µM. Additionally, during elongation NTP concentrations were increased to 2.7 mM (ATP), 1.4 mM (UTP), 0.7 mM (CTP), and 1.1 mM (ITP). Rho concentrations were saturating (28 µM) under these conditions.

RESULTS

Overexpression of M. luteus des(60–300) Rho—To address the potential role of the arginine-rich amino acid extra sequence in the M. luteus Rho protein, a mutant form of the factor, M. luteus des(60–300) Rho, which lacks most of the extra sequence was prepared and used for functional assays. To allow rapid purification and separation from endogenous E. coli Rho, the gene for expressing M. luteus des(60–300) Rho was engineered to encode an N-terminal hexahistidine sequence. After expression from a T7 RNA polymerase promoter by induction with IPTG, this variant of M. luteus Rho comprised greater than 40% of the total cellular protein (data not shown).

Purification to homogeneity was achieved by cation exchange chromatography over Biorex-70 and metal ion affinity chromatography on a nickel-nitrilotriacetic acid-agarose. Table I shows the summary of the purification of the factor. M. luteus des(60–300) Rho protein from 1 g of induced cells.

Fractions containing poly(C)-dependent ATPase activity were pooled, concentrated with a Centricon-100 (Amicon), and dialyzed overnight against 1 liter of buffer F (50 mM Tris-HCl, pH 7.8, 0.15 M NaCl, 0.1 mM EDTA, 0.1 mM dithiothreitol, and 50% glycerol). The protein was stored at −20 °C.
M. luteus Rho Subdomain Function

Summary of purification of M. luteus des(60–300) Rho

| Step            | Protein | Activity* | Yield |
|-----------------|---------|-----------|-------|
| Crude extract   | 23.0    | 198,000   | 8.5   | 100  |
| Biorex-70 pool  | 3.8     | 36,800    | 9.7   | 19   |
| Ni-NTA pool     | 1.5     | 30,000    | 20.2  | 15   |
| Dialyzed protein| 0.77    | 11,700    | 23.0  | 6    |

*One unit corresponds to the release of 1 nmol of P_i per min at 37 °C.

M. luteus Rho protein migrated anomalously at the position for a 95-kDa protein (Fig. 1, lane 4), presumably due to the arginine-rich composition of its extra sequence (13).

M. luteus des(60–300) Rho Retains RNA-dependent ATPase Activity—To determine if the 241-amino acid deletion from within the RNA binding domain of the M. luteus protein significantly altered the ATPase activity of M. luteus des(60–300) Rho, ATPase assays were performed using homopolymer RNA cofactors which had been used previously to characterize E. coli Rho (14) and M. luteus Rho (5). The results (Table II) indicated that removal of the insert had no effect of the specific activity of M. luteus des(60–300) Rho compared to the wild-type protein when poly(C) was the cofactor. Furthermore, M. luteus des(60–300) Rho was activated to nearly the same extents as the wild-type protein by the other homopolymers tested. Finally, removal of the insert did not abolish the requirement for an RNA cofactor as no ATPase activity was detected in the absence of RNA.

M. luteus des(60–300) Rho was indistinguishable from the wild-type M. luteus Rho with respect to its ability to hydrolyze the three other NTPs and dATP and its sensitivity to the Rho-specific inhibitor, bicyclomycin (data not shown).

M. luteus des(60–300) Rho Terminates Transcription—To determine what effect the alteration of the RNA binding domain had on the ability of the protein to terminate transcription, we tested its function in vitro with E. coli RNA polymerase transcribing a λ cro DNA fragment, which has the Rho-dependent terminator, tr1 (15). Strong E. coli Rho termination stop points occur at sites I (226 nucleotides), II (318 nucleotides), and III (351 nucleotides) within the tr1 terminator. When a saturating amount of M. luteus des(60–300) Rho was added to the transcription mixture, RNA transcripts were synthesized that ended at several positions, but primarily at nt 240 and nt 260 (Fig. 2a, lane 4). These preferred end point positions were very different from those for the transcripts made when wild-type M. luteus Rho was present (lane 6). This result shows that the des(60–300) Rho has termination activity but with a different specificity than that of the full-length M. luteus Rho. Its specificity also differed from that of E. coli Rho (Fig. 2a, lane 2) but was very similar to that of E. coli Rho with NusG present (lane 3). Although M. luteus des(60–300) Rho caused termination primarily at more upstream sites, it was able to recognize signals within tr1, causing RNA polymerase molecules that had passed through those upstream sites to terminate transcription at sites I, II, and III (Fig. 2, lane 4). We do not know whether the observation of the close similarity of the termination specificity of M. luteus des(60–300) Rho and E. coli Rho with NusG present is merely a coincidence or is an indication that M. luteus Rho normally circumvents the requirement for NusG. E. coli NusG had very little effect on the termination specificity with M. luteus des(60–300) Rho or with M. luteus Rho (Fig. 2a, lanes 5 and 7). We did not have M. luteus NusG available to test its function. However, in further experiments

Table I

Summary of purification of M. luteus des(60–300) Rho

| Step            | Protein | Activity* | Specific activity | Yield |
|-----------------|---------|-----------|------------------|-------|
| Crude extract   | 23.0    | 198,000   | 8.5              | 100   |
| Biorex-70 pool  | 3.8     | 36,800    | 9.7              | 19    |
| Ni-NTA pool     | 1.5     | 30,000    | 20.2             | 15    |
| Dialyzed protein| 0.77    | 11,700    | 23.0             | 6     |

*One unit corresponds to the release of 1 nmol of P_i per min at 37 °C.

Table II

Activation of Rho ATPase with different RNA homopolymers

| Polynucleotide | E. coli Rho | M. luteus des(60–300) Rho | M. luteus Rho |
|----------------|-------------|---------------------------|---------------|
| ATPase rate    | μmol ATP hydrolyzed min⁻¹ nmol⁻¹ | μmol ATP hydrolyzed min⁻¹ nmol⁻¹ | μmol ATP hydrolyzed min⁻¹ nmol⁻¹ |
| None           | <0.1        | <0.1                      | <0.1          |
| Poly(C)        | 1.2 ± 0.3   | 14.2 ± 0.3                | 14.5 ± 0.1    |
| Poly(U)        | 2.2 ± 0.1   | 7.7 ± 0.4                 | 6.2 ± 0.3     |
| Poly(A)        | <0.1        | 2.4 ± 0.1                 | 5.0 ± 0.1     |
| Poly(I)        | 0.2 ± 0.1   | 1.1 ± 0.1                 | 1.1 ± 0.2     |
(16) termination by M. luteus Rhos could be enhanced by E. coli NusG, but not nearly to the same extent as E. coli Rho.

We verified that the shorter transcripts synthesized in the presence of the Rho factors were not produced by RNase degradation by doing control experiments in which the factors were added to reaction mixtures which had synthesized full-length runoff transcripts prior to adding the factors (data not shown). Titrations of the individual Rho factors revealed that the amount of Rho that was half-maximal for termination activity was approximately 2 nM for each of the proteins (data not shown). The ability of these three Rho factors to terminate transcription was also assessed for tZ1, the first intragenic terminator in lacZ (17) and the results closely paralleled those for the λ tR1 terminator; M. luteus Rho caused transcripts to terminate at points well upstream from the first stop points used by E. coli Rho, and the M. luteus des(60–300) Rho terminated transcription at points just slightly upstream from the E. coli stop points (data not shown). This indicates that the extra sequence in M. luteus Rho has a general effect on the specificity of termination.

Transcription of Inosine-substituted RNA Allows Rho-dependent Termination of Smaller RNA Transcripts—To determine whether the differences in the specificities of the three Rho factors is dependent on the degree of stability of the base-paired secondary structures in the nascent RNAs, we tested their function on the transcription of λ cro DNA in a reaction mixture containing ITP in place of GTP. ITP can replace GTP as a substrate for E. coli RNA polymerase (18) and because inosine forms only two hydrogen bonds with cytosine, the base-paired secondary structures in inosine-substituted RNA will be less stable than the corresponding structures in RNA with guanosine residues (18, 19). The effect of the substitution of GTP with ITP on Rho action was first recognized by Adhya et al. (19), and Morgan et al. (20, 21) showed that the substitution caused E. coli Rho to terminate transcription at new sites well upstream from the normal sites on ω cro DNA.

Since the efficiency of transcription termination at a particular site is very sensitive to the RNA chain elongation rate (22), and since that rate is about 5-fold lower in reaction mixtures containing ITP than in reaction mixtures containing GTP at the same concentration (16), we increased the levels of NTP concentrations in the reaction mixtures for our termination studies to a level that allowed the RNA chains to grow at the same average rate as in the standard reaction mixtures with GTP. With these conditions E. coli Rho terminated transcription at more upstream sites than were used during synthesis of guanosine-containing RNAs (compare Fig. 2, panel b, lane 1, with panel a, lane 2). The sizes of these transcripts, as estimated by gel migration, were similar to those reported by Morgan et al. (20). Likewise, smaller, novel transcripts were produced when M. luteus des(60–300) Rho was present for the synthesis of inosine-containing RNA than for the synthesis of guanosine-containing RNA (Fig. 2, panel b, lane 2). The termination pattern was in fact very similar to that produced by E. coli Rho (compare Fig. 2, panel b, lanes 1 and 2), although there was a slightly greater preference for terminating at earlier points. The wild-type M. luteus protein was also functional on inosine-substituted RNA and the overall preference of termination stop points with it was similar to that produced with the guanosine-containing RNA at nucleotide positions 96, 120, 145, 170, and 240 (compare Fig. 2, panel a, lane 6, with panel b, lane 3, which has a darker exposure). A control reaction in which no Rho factor was added indicated that most of the polymerase molecules were able to read through the template to produce the run-off cro transcript (Fig. 2, panel b, lane 4).

The effect of ITP substitution was also assessed with the tZ1 terminator in lacZ. The results (data not shown) closely paralleled those for the cro template. When transcription was carried out with ITP in place of GTP, termination was shifted to more upstream positions with des(60–300) Rho and E. coli Rho but not with M. luteus Rho.

RNA Binding Properties—To determine if the inability of M. luteus des(60–300) Rho to terminate at the upstream sites used by the wild-type M. luteus protein during transcription with GTP was due to a lack of protein-RNA interaction in the highly structured 5’ end of the RNA molecule, RNA binding studies with λ cro RNAs of various lengths and compositions were performed. Using the same procedures as described by Faus and Richardson (11), we confirmed that the equilibrium dissociation constant for the complexes of E. coli Rho with the full-length, 378-nucleotide λ cro RNA was approximately 1 nM (Table III). With M. luteus Rho and M. luteus des(60–300) Rhos, the Kd values for their complexes with the same RNA were both about 10-fold lower (Table III). When the RNA was a partial transcript of the cro gene of only 115 nucleotides, which is about the size of the shorter transcripts produced by action of M. luteus Rho but shorter than the transcript terminated by the other Rhos, M. luteus Rho bound to it with the same affinity as to the full-length cro transcript, while M. luteus des(60–300) Rho bound to it with nearly 20-fold lower affinity, and E. coli Rho gave barely detectable binding (Table III).

However, when a 115-nt partial cro transcript which has been synthesized using ITP instead of GTP was used, M. luteus Rho again bound to it with the same affinity as to the guanosine-containing transcript, while M. luteus des(60–300) Rho and E. coli Rho were able to bind to it almost as well as the M. luteus Rho (Table III). These results strongly suggest that higher ordered nucleic acid structure near the 5’ end of the cro RNA is inhibitory to binding of Rho factors lacking the arginine-rich insertion within the RNA-binding domain.

### DISCUSSION

We have shown that the arginine-rich subdomain of M. luteus Rho confers on it the ability to bind RNA molecules that do not interact well with two Rho proteins which lack that subdomain, E. coli and M. luteus des(60–300) Rhos, and that this difference in RNA binding properties accounts for the altered transcription termination specificity of M. luteus Rho. We then showed that the differences in RNA binding and termination selectivity are largely eliminated when the RNA transcripts contain inosine residues instead of guanosine residues. Together, these results support the contention that the subdomain in M. luteus Rho gives it the ability to terminate transcription of its G-rich transcripts.

To date the rho genes from 15 organisms from several subgroups of the bacteria have been sequenced. These genes contain a number of highly conserved sequence motifs. The argi-
nine-rich subdomain of M. luteus Rho is an unusual, extended sequence in an unconserved segment between two well conserved sequences in the RNA binding domain. So far, similar extended insert-like sequences have been found only in the proteins encoded by genes from organisms in the same phylogenetic group that includes M. luteus, the high G + C Gram-positive group. Sequences for four members of that group have been reported and all have similar extra sequences. They are similar in having greater than 150 residues, in being rich in Arg residues with balancing Glu and Asp residues, in being poor in hydrophobic residues, and in being in the same position, just preceding the highly conserved RNPI-like sequence. However, the four sequences show very little conservation among themselves with the greatest similarity in the Arg- and Gly-rich C-terminal parts (6). In spite of the lack of sequence conservation, the presence of this similar extra subdomain in all of the known putative Rho factors in members of this phylogenetic group suggests that it arose as an evolutionary adaptation.

This extra sequence is apparently not a characteristic of all the Gram-positive bacteria nor of all organisms with a high G + C content, because it is not present in the putative Rho homologs from the low G + C Gram-positive bacterium, Bacillus subtilis (23), or from Rhodobacter sphaeroides (24), a Gram-negative organism with a G + C content of 69%. Rather, the extra sequence appears to have evolved along the lineage of the high G + C Gram-positive bacteria.

The abundance of arginine residues in the extra sequence of some Rho factors make those factors members of the argininerich motif family of RNA-binding protein. Even though these extra sequences are also rich in Gly residues, they do not have many multiples of the RGG motif, thus they are not members of the family with that feature. Since the Rho factors with the extra sequences are also rich in Gly residues, they do not have some Rho factors make those factors members of the arginine-rich motif family of RNA-binding protein. Even though these extra sequences are also rich in Gly residues, they do not have many multiples of the RGG motif, thus they are not members of the family with that feature. Since the Rho factors with the extra sequence also contain all of the highly conserved RNPI-like sequence motifs found in all Rho factors, they are also members of the RNP motif family (25). The finding that both forms of M. luteus Rho could bind to the full-length λcro RNA with a higher affinity than E. coli Rho could indicate that an important part of the difference in termination selectivity between M. luteus and E. coli Rho resides in the sequences that were still present in the des(60–300) derivative, that is within the RNPI-like sequences. The residual difference in binding affinity is probably the reason for the ability of M. luteus des(60–300) Rho to terminate transcription at sites upstream from those used by E. coli Rho in the absence of NusG.

Arginine residues in RNA-binding proteins are known to interact with specific RNA sequence elements (26) and nonspecifically with the RNA phosphate backbone (27). Although the arginines within the subdomain could be providing either or both of these interactions to the RNA binding properties of M. luteus Rho, we suggest they could have another possible function. The lack of hydrophobic residues, which are important organizing elements for α-helical and β-strand secondary structures in globular proteins, indicates that the subdomain does not have an ordinary globular structure but instead has a loosely ordered structure of largely solvent accessible residues stabilized by salt bridges. Thus, this subdomain could be providing a local solvent environment that could affect the properties of the RNA as the protein is making initial contacts with it through the conserved elements of the RNA binding domain. Taking into consideration the partial specific volumes of the residues included in the extra sequence, we estimate that the concentration of guanido groups from the arginine residues in this 3.4 × 10⁻²⁰ cm³ subdomain to be approximately 2.8 m. At this concentration these guanido groups could be acting as a localized general denaturant causing the disruption of base-paired RNA secondary structure. The disruption of base-paired structures could then allow M. luteus Rho to make strong functional contacts with single-stranded segments of the RNA using the conserved elements of the RNA binding domain. Studies on how the subdomain affects the structure of the bound RNA and on how specific Arg residues affect the binding specificity should be able to distinguish whether the subdomain is merely serving to alter the local solvent properties or is contributing more directly to the binding process through side-chain-nucleotide interactions.

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REFERENCES
1. Opperman, T., and Richardson, J. P. (1994) J. Bacteriol. 176, 5033–5043
2. Platt, T., and Richardson, J. P. (1995) in Transcriptional Regulation, (McKnight, S. L., and Yamamoto, K. R., eds) pp. 365–388, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
3. Dombroski, A. J., and Platt, T. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 2558–2562
4. Dolan, J. W., Marshall, N. F., and Richardson, J. P. (1990) J. Biol. Chem. 265, 5747–5754
5. Nowakcze, W. L., and Richardson, J. P. (1996) J. Biol. Chem. 271, 742–747
6. Ingham, C. J., Hunter, I. S., and Smith, M. C. M. (1996) J. Biol. Chem. 271, 21803–21807
7. Faus, I., and Richardson, J. P. (1990) J. Mol. Biol. 212, 53–66
8. Nowakcze, W. L., Richardson, J. Y., and Richardson, J. P. (1996) Methods Enzymol. 274, 353–363
9. Martinez, A. (1993) A Mutational Analysis of the RNA Binding Domain of Transcription Termination Factor Rho of Escherichia coli. Ph.D. dissertation, Indiana University
10. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
11. Faus, I., and Richardson, J. P. (1989) Biochemistry 28, 3510–3517
12. Witherell, G. W., and Uhlenbeck, O. C. (1989) Biochemistry 28, 71–76
13. Hu, C. C., and Ghahrial, S. A. (1995) J. Virol. Methods 55, 367–379
14. Lowrey, C., and Richardson, J. P. (1977) J. Biol. Chem. 252, 1375–1380
15. Lau, L. F., Roberts, J. W., and Wu, R. (1983) J. Biol. Chem. 258, 9391–9397
16. Burns, C. M. (1986) Nus Factor Modulation of RNA Chain Elongation and Rho-dependent Transcription Termination in Escherichia coli. Ph.D. dissertation, Indiana University
17. Burns, C. M., and Richardson, J. P. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 4738–4742
18. Chamberlin, M. J. (1965) Fed. Proc. 24, 1446–1457
19. Adhya, S., Sarkar, P., Valenzuela, D., and Maitra, U. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 1613–1617
20. Morgan, W. D., Bear, D. G., and von Hippel, P. H. (1983) J. Biol. Chem. 258, 9553–9564
21. Morgan, W. D., Bear, D. G., and von Hippel, P. H. (1983) J. Biol. Chem. 258, 9565–9574
22. Jin, D. J., Burgess, R. R., Richardson, J. P., and Gross, C. A. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 1453–1457
23. Quirk, P. G., Dunkley, E. A., Lee, F., and Krulwich, T. A. (1993) J. Bacteriol. 75, 674–675
24. Gomesky, M., and Kaplan, S. (1966) J. Bacteriol. 92, 680–685
25. Mattai, I. W. (1993) Cell 73, 837–840
26. Cramer, B. J., Tidor, B., Biancalana, S., Hudson, D., and Frankel, A. D. (1991) Science 252, 1167–1171
27. Burd, C. G., and Dreyfuss, G. (1994) Science 265, 615–621
28. Laemmli, U. K. (1970) Nature 227, 680–685