Sequence-specific Interactions in the RNA-binding Domain of Escherichia coli Transcription Termination Factor Rho*

Received for publication, June 2, 2006, and in revised form, August 11, 2006. Published, JBC Papers in Press, August 14, 2006, DOI 10.1074/jbc.M605312200

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Rho factor is an essential protein that causes termination of transcription in a wide variety of bacteria by an RNA-dependent helicase activity. Rho is activated by transcripts that contain a high proportion of cytidine residues. The interaction between Rho and two adjacent cytidine residues within the bound RNA has been identified by previous crystallographic studies (Skordalakes, E., and Berger, J. M. (2003) Cell 114, 135–146). In this study, NMR methods were used to investigate the sequence dependence of the binding of oligonucleotides to the RNA-binding domain of Rho protein (rho130). A comparison of the NMR spectra obtained for rho130 bound to single-stranded oligonucleotides ACTTCCA or ATTCCA showed that the 5′-cytidine residue interacts with Rho at a site that is distinct from the CC binding site identified by crystallographic studies. Two amino acid residues within this new cytidine binding site, Arg88 and Phe89, were altered to Glu and Ser, respectively. These mutant forms of Rho were defective in transcriptional termination, suggesting that those residues play an important role in the activation of Rho by bound RNA.

The termination of a number of transcripts in Escherichia coli, as well as in a number of other bacterial species (1), is the result of the action of Rho protein (see Ref. 2 for review). Rho is an essential protein that is required for the release of nascent messenger RNA at specific Rho-dependent termination sites (3). Rho is hexameric with identical subunits and binds to exposed regions of mRNA that are rich in cytidine (see Fig. 1). The affinity of Rho to poly(rC) is 10-fold higher than to poly(rU) (4) and 100-fold higher than to poly(rA) (5).

Rho contains two distinct classes of binding sites for nucleic acid. The primary site can bind either single-stranded DNA or RNA with similar specificity and affinity, whereas the secondary site binds only RNA. There are six binding sites of each type in the Rho hexamer (6), suggesting that there is one primary and one secondary site on each subunit. The binding of RNA to the primary site, with its subsequent binding to the secondary site, leads to the activation of Rho as a helicase. Although the binding of DNA to the primary site is insufficient for the activation of Rho, it does reduce the concentration of RNA required for activation (7–9). Once activated, Rho couples ATP hydrolysis to its movement in the 5′ → 3′ direction on the mRNA. When the transcriptional complex is reached, Rho causes release of RNA polymerase from the DNA by an unknown mechanism. A number of termination sites require accessory proteins such as NusG for efficient termination (10).

Rho protein can be divided into two structural domains, each of which provides one of the nucleotide binding sites. The amino-terminal 130 residues comprise the primary RNA-binding domain (11), whereas residues 131–419 contain the secondary RNA-binding site as well as the site for the coupling of ATP hydrolysis to the translocation of Rho along the RNA. The primary RNA-binding domain consists of a three-helix bundle (residues 1–50) followed by a β-domain that is a member of the OB-fold family (12). Crystal structures of the primary RNA-binding domain (13), as well as intact hexameric Rho (14) complexed with nucleic acid, have provided considerable insight into the interaction of Rho with RNA bound at the primary site. In the case of the isolated RNA-binding domain, the reported structure was obtained using rC9 as the ligand. The reported structures of intact Rho were obtained by using either DNA (AACCAAGAACCCAA) or RNA ([CU]4) as the ligand. Although studies suggest that the optimal length for nucleotide binding to the primary site appears to be 8–10 cytosine residues (7), the structures of Rho-nucleic acid complexes show interactions with only two adjacent cytidines. The first cytidine base fits tightly within a hydrophobic pocket formed by Phe62 and Tyr80, and it also contacts residues Glu108, Arg109, and Tyr110 in the RNA-binding domain. The second cytidine forms specific hydrogen bonds with Arg68 and Asp78 and stacks on the aromatic side chain of Phe64. In these structures there are no interactions between Rho and the 2′-OH group of the bound RNA, explaining why the primary binding site does not discriminate between RNA and single-stranded DNA. On the basis of these crystal structures, the binding of DNA or RNA does not appear to change the structure of the RNA-binding domain.

The crystal structure of intact Rho, complexed with either single-stranded DNA or RNA bound to the primary site, has illuminated a number of features associated with the function of Rho (14). In these structures the six subunits are related by a pseudo-6-fold screw axis, generating a lock-washer-like topology (see Fig. 1). The primary RNA-binding site is found on the inside surface of the hexamer and oriented in such a manner that the 3′-end of the bound RNA points downward toward the ATPase domain. This model explains how Rho protein can load
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![Illustration of the interaction of hexameric Rho with RNA during loading of the RNA](image)

FIGURE 1. Illustration of the interaction of hexameric Rho with RNA during loading of the RNA. Each subunit is composed of an ATPase domain (residues 131–419), colored red, and an RNA-binding domain (residues 1–130), colored purple. The general region of the primary RNA binding site within the RNA-binding domain is shaded yellow. The bound cytidine-rich RNA is indicated by the green line.

on a circular nucleic acid template (15); after the RNA binds to the primary site, it can associate with the secondary site by sliding through the gap in the lock-washer. This gap then presumably closes, trapping the RNA in the central part of the hexamer as Rho forms the catalytically competent form for helicase activity. The location of the secondary binding site for RNA within the central core of the hexamer is supported by the cross-linking and chemical protection studies reported by Burgess and Richardson (16) and Wei and Richardson (17), respectively.

The kinetic pathway for the activation of Rho by poly(rC) has been investigated by stop-flow methods (18). The initial binding of RNA to the primary site is diffusion-limited, occurring at a rate of $8 \times 10^8 \text{M}^{-1} \text{s}^{-1}$. The Rho-RNA complex then presumably undergoes ring opening at a rate of $21 \text{s}^{-1}$, permitting the RNA to enter the center of the hexamer. This intermediate is likely the complex obtained by Skordalakes and Berger (14). This step is followed by conversion to a third form, with a rate constant of $32 \text{s}^{-1}$, followed by conversion to the final active form, with a rate constant of $4.1 \text{s}^{-1}$.

Although the crystal structures determined by Berger and colleagues (13, 14) and studies of the kinetic pathway by Kim and Patel (18) have resolved a number of questions regarding the function of Rho, there remain a number of unanswered questions. Among these is the nature of the primary RNA-binding site. Crystallographic studies have identified important interactions between Rho and two bound cytidine residues. However, binding data in the literature suggest that more than two cytidine residues are required for the binding of short oligonucleotides. For example, there is an $\sim 10$-fold decrease in the binding affinity of rC$_n$ versus rU$_3$C$_3$, and rU$_5$C$_3$ binds so weakly that it is not possible to measure the binding affinity by gel-shift assays (9). Assuming that productive interactions only occur between Rho and the cytidine residues, a reduction of the number of cytidine residues will reduce the kinetic on-rate by a factor that is proportional to the number of potential binding sites available in the oligonucleotide. An additional decrease in the association constant ($1/K_D$) will occur if Rho cannot form productive interactions with the nucleotide base, leading to an increase in the off-rate. The 10-fold difference in affinity between rC$_n$ and rU$_3$C$_3$ can be largely accounted for by predicted changes in only the on-rate. In contrast, the low binding of rU$_5$C$_3$ appears to require both a decrease in the on-rate and an increase in the off-rate. This suggests that more than two cytidine residues are involved in binding to the primary nucleic acid binding site of Rho.

In this article we have used NMR chemical shift and NMR-derived kinetic binding parameters to probe the binding properties of nucleic acid to the isolated 130 amino-terminal residue of the RNA-binding domain of Rho (rho130). In contrast to other studies, we have identified additional residues in rho130 that contact the bound nucleic acid. We further show that alterations of these residues within the intact Rho hexamer have deleterious effects on transcription termination, even in the presence of NusG.

EXPERIMENTAL PROCEDURES

rho130 Preparation—Isotopically labeled rho130 was produced as described previously (19). Briefly, *E. coli* (BL21(DE3)pLysS) cells containing the expression vector were grown on minimal media with 1 g/liter (15NH$_4$)$_2$SO$_4$ and 4 g/liter [13C]glucose (uniform labeling) or with 50 mg/liter [13C]Phe labeled at the β-carbon (Phe-labeled sample). All stable isotopes were purchased from Cambridge Isotopes (Andover, MA). When the cells reached an $A_{550}$ of 0.8, isopropyl thigalactopyranoside was added to a concentration of 1 mM. Improved yields were obtained with the addition of guanosine, cytosine, and uridine, each at 50 mg/liter, at the time of induction. After 3–4 h of induction, the cells were harvested and lysed by sonication, and the lysate was clarified by centrifugation at 5 °C (20,000 rpm, Beckman Ti70 rotor, 20 min). All subsequent purification steps were performed at room temperature. The lysate was then chromatographed on a CM Sephadex column using a linear gradient of 0.0–0.8 M NaCl in 2 mM EDTA, 50 mM Tris-HCl buffer, pH 7.5. Sufficient potassium sulfate was added to the protein-containing fractions to raise the sulfate concentration to 150 mM. The pooled fractions were concentrated with Centricon-10 ultracentrifugation devices (Amicon) and chromatographed on a Sephadex G-50 gel filtration column. Following extensive dialysis against buffer (2 mM EDTA, 25 mM Tris-HCl, pH 7.5), the protein was bound to a DEAE-agarose column. The rho130 was eluted with a linear 0.0–0.4 M NaCl gradient in the same buffer used for loading. Purified rho130 was dialyzed against 10 mM potassium phosphate buffer, pH 7.0, containing either 150 mM potassium sulfate or 100 mM potassium chloride. The samples were then concentrated by ultracentrifugation for NMR experiments.

Oligonucleotide Preparation and Purification—DNA oligonucleotides dC$_n$ and dAC$_n$A were synthesized in-house using

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2 The abbreviations used are: rho130, the first 130 amino acids of Rho; HSQC, heteronuclear single quantum coherence.
standard phosphoramidite chemistry (20). High-loading CPG and other phosphoramidite reagents were purchased from Glen Research (Sterling, VA). Anhydrous acetonitrile was purchased from Sigma-Aldrich. Because these oligonucleotides are relatively short, the “capping” step was omitted from the synthesis. The dATTTCCA, dACTTCCA, and dATCTCCA nucleotides were purchased as custom syntheses from Synthegen, LLC (Houston). In all cases, the oligonucleotides were purified by binding the crude product to DEAE-agarose followed by elution with a linear 0.01–1.0 M ammonium acetate gradient. Fractions that contained the desired product were lyophilized several times to remove residual ammonium acetate. The desired full-length product was confirmed by electrospray mass spectrometry.

**NMR Spectroscopy**—NMR spectra were recorded at 600 MHz using a Bruker DRX spectrometer equipped with a Bruker triple resonance probe with triple axis pulsed-field gradient coils. All NMR spectra were recorded at 298 K as described previously (19). The chemical shift assignments for rho130 bound to dC6 were determined using methods similar to those previously (19). The chemical shift assignments for rho130 when other ligands were bound could generally be obtained by following the change in chemical shifts during titration of nucleotide ligands. In many cases, HNCA spectra (21) were recorded for rho130 bound to different nucleotide ligands to confirm chemical shift assignments.

**Nucleotide Titrations**—DNA concentrations were determined spectrophotometrically by measuring solution absorbance at λ = 260 nm. Extinction coefficients were determined by summing the coefficient for each residue of the oligonucleotide. Prior to DNA titrations, the oligonucleotides were aliquoted and lyophilized. DNA was added to the sample by using the protein solution to dissolve the lyophilized DNA. Protein concentrations were typically 0.25 mM. After each incremental addition of DNA was performed until single titration to minimize loss of sample during transfers. For recorded on the samples. The same glassware was used for a

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**Nucleic Acid Binding Kinetics**

**ATPase and RNA Binding Assay Methods for Full Length Rho**—Poly(C)-activated ATPase activities of the Rho factors were determined by the colorimetric procedure of Nowatzke et al. (27). ATPase activities with λcro RNA were determined as described by Faus and Richardson (29) with saturating levels of RNA (15 with 4 nM Rho hexamers). The procedure was modified to detect the P1 product colorimetrically.

**RESULTS**

**Nucleic Acid Binding Kinetics**—Given that intact Rho and rho130 bind both RNA and single-stranded DNA with equal
affinity and specificity, the binding studies with rho130 were carried out using DNA because of its enhanced chemical stability. Our initial studies were directed at observing DNA-induced changes in the structure of rho130, employing dC₆ as the ligand. Although HSQC spectra of the amide groups gave spectra of reasonable quality, the spectra obtained for side-chain resonances suggested that considerable chemical exchange was present in the complex. In the absence of bound DNA the HβCβ signals in a carbon-proton-correlated spectrum of [β-¹³C]Phe-labeled rho130 clearly show resonance peaks from all seven Phe residues in the protein (Fig. 2A). In contrast, when the complex with dC₆ is formed, the spectra show multiple peaks, many of which are broadened by chemical exchange (Fig. 2B). The appearance of the spectrum obtained in the presence of dC₆ suggests that the time scale for the interconversion between binding modes is on the slow to intermediate NMR time scale. The exchange rate is ~100–1000 s⁻¹, based on chemical shift differences between the resonance lines in the bound and free forms of the protein.

Exchange broadening in the dC₆ complex is due to multiple binding modes, i.e., different chemical shifts are obtained when rho130 binds at the end of the oligonucleotide versus the middle of the oligonucleotide. In principle, exchange broadening could be minimized by simply increasing the length of the oligonucleotide. However, rho130 forms oligomers on longer oligonucleotides (19), severely degrading the quality of the NMR spectra. Therefore, multiple binding modes and, consequently, exchange broadening were reduced by the addition of adenosine, a nucleotide that is known to have low affinity to Rho protein, at both ends of the oligonucleotide. In the case of Rho bound to dAC₅A, the resultant spectra indicate that a single binding mode is predominant (see Fig. 2D). Presumably the larger purine residue prevents rho130 from binding at the very end of the oligonucleotide.

The kinetic on- and off-rates of the binding of dC₆ and dAC₇A, where n = 3, 4, or 5, are presented in Table 1. These data were obtained by fitting the proton resonance line of Phe⁶₂ to Equation 1 at different ligand concentrations (see Fig. 3). The on- and off-rates indicate that the binding of dAC₇A is essentially equivalent to the binding of dC₆; the kinetic on-rates are both near the diffusion limit, and the equilibrium binding constants for these two ligands are similar. A diffusion-limited on-rate was also observed for the binding of poly(rC) to intact Rho (18), suggesting that the binding of nucleic acid to both forms of Rho follow a similar mechanism. The measured off-rate of 2600 s⁻¹ suggests that unbinding followed by rebinding, in a different configuration, is responsible for the appearance of the spectra obtained in the presence of dC₆.

The measured binding constants show that reducing the number of C residues results in a systematic decrease in the kinetic on-rate and, at least for dAC₇A, a significant increase

**TABLE 1** Kinetic on- and off-rates
Data were acquired at 25 mM KCl to increase the binding to dAC₃A. Kinetic on- and off-rates were obtained by nonlinear least squares fit of the proton resonance line from Phe⁶₂ at different ligand concentrations to Equation 1. The error ranges correspond to 1 S.D. for each parameter. 

| Ligand     | \( k_{on} \times 10^{-7} \) | \( k_{off} \times 10^{-7} \) | \( K_D \) |
|------------|-----------------------------|-----------------------------|----------|
| dAC₃A      | 0.8 ± 0.5                   | 4.7 ± 1.4                   | 550      |
| dAC₄A      | 1.8 ± 0.2                   | 2.2 ± 1.0                   | 120      |
| dAC₅A      | 6.2 ± 2.5                   | 2.8 ± 1.2                   | 45       |
| (dC)₆      | 9.8 ± 0.3                   | 2.6 ± 2.0                   | 16       |

**FIGURE 2.** ¹H-¹³C HSQC spectra of Rho labeled with [¹³C]phenylalanine (β-¹³C). A, rho130 in the absence of ligand. B, the spectrum for the rho130-dC₆ complex. C, the spectrum for the rho130-dAC₅A complex. D, the spectrum for the rho130-dAC₅A complex. In all cases the protein was saturated with the indicated ligand ([DNA] > 10 \( K_D \)).

**FIGURE 3.** Typical DNA titration data. The intensity versus frequency data for Phe⁶₂ were extracted from two-dimensional ¹H-¹⁵N HSQC spectra of rho130 acquired during the titration of rho130 with dC₆. Only a subset of the peaks is shown in this figure for clarity. The lines through the data points represent the fit to Equation 1. The concentration of protein was 0.25 mM, and the DNA concentrations were 0, 0.117, 0.312, and 1.093 mM (left curve to right curve).
in the off-rate. The combination of these two factors leads to a 12-fold increase in the dissociation constant ($K_D$) when $dAC_3A$ is compared with $dAC_5A$. It is possible that this decrease in binding affinity is simply due to a decrease in the length of the oligonucleotides. However, experiments that measured the binding of oligonucleotides of constant length show a large decrease in binding affinity between $U_1C_3$ and $U_2C_3$ (7). Together, these data suggest that the primary RNA binding site of Rho interacts with more than the two cytidines identified in crystallographic studies.

To define which cytidine position within $dAC_5A$ interacts with rho130, we obtained proton-nitrogen-correlated HSQC spectra in the presence of $dATTTCCA$, $dATCTCCA$, and $dACTTCCA$ (see Fig. 4A). In these oligonucleotides, the 3’ pair of cytidines was designed to interact with the dicytidine binding site identified in the crystallographic studies. The spectra obtained with $dATTTCCA$ and $dACTTCCA$ are essentially identical (not shown), indicating that rho130 does not distinguish between a T or a C at the second position within the C$_5$ stretch. In marked contrast, the HSQC spectrum obtained with $dACTTCCA$ showed chemical shift changes (relative to $dATTTCCA$) for a number of residues, suggesting that rho130 interacts selectively with the first cytidine within the C$_5$ stretch of AC$_5$A. These residues included Val$^{81}$, Ser$^{82}$, Ser$^{84}$, Gln$^{85}$, Ile$^{86}$, Arg$^{87}$, Phe$^{89}$, Lys$^{100}$, Leu$^{114}$, Lys$^{115}$, and Asn$^{117}$ (see Fig. 4A). Arg$^{88}$, although located between Arg$^{87}$ and Phe$^{89}$ in the primary sequence, showed a similar chemical shift change in the presence of either $dACTTCCA$ or $dATTTCCA$.

On the basis of the preceding observation, one would predict that spectral changes due to the binding of $dAC_5A$ should be similar to the changes that occur when $dATTTCCA$ binds. This is indeed the case for 9 of the 11 residues that show different chemical shifts in the $dACTTCCA$ complex versus the $dATTTCCA$ complex. The chemical shift changes of these residues result from the binding of $dACTTCCA$ and $dAC_5A$ are similar in direction and magnitude for both the amide proton and nitrogen atoms (see Fig. 4B). These residues map to a single region of rho130, as shown in Fig. 5.

Effect of rho Mutations on Termination—The location of residues with altered amide chemical shifts in the $dACTTCCA$ complex suggests a region of rho130 that could potentially interact with the additional 5’-cytidine (see Fig. 5). Two residues within this region, Arg$^{88}$ and Phe$^{89}$, were selected for mutagenesis studies because their side chains project into the putative RNA-binding region, and residues of these types are often involved in protein-nucleic acid interactions. Arg$^{88}$ and Phe$^{89}$ were converted to glutamic acid and serine, respectively. Although both mutants

FIGURE 5. Location of the cytidine binding sites on rho130. The structure of rho130 is shown as a stereo ribbon diagram. The two bound cytidine residues identified by Bogden et al. (13) in the crystal structure of the complex between the RNA-binding domain of Rho and rC$_3$ are rendered in ball-and-stick form and labeled as rCC. Phe$^{69}$, which stacks on the 3’-cytosine base, is colored purple, covered with a dotted surface, and labeled. The location of residues in which amides show similar chemical shift changes when bound to either $dAC_5A$ or $dACTTCCA$ is indicated by red coloring of the ribbon. Residues in the vicinity of the cytidine-binding domain (Ser$^{84}$, Arg$^{87}$, Arg$^{88}$, Phe$^{89}$, Asn$^{117}$) are labeled. The side chains of Arg$^{88}$ and Phe$^{89}$ are shown as covered with a red dotted surface. In this figure, the stereo images occupy approximately the same relative location as the adjacent RNA-binding domain in intact Rho, i.e. residues Arg$^{88}$ and Phe$^{89}$ are close to the interface between Rho monomers. The RNA-binding domain is oriented such that the ATPase domain would be found below the RNA-binding domain in the hexamer.

FIGURE 4. Effect of DNA sequence on HSQC spectra of rho130. A, the HSQC spectrum of the rho130-$dATTTCCA$ complex (blue contours) overlaid with the HSQC spectrum of the rho130-$dACTTCCA$ complex (red contours). Labeled peaks showed a greater than 15-Hz difference between the chemical shift in the $dACTTCCA$-rho130 complex versus the $dATTTCCA$-rho130 complex. The peaks labeled with bold letters indicate residues that show similar chemical shift changes when either $dAC_5A$ or $dACTTCCA$ is bound to the protein (see B). The two remaining labeled peaks (Leu$^{114}$ and Ile$^{86}$) are residues in which the chemical shift in the $dACTTCCA$ complex differs from the shift in either the $dAC_5A$ or the $dATTTCCA$ complex. B, illustrates the chemical shift changes in the HSQC peak due to the binding of $dAC_5A$ (black), $dACTTCCA$ (red), $dATTTCCA$ (blue), or $dATCTCCA$ (green) for Phe$^{89}$, Phe$^{89}$, and Lys$^{115}$. The open circle is the position of the peak in the unliganded sample, and the closed circles indicate the peak in the DNA-rho130 complex. Phe$^{89}$ is not sensitive to the sequence of the bound ligand and shows a uniform chemical shift change for all four DNA sequences. In contrast, for both Phe$^{89}$ and Lys$^{115}$, the chemical shift change due to binding of $dAC_5A$ is similar to that seen for $dACTTCCA$. Similar plots were observed for Val$^{81}$, Ser$^{82}$, Ser$^{84}$, Gln$^{85}$, Arg$^{87}$, Phe$^{89}$, Lys$^{100}$, Leu$^{114}$, Lys$^{115}$, and Asn$^{117}$ (see Fig. 4A). Arg$^{88}$, although located between Arg$^{87}$ and Phe$^{89}$ in the primary sequence, showed a similar chemical shift change in the presence of either $dACTTCCA$ or $dATTTCCA$. The location of residues in which amides show similar chemical shift changes when bound to either $dAC_5A$ or $dACTTCCA$ is indicated by red coloring of the ribbon. Residues in the vicinity of the cytidine-binding domain (Ser$^{84}$, Arg$^{87}$, Arg$^{88}$, Phe$^{89}$, Asn$^{117}$) are labeled. The side chains of Arg$^{88}$ and Phe$^{89}$ are shown as covered with a red dotted surface. In this figure, the stereo images occupy approximately the same relative location as the adjacent RNA-binding domain in intact Rho, i.e. residues Arg$^{88}$ and Phe$^{89}$ are close to the interface between Rho monomers. The RNA-binding domain is oriented such that the ATPase domain would be found below the RNA-binding domain in the hexamer.
were readily expressed in soluble form, the fraction of each that was active in binding RNA (0.2) was significantly lower than that for the wild-type Rho (0.68, Table 2), suggesting that these mutants were not as stable as the wild-type protein. When corrected for these differences, the $K_D$ values of F89S and R88E Rhos for binding to $\lambda$cro RNA were increased 4- and 10-fold, respectively.

The effects of these mutations on the termination of $\lambda$cro RNA is shown in Fig. 6. Even in great excess, R88E Rho was inactive in termination and had only slight activity when the NusG termination enhancer protein was added. F89S Rho, however, was only partially defective in termination function, as expected from its partial loss of RNA binding affinity and intermediate level of ATPase with $\lambda$cro RNA (Table 2).

**DISCUSSION**

On the basis of binding affinities and chemical shift changes, we have identified another region of the primary RNA binding site in Rho that interacts with a cytidine residue. This additional binding site is 5' from the previously identified site that binds two adjacent cytidine residues. This additional interaction was not observed in crystallographic studies of the complex between the RNA-binding domain and rCD described by Bogden et al. (13). However, because the contribution of the 5'-cytidine to the overall binding affinity is small as suggested by the small change in off-rates, this binding site may not have sufficiently high occupancy to be observed in the crystal structure.

In intact Rho protein, the binding pocket for the 5'-cytidine residue is located close to the contact point between adjacent RNA-binding domains as described in the structure by Skordalakes and Berger (14) (see Fig. 5). Consequently, it would be difficult for nucleic acid to occupy this site in the observed structure of Rho. Nevertheless, alteration of the residues within the newly identified 5'-cytidine binding pocket (Arg88, Phe89) has a great effect on the ability of Rho to terminate transcription. This effect could be due to the reduced binding of the RNA to the protein. However, the decreased binding does not appear to be sufficient to explain the deficiency in transcriptional termination of $\lambda$cro transcripts at high concentrations of Rho protein (Fig. 6). Accordingly, interactions between the bound RNA and the new 5'-cytidine binding site in rho130 may represent an essential, but transient, interaction that is important for subsequent conformational changes in Rho, such as ring closure, that lead to the activation of Rho helicase activity.

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