A Mutation in the ATP Binding Domain of Rho Alters Its RNA Binding Properties and Uncouples ATP Hydrolysis from Helicase Activity*

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Shalini Pereira and Terry Platt
From the Department of Biochemistry, University of Rochester Medical Center, Rochester, New York 14624

The Escherichia coli mutant rho201 was originally isolated in a genetic screen for defects in rho-dependent termination. Cloning and sequencing of this gene reveals a single phenylalanine to cysteine mutation at residue 232 in the ATP binding domain of the protein. This mutation significantly alters its RNA binding properties so that it binds trp tRNA 100-fold weaker than the wild type protein, with a Kd of approximately 1.3 nM. Rho201 binds nonspecific RNA only 3-4-fold less tightly than it binds trp tRNA, while the wild type differential for these same RNAs is 10-20-fold. Curiously, rho201 displays increased secondary site RNA activation, with a Km for ribo(C)10 of 0.6 μM, compared to the wild type value of 3-4 μM. Although rho201 and the wild type protein hydrolyze ATP similarly with poly(C), or trp tRNA, as cofactors, rho201 has a higher ATPase activity when activated by nonspecific RNA. Physically, rho201 displays an abnormal conformation detectable by mild trypsin digestion. Despite effective ATP hydrolysis, the rho201 mutant is a poor RNA:DNA helicase and terminates inefficiently on trp tRNA. The single F232C mutation thus appears to uncouple the protein's ATPase activity from its helicase function, so rho can no longer harness available energy for use in subsequent reactions.

Rho is an essential cellular protein required for certain transcription termination events in Escherichia coli. It is a highly complex molecule with multiple activities that are essential to its role as a transcription terminator. It has both primary and secondary nucleic acid binding sites, which differ in the specificity and the relative affinity with which they bind polynucleotides (Richardson, 1982). The RNA-dependent ATPase activity of rho (Lowery-Goldhammer and Richardson, 1976; Galluppi et al., 1976) is essential for its 5'-3' RNA:DNA helicase activity (Brennan et al., 1987). Models for transcription termination propose that rho binds target sites on a nascent RNA, translocates in a 5'-3' direction, and disrupts any RNA:DNA helix present at the transcription bubble, utilizing the energy released by ATP hydrolysis to facilitate release of the DNA helix present at the transcription bubble, utilizing the available energy for use in subsequent reactions.

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†To whom correspondence should be addressed. Tel.: 716-275-8244; Fax: 716-271-2683; E-mail: tpla@uhura.cc.rochester.edu.

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not been determined. Steinmetz et al. (1990) proposed the existence of a single RNA binding site on each subunit, which could alternate between primary and secondary site character dependent on conformational state. Such a “two-state” model for a single site would account for the inability to separate chemically the primary and secondary RNA binding sites in wild type rho (Dolan et al., 1990).

A second domain of rho extends from amino acids 160 to 340 and shows extensive sequence similarity with the E. coli F$_1$ ATPase $\alpha$ and $\beta$ subunits as well as adenylate kinase, proteins known to be involved in nucleotide binding and hydrolysis. The similarity strongly suggested that these elements within rho were involved in NTP binding (Dombroski and Platt, 1988), a prediction that was supported by affinity labeling studies with proteins known to be involved in nucleotide binding and hydrolysis. The ATPase activity of rho has not been determined. Steinmetz et al. (1977) showed extensive sequence similarity with the rho helicase and showed that the primary and secondary RNA binding sites in rho are involved in nucleotide binding and hydrolysis. The existence of a single RNA binding site on each subunit, which may also play a role in rho's secondary site interactions (Zheng et al., 1992), suggests that these residues might be involved in the assembly of rho subunits (Opperman et al., 1995). Furthermore, studies with the known secondary site mutant, rho suA1, have identified its single mutation as a lysine to glutamic acid change at amino acid 352 (K352E), raising the possibility that this region may play a role in rho's secondary site interactions (Zheng and Friedman, 1994; Pereira and Platt, 1995).

Finally, rho itself appears to assemble as a trimer of asymmetric dimers, with two types of both ATP and RNA binding sites within the active hexamer (Stitt, 1988; Geissmann and von Hippel, 1992; Geissmann et al., 1992; Wang and von Hippel, 1993). Current models for rho action thus require: 1) binding of RNA to both primary and secondary sites in order to elicit ATP hydrolysis by the ATP-binding domain (Richardson, 1992; Seifried et al., 1992); and 2) “coupling” of this hydrolysis, in turn, back to an RNA bind-and-release cycle to obtain directed helicase/termination function (Geissmann et al., 1993; Platt, 1994). Communication between the monomers of the rho hexamer is thus an essential component of the coupling reactions between ATP hydrolysis and the subsequent translocation, helicase, and termination activities (Seifried et al., 1992; Geissmann et al., 1993). Although rho factor has been extensively characterized, the elements within the protein that are involved in this “coupling reaction” remain unknown.

Here we report the sequencing of the gene and the characterization of the mutant rho201 protein, which displays significantly altered primary site RNA binding whereas its single F232C mutation lies outside the core RNA binding amino-terminal third of the protein. Despite a much weaker affinity for RNA than wild type protein, this mutant has an efficient RNA-dependent ATPase activity. Nevertheless its helicase activity is significantly lower than that of the wild type, suggesting that the mutation has uncoupled these two activities.

**Materials and Methods**

Identification of the Rho201 Mutation and Purification of the Mutation Protein—Genomic DNA for PCR amplification was obtained from strain X8605 rho201 (Guarente et al., 1977; Guarente and Beckwith, 1978), which was tested for mutant rho activity as follows. E. coli strains X8605 and X8605 rho201 (in which the lac genes have been transposed to a chromosomal location downstream of the trp operon) were grown on LB plates containing 0.02 mg/ml 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside. The X8605 rho201 colonies displayed blue color, due to transcriptional readthrough beyond the trp t region into the lac operon (Geiselmann et al., 1977), while the X8605 colonies remained white. The rho201 gene carried three mutations, identified by sequencing multiple clones of rho201-specific PCR products subcloned into pGEM3Z(+) (Promega). One of the mutations, a UUU to UGU resulted in an amino acid change at position 232, leading to phenylalanine being replaced by cysteine (F232C). The other two were silent mutations in the codons for isoleucine 38 and leucine 39. Since the PCR products carried Tag polymerase, the mutations in addition to the one at amino acid 232 elicit ATP hydrolysis by the ATP-binding domain (Richardson, 1992; von Hippel, 1992; Geiselmann et al., 1992; Wang and von Hippel, 1993) and show extensive sequence similarity with the rho helicase and termination activities (Seifried et al., 1992; Guarente and Beckwith, 1978), communication between the monomers of the rho hexamer was elicit ATP hydrolysis by the ATP-binding domain (Richardson, 1992; Seifried et al., 1992; Guarente and Beckwith, 1978), and show extensive sequence similarity with the rho helicase and termination activities (Seifried et al., 1992; Guarente and Beckwith, 1978).

**Protein—**

Expression of the mutant rho201 protein was overexpressed, purified, and the percent active protein estimated at 75% (as measured by the ratio of rho active for RNA binding) according to the method of Witherell and Uhlenbeck (1989) and as described by Brennan and Platt (1991). The wild type protein was isolated from DH5α cells carrying pCIRE (the expression vector carrying a wild type copy of the rho gene active). Communication between the monomers of the rho hexamer was thus an essential component of the coupling reactions between ATP hydrolysis and the subsequent translocation, helicase, and termination activities (Seifried et al., 1992; Geissmann et al., 1993). Although rho factor has been extensively characterized, the elements within the protein that are involved in this “coupling reaction” remain unknown.

Here we report the sequencing of the gene and the characterization of the mutant rho201 protein, which displays significantly altered primary site RNA binding whereas its single F232C mutation lies outside the core RNA binding amino-terminal third of the protein. Despite a much weaker affinity for RNA than wild type protein, this mutant has an efficient RNA-dependent ATPase activity. Nevertheless its helicase activity is significantly lower than that of the wild type, suggesting that the mutation has uncoupled these two activities.
Summary of the RNA binding and ATPase activity data for the wild type and mutant rho201 proteins with different RNA cofactors

|                      | rho wt | rho201 |
|----------------------|--------|--------|
| ATPase with poly(C)  | 4 ± 1  | 4.5 ± 1|
| ATPase with trp t'   | 1.2 ± 0.3| 1.5 ± 0.2|
| ATPase with T/H      | 0.05 ± 0.01| 0.74 ± 0.1|
| ATPase with Gal 7    | 0.26 ± 0.1| 1.2 ± 0.2|
| $K_d$ with trp t'    | 0.012 ± 0.005 μM | 1.35 ± 0.15 μM |
| $K_d$ with T/H       | 0.15 ± 0.05 μM | 5.0 ± 0.5 μM |
| $K_d$ with Gal 7     | 0.25 ± 0.05 μM | 6.0 ± 0.5 μM |

RESULTS

Purification of the Mutant Rho201 Protein—Rho201 was originally isolated as a termination-defective allele of rho in vivo (Guarente et al., 1977). After establishing that it was defective at the trp t' rho-dependent termination site in vivo, we amplified the chromosomal copy of the mutant rho gene using PCR, cloned these products into pGEM3zf(+), and sequenced multiple progeny clones (as described under “Materials and Methods”). We determined that the rho201 defect is due to a single mutation in rho’s predicted ATP-binding domain, a phenylalanine to cysteine change as a result of a UUU to UGU codon change at position 232 (F232C). Since the PCR products carried additional Taq polymerase induced mutations, we introduced the single F232C mutation into the wild type copy of the rho gene using site-directed mutagenesis, and cloned the mutated gene into the overproducing vector pCI1R (Dombroski and Platt, 1990). After overexpressing and purifying both rho201 and the wild type proteins, we determined the fraction of active protein in the preparations according to the method described by Witherell and Uhlenbeck (1989), and have expressed all further values as concentration of active hexamer (see “Materials and Methods”).

Rho201 Hydrolyses ATP Very Effciently with Both Poly(C) and trp t'—Rho201 was defective at terminating at the trp t' site in vivo, and its single mutation was determined to be in the putative ATP-binding domain. In order to determine whether decreased ATPase activity could be responsible for its reduced termination capability at the trp t' site in vivo, we examined its poly(C) and trp t'-dependent ATPase. Rho201 hydrolyzes ATP as well as the wild type protein when using poly(C) as a cofactor (data not shown). Surprisingly, there was no decrease in its trp t'-dependent ATPase activity, as compared to the wild type protein. The specific activities of both proteins with each cofactor are summarized in Table I. The efficient ATPase activity of the rho201 protein indicates that the F232C mutation in its ATP-binding domain does not interfere with its ability to hydrolyze ATP.

The Primary Binding Site of Rho201 Has a Weakened Association with RNA—Since the rho201 mutant protein could efficiently hydrolyze ATP using trp t' as a cofactor, we examined the interaction of the protein’s primary binding sites with trp t' RNA. Labeled trp t' at 0.005 nM was incubated with varying concentrations of rho, ranging from 0.008 to 0.1 nM for the wild type protein, and from 0.25 to 5.0 nM for rho201. Nitrocellulose filter retention of the labeled RNA by rho was used to determine the $K_d$ of the protein-RNA complexes, via Scatchard analysis (Fig. 1, A and B). We observed that rho201 bound trp t' RNA with an apparent $K_d$ of approximately 1.3 nM, 100-fold less tightly than did the wild type, which has a $K_d$ of approximately 0.012 nM (Table I). The binding data thus indicates that rho201 is defective in primary site RNA binding, although the single amino acid change in the protein lies in the domain defined primarily as ATP binding (Dombroski and Platt, 1988).

Rho201 Is a Better ATPase Than the Wild Type Using Non-specific RNA as a Cofactor—Since rho201’s ability to hydrolyze ATP with trp t’ as a cofactor (at saturating concentrations) was unaffected by its decreased affinity for trp t’ RNA, we decided to investigate the capacity of two RNA heteropolymers, which are not normally substrates in its transcription termination reaction (nonspecific RNAs), to act as cofactors in an ATPase reaction. We used T/H RNA, made up of E. coli vector sequence (Steinmetz et al., 1990), and an RNA derived from the yeast GAL7 3’-untranslated region (Butler et al., 1990). These two RNAs are nearly the same length as trp t’, i.e. approximately 240 nucleotides each, and have about the same cytosine content (50-60 cytosine residues). A comparison of the specific activity
expressed as nanomoles of ATP hydrolyzed/min/pmol of rho of each protein with the different cofactors is presented in Table I. Rho201 is a very efficient ATPase with both T/H and GAL7 RNA displaying a specific activity of 0.74 and 1.2 for each RNA respectively (Table I). The wild type protein on the other hand, discriminates between substrate and nonspecific RNA, exhibiting a much lower rate of ATPase activity with T/H and GAL7 as compared with trp t’ (specific activity of 0.05 and 0.26 versus 1.2; see also Table I). To test whether rho201’s ATPase activity had become cofactor-independent we carried out the assay in the absence of RNA, and found no detectable hydrolysis of ATP over a 1-h period (data not shown). The ATPase activity of the rho201 protein with the T/H and GAL7 RNAs suggests that the single F232C mutation causes it to hydrolyze ATP efficiently when bound to either substrate (trp t’) or nonspecific (T/H and GAL7) RNA.

Binding of the Wild Type Rho and Rho201 to Nonspecific RNA—Based on the finding that rho201 hydrolyzes ATP very efficiently with both substrate and nonspecific RNA and that it binds one of its natural cellular substrates, trp t’, very weakly as compared to the wild type protein, we postulated that it may have lost its specificity for binding RNA at its primary site. To test this hypothesis we carried out nitrocellulose filter binding assays of both the wild type and the mutant protein with the two nonspecific RNAs, T/H and GAL7. The reactions were carried out at RNA concentrations of 0.05 nm and rho concentrations ranging from 0.008 to 2.0 nm for the wild type protein and from 0.25 to 20 nm for rho201 (data not shown) with the results summarized in Table I. The wild type protein binds T/H with a 12-fold weaker affinity than trp t’ with a $K_d$ value of 0.15 nm; the binding to GAL7 RNA is about 20-fold weaker, the $K_d$ being 0.25 nm. Rho201, on the other hand, binds both T/H and GAL7 RNA only 3–4-fold less tightly than it binds trp t’, with apparent $K_d$ values of 5.0 and 6.0 nm, respectively. The binding data thus suggest that rho201 is not only defective for binding RNA at the primary site as compared to the wild type, as is demonstrated by its increased $K_d$ values for all the RNAs tested, but that its ability to discriminate between substrate and nonspecific RNA in the binding reaction has been compromised.

Secondary Site Activation of the Rho Proteins—To test the hypothesis that rho201’s increased ATPase activity, despite poor primary site binding to RNA, could be due to an enhancement of its transient secondary site interactions, we carried out the experiments shown in Fig. 2. Rho has been demonstrated to bind single stranded DNA efficiently, but is unable to use it as a cofactor in the ATPase reaction (Richardson, 1982). The primary binding site of both wild type rho and rho201 were saturated with a fixed concentration of poly(dC)$_{34}$. The ATPase activity was stimulated by the addition of short ribo(C)$_{10}$ oligos at varying concentrations. Lineweaver-Burk plots of the resulting data allowed the $K_m$ value of the ribonucleotide stimulation at the secondary site to be determined (Richardson and Carey, 1982). Richardson (1982) has determined that in the absence of poly(dC) the $K_m$ value for the short oligoribonucleotides activating the ATPase of wild type rho is 20–25 $\mu$M. The $K_m$ value for ribo(C)$_{10}$ in the presence of poly(dC)$_{34}$ at saturating concentrations was 3–4 $\mu$M for the wild type protein (Fig. 4), in agreement with Richardson and Carey (1982). In contrast, for rho201 the $K_m$ was 0.6–0.7 $\mu$M (Fig. 4), indicating that it has an enhanced affinity for RNA at its secondary binding site. Both proteins demonstrate a similar $V_{max}$ at 25–30% of the value obtained when they hydrolyze ATP using poly(C) as a cofactor, as reported previously for wild type rho (Pereira and Platt, 1995). Thus the F232C mutation affects both RNA binding sites, such that the affinity for RNA at the primary site is reduced, while the avidity of the secondary site interaction is simultaneously increased.

Transcription Termination Reactions on Wild Type and Defective Templates—Rho201 was isolated in an in vivo screen that demanded transcriptional readthrough beyond trp t’ into the lac operon (Guarente et al., 1977). Although we have shown that it binds RNA in vitro with a lower affinity than the wild type protein, once bound it hydrolyzes ATP very efficiently using both substrate and nonspecific RNA. In order to determine whether this enhanced ATPase activity would extend to its termination activity in vitro, we examined its transcription termination efficiency on wild type, nonspecific, and defective RNA substrates. The DNA templates used carried the strong T7A1 E. coli promoter followed by either trp t’, two tandem repeats of T/H (nonspecific), or 11a, consisting of the first 104 nucleotides of trp t’ harboring 11 cytosine-to-uracil mutations, and which has been shown to be severely defective for rho-dependent transcription termination in vitro (Zalatan and Platt, 1992; Nehrke et al., 1993).

The results of these experiments are shown in Fig. 3. With trp t’, the wild type protein (lane 2) produces terminated products 130–155 nucleotides in length. Rho201, in keeping with its characteristics as a defective termination factor in vivo, does not terminate trp t’ as efficiently. It is defective at causing termination at the earlier end points; allowing RNA polymerase to transcribe further along the DNA before termination occurs (lane 3). Neither the wild type nor the 201 protein terminates very well on templates carrying either T/H or 11a (lanes 5, 6, 8 and 9). In T/H the wild type protein is found to enhance termination at an unmapped site$^{3}$ within the second copy of T/H in the tandem construct (Fig. 3, lane 5). Rho201

$^{3}$K. Nehrke, unpublished data.
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**Fig. 3.** Characterization of the in vitro transcription termination activities of the wild type and rho201 proteins with wild type and defective substrates. Wild type rho and rho201 were tested for in vitro transcription termination activities with trp t′ (wild type RNA substrate), 2T/H which contains random E. coli vector sequences, and 11A which consists of a mutated defective substrate carrying the first 104 nucleotides of trp t′ with 11 C to U changes. Transcription was initiated from the strong T7A1 promoter. RT indicates readthrough transcription by RNA polymerase. The boxed in area represents rho-dependent termination giving rise to truncated transcripts. The first 104 nucleotides of trp t′ are terminated by rho at the same end points as the entire 220 nucleotides of trp t′ so that truncated transcripts of the same length are produced in both cases (data not shown).

seems defective at this site as well. Therefore, despite an increased ability to use substrate or nonspecific RNA as cofactors in the ATPase reaction, rho201 cannot terminate efficiently on wild type or defective templates in vitro, as occurs in vivo with trp t′. This suggests that the rho201 defect in primary site binding cannot be compensated for by either efficient ATPase activity or enhanced secondary site binding in order to bring about transcription termination.

**Trypsin Analysis Reveals a Conformational Change in Rho201**—The mutation in rho201 lies in the putative ATP binding domain, well outside the core NH2-terminal RNA binding domain, but nonetheless it has striking effects on the protein's RNA binding characteristics. One possibility is that the F232C mutation causes a global conformational change within the protein. Since changes in conformation can be detected in mutant forms of rho by probing with mild trypsin digestion (Dombroski et al., 1988a), we carried out a partial trypsin digestion of both the wild type and rho201 proteins as shown (Fig. 4). F1 and F2 are the initial products obtained as a result of hydrolysis of the peptide bond between amino acids 283 and 284 (Bear et al., 1985; Dombroski et al., 1988a; Brennan et al., 1990; Dolan et al., 1990). Further digestion over time yields smaller products. Rho201 is completely digested in the absence of any cofactor as early as 30 min into the reaction (lane 4), indicating a more open conformation than the wild type protein. The presence of ATP and poly(C) protects both proteins (lanes 7–10). This is observed as an increased protection of the F1 fragment, which in the case of the wild type protein remains intact even after 60 min (lanes 7 and 9). In rho201, although the protein is less susceptible to overall digestion in the presence of ATP and poly(C) (lanes 8 and 10), the F1 fragment remains labile, being digested to smaller products within 30 min of the start of the reaction (lane 8). Both proteins used in this work contain the wild type glutamic acid at position 155 (Nehrke et al., 1992); the protein with glutamic acid at this position displays differences in the pattern obtained on trypsin digestion (lanes 3, 5, 7, and 9) compared to the rho protein with lysine at the same position, previously thought to be "wild type" (Bear et al., 1985; Brennan et al., 1990).

In order to test if the change in rho201's conformation would affect its capacity to assemble normally, the wild type and the rho201 proteins were subjected to sedimentation on a 5–20% sucrose gradient (Dombroski et al., 1988a) alongside molecular weight markers. Both proteins sedimented in the fractions corresponding to the hexameric form of the protein (data not shown), indicating that the conformational change does not interfere with rho201's ability to form active hexamers. From the trypsin digestion pattern of the two proteins, we conclude that the F232C mutation in rho201 causes a conformational change in the protein significant enough to be detected by mild trypsin degradation.

**Rho201 Is Defective in Disrupting an RNA:DNA Helix**—The 5′-3′ RNA:DNA helicase activity of rho has been postulated to play a role in transcription termination (Brennan et al., 1987). Despite the observation that rho201 was defective in the termination reaction, we decided to test rho201's ability to disrupt an RNA:DNA helix, to determine if its lower affinity for RNA at the primary site would also lead to the catalytic behavior observed with the F62A/F66A mutant, which binds trp t′ 160-fold less tightly than the wild type protein (Brennan and Platt, 1991).

The helix was formed by annealing the last 28 nucleotides of labeled trp t′ RNA to a complementary polylinker region on single stranded M13 mp11. Both the wild type and the rho201 proteins, at a concentration of 10 nM, were incubated in separate reactions with the helicase substrate at 1 nM and the amount of RNA released over time was monitored. As shown in Fig. 5, the wild type protein shows a very fast initial rate of helix disruption, with most of the RNA being released within the first 2 min. Rho201, however, shows reduced helicase activity and incubation with the helix for increased lengths of time does not increase the activity significantly, nor does it exhibit catalytic behavior (data not shown). Thus the rho201

* K. Nehrke, unpublished results.
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The mutant rho201 allele was isolated using a genetic screen, which detected transcriptional readthrough into a reporter gene due to defective termination (Guarente et al., 1977). Our sequencing of the mutant rho gene revealed a single amino acid change within the predicted β strand of the ATP binding domain of rho (Dombroski and Platt, 1989). Although this change (F232C) lies in the ATP domain, it weakens primary site RNA binding by 100-fold, which is accompanied by decreased discrimination between RNA substrates. Surprisingly, the secondary RNA site activation characteristics have become more sensitive, in responding to oligo r(C)10 stimulation at 5-fold lower concentrations than wild type protein. The mutant protein is a highly efficient ATPase, equalling or surpassing wild type levels, particularly with nonspecific RNA molecules. The rho201 protein is nevertheless very defective in RNA:DNA helicase activity and transcription termination, in agreement with in vivo observations. This protein therefore harbors an unusual mutation in which the energy generated by ATP hydrolysis has become uncoupled from helicase activity, and hence termination.

In contrast to rho201, other rho proteins with amino acid changes in the ATP binding domain are relatively unaffected in their RNA binding function. For example, the K181Q, K181Q/K184Q, and D265N mutants have near normal RNA binding ability, although altered in their ATPase activities and subsequent reactions that are dependent on ATP hydrolysis (Dombroski et al., 1988a). In another case, the termination defective mutant rho ts702 has the mutation A304T within the ATP binding domain (see Platt and Richardson (1992)), and shows wild type ATPase activity with poly(C), although it is severely defective with poly(U) and poly(A) RNA (Shigesada and Imai, 1978). The mutant is postulated to be defective in its secondary site interactions with RNA. A direct measurement of primary site RNA binding has not been reported and we think it is likely that rho ts702, despite defective ATPase activity with non(C) homooligoribopolymers, binds RNA normally, similar to rho suA1 and rho G99V, which are unaffected for binding RNA at the primary binding site and have wild type ATPase activities with poly(C), but are defective with T7 and trp t' RNA (Richardson and Carey, 1982; Pereira and Platt, 1995).

Whether the alteration in the RNA binding properties or the uncoupling between the ATPase and helicase activities, or both, are a direct result of the conformational change in the rho201 protein remains to be determined. Brennan et al. (1990) observed that the trypsin pattern with wild type rho bound to poly(C) differed depending on which NTP was present, and that the energy coupling to RNA release in the helicase assay was two to four times more efficient for ATP than for GTP, UTP, and CTP, suggesting some correlation between coupling and conformational changes. However, studies by Dombroski et al. (1988a) with the K181Q mutant protein, which displays an altered conformation on probing with trypsin but does not uncouple ATPase from helicase action, show that not all conformational changes have such effects. Perhaps amino acids in the β strand are part of the region involved in the coupling reaction, and if this were the true conformational change might be incidental to, rather than the cause of the uncoupling effect.

The phenylalanine residue at position 232, while absolutely conserved in rho homologs from evolutionarily divergent bacteria (Opperman and Richardson, 1994), is at one of the nonconserved positions at the end of the β strand when compared with other ATP binding proteins, such as adenylate kinase and the α and β subunits of F1 ATPase (Dombroski and Platt, 1989). The conformational change in the rho201 (F232C) protein could result from the formation of an internal disulfide bond. According to the predicted structure for rho's ATP binding domain (Dombroski and Platt, 1989) the only other cysteine residue within rho (at position 202) lies at the opposite end of the ATP binding pocket in the β strand, making the distance between these two cysteines too great for intramolecular bond formation. To ask whether intersubunit disulfide bonds might be affecting the activity, we tested both the wild type protein and rho201 in ATPase assays with poly(C) and trp t' as well as in helicase assays, either in the absence of dithiothreitol or in the presence of 10 or 100 nm dithiothreitol. We saw no difference in the results obtained (data not shown). Furthermore, although the trypsin digestion experiments were done in the presence of dithiothreitol, rho201 still revealed a conformational change. The simplest interpretation of these observations is that neither the conformational change nor the altered activities of the mutant protein are due to the formation of any disulfide bonds.

One surprising observation that contrasts with other mutant rho proteins tested is that rho201's termination activity is unaffected by the presence of the accessory E. coli protein NusG in vitro (data not shown). NusG, originally identified as a participant in the λN mediated anti-termination complex (Horwitz et al., 1987; Mason and Greenblatt, 1991; Li et al., 1992) is also necessary for rho-dependent termination at some sites in vivo (Sullivan and Gottesman, 1992) and enhances rho-dependent termination in vitro with both defective RNA substrates (Nehrke et al., 1993) and with mutant rho proteins (Pereira and Platt, 1995). NusG acts in part by slowing the off-rate of rho from nascent RNA in stalled elongation complexes (Nehrke and Platt, 1994), and thus would be predicted to...
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The rho specific downstream PCR oligo was a kind gift from Sayeeda Zain.

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improve the termination efficiency of mutants with weakened primary site binding such as rho201 and F62A/F64A, which display Kₗₚ values of 1.3 and 1.6 nM for trp t mRNA, respectively (this work and Brennan and Platt, 1991). Indeed with the F62A/F64A mutant, NusG has such an effect. In the case of rho201, since the helicase activity appears to be uncoupled from ATP hydrolysis, even if NusG did increase the “dwell time” of rho on the RNA, the inability to harness the energy obtained from ATP hydrolysis to subsequent function would explain rho201’s lack of response to NusG.

Our long-term goal is a mutational and biochemical dissection of the overall process of termination, which will entail understanding each of the individual steps along the pathway. Thus far, a number of mutations have been analyzed in our laboratory and elsewhere that address the first stages involving RNA binding and ATPase activation. These include K181Q/K184Q, K181Q, and D265N, which reduce or eliminate the ATPase activity of rho without any apparent effect on the RNA binding properties (Dombroski and Platt, 1988a). Another class of mutations, represented by K352E (rhoSU1A) and G99V (derived from rho115) display defects in activating the secondary RNA site of rho, with little significant effect on primary site RNA binding (Richardson and Carey, 1982; Pereira and Platt, 1995). In these cases termination defects correlate with the deficiencies in ATPase activation at the secondary site. In all mutants tested, with the exception mentioned below, the mutational effects are manifested in the levels of ATPase activation, such that poor ATP hydrolysis correlates with poor helix and termination activities.

The first suggestion that the energy coupling step in the pathway could be affected was provided by the rho nita18 mutant, which carries an unmapped mutation leading to significantly higher ATPase activity than the wild type protein with different RNA substrates (Shigesada and Imai, 1978). Although its helicase activity was not determined, it had moderate termination activity ranging from 35 to 90% of the wild type efficiency depending on the template used. We have now reported and characterized here a mutational change in the rho201 protein that appears to result in a severe defect in this second critical step in the pathway, the ability to harness ATP hydrolysis to helicase and termination ability. Recently, Miwa et al. (1995) have characterized 14 mutations in the COOH-terminal 100 amino acids of rho, and found that many of these also have “coupling” effects on the RNA binding, ATP binding and hydrolysis, and termination activities of rho factor in vitro. The characterization of these and other mutants, in concert with biochemical analysis of rho’s structure-function relationships, should help to decipher the mechanism by which RNA binding, ATP hydrolysis, and helicase activity are coupled together to bring about termination of transcription by E. coli RNA polymerase.

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A Mutation in the ATP Binding Domain of Rho Alters Its RNA Binding Properties and Uncouples ATP Hydrolysis from Helicase Activity
Shalini Pereira and Terry Platt

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