Transcription Termination Factor Rho Activity Is Altered in Escherichia coli with suA Gene Mutations
(RNA-dependent ATPase/RNA polymerase/polarity)

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ABSTRACT Rho factor has been purified from a strain of E. coli containing the Su78 mutation in the suA gene and assayed in another strain with an amber mutation in the suA gene. The rho from the Su78 mutant strain is present in normal amounts but has altered termination function; it does not terminate transcription at some sites that are recognized effectively by the rho factor from the isogenic wild-type strain. Rho in cells with an amber mutation in the suA gene has been assayed by its RNA-dependent ATPase activity. Extracts of cells of this strain have only 9% as much of this rho activity as extracts of cells of the isogenic wild-type strain. These results suggest that the rho is the product of the suA gene. Since mutations in the suA gene are known to decrease polar effects of mutations in other genes, it is also suggested that rho factor is at least partially responsible for polar effects.

Many nonsense and frameshift mutations in one gene of an operon pleiotropically reduce the level of expression of the genes in the operon that lie on the operator-distal side of the mutant gene (1). This polar effect is evident in the levels of both the messenger RNA sequences corresponding to the affected genes and the protein products (2, 3). In Escherichia coli, second-site mutations in the suA gene can partially relieve the polar effect of the original mutation without suppressing the original mutation (4, 5). Since these suA gene mutations are recessive to the wild-type allele (5) and since amber (nonsense) suA mutations have been found (6), it has been suggested that the product of the wild-type suA gene is a protein required for the full polar effect (6). In this paper we present evidence that the product of the suA gene is the RNA transcription termination factor.

Rho factor was first isolated and purified from E. coli K12 by Roberts, who showed that it causes specific termination of the synthesis of λ RNA molecules in vitro (7). It has since been shown that rho is active in terminating transcription from a large number of natural DNA templates (8). Roberts also showed that rho is not a ribonuclease; it does not cleave or degrade large, isolated λ RNA molecules even in complete RNA polymerase reaction mixtures (7). We now know that it does not catalyze the degradation of nascent RNA molecules either (9). On the other hand, when RNA molecules are present, rho does catalyze the hydrolysis of nucleoside triphosphates to nucleoside diphosphates and orthophosphate (9). Although the significance of this ATPase activity is not yet clear, it can be used for a convenient quantitative assay of rho factor. We have used this assay to identify and purify rho factor from cells with mutations in the suA gene.

RESULTS
The rho proteins from a suA− strain isolated by Carter and Newton (Su78) (10) and an isogenic strain with the wild-type allele for the suA gene have been purified to homogeneity. The yields in both cases are the same: about 0.12 mg of protein from 20 g of cells, which is similar to yields from other cells by means of the Roberts purification procedure. The two pure proteins have the same specific activities for the poly(C)-activated hydrolysis of ATP—20 μmol of ATP hydrolyzed min⁻¹ per mg—and the same mobilities when electrophoresed on polyacrylamide gels containing sodium dodecyl sulfate (Fig. 1). However, these rho factors differ significantly in their abilities to depress the yields of RNA synthesized in vitro with purified RNA polymerases from several DNA templates (Table 1). For each DNA, the rho from the suA mutant strain (Su78) reduces the yield of RNA synthesized less than the rho from the cells with the wild-type allele. The magnitude of this difference depends on the DNA used. With T4 DNA, the Su78 rho caused only an 8% depression, compared with the early 50% depression with the wild-type rho. In contrast, with PM2 DNA the Su78 rho caused a 33% depression, whereas the wild-type rho still only caused a 50% depression. These differences are not because of limitations in the amount of rho used; the reactions were saturated with the rho used in each case.

Analyses of the effects of these factors on the size of the RNA made on T4 and T7 DNA templates confirm the interpretation that the depression of yields of RNA synthesis given in Table 1 is an accurate indication of the relative termination activities of the factors. With T7 DNA, for example, the sedimentation profile of the RNA synthesized in the absence of rho has a peak indicating a sedimentation coefficient of 17.8 S in 1.1 M formaldehyde (Fig. 2), which corresponds to an RNA with a molecular weight of 2.2 × 10⁴ (11). When the rho from the wild-type cells is present, the RNA peak is at 9.8 S, which corresponds to an RNA with a molecular weight of 0.5 × 10⁴. However, when rho from Su78 is present, most of the RNA molecules synthesized are much larger than those synthesized in the presence of the wild-type rho; the main peak is at 16.8 S, but there are also peaks at 14.5 S and 12.0 S. Thus the Su78 rho does not terminate T7 transcription at the site recognized by the wild-type rho factor, although it does appear to terminate the transcription of some RNA molecules at other sites that are reached later by RNA polymerase.

Although the two rho factors are identical with respect to their poly(C)-activated ATP hydrolysis activities, they do show different rates of ATP hydrolysis when activated by nascent RNA molecules. In complete RNA polymerase reaction mixtures containing T4 DNA or T7 DNA, only the rho that causes extensive termination also catalyzes the release of Pi from [γ-32P]ATP (Table 2). This observation is consistent with others we have made (C. L. and J.P.R., unpublished ex-
Fig. 1. Polyacrylamide gel electrophoresis of purified rho proteins. Rho protein was isolated by the procedure of Roberts (7) from E. coli strains T82 (Su78) and T83 (suA +) supplied by Austin Newton. These strains are isogenic P1 transductants of an rbs- derivative of MU118 (z-asuA) (13). The original Su78 strain was described by Carter and Newton (10). The proteins were electrophoresed in a discontinuous slab gel (22), using a 3.5% stacking gel and a 10% running gel of polyacrylamide in the sodium dodecyl sulfate/Tris-HCl buffer system described by Laemmli (23). The proteins were stained with Coomassie brilliant blue using the conditions of Laemmli (23); (A) 0.2 μg of rho from T83 (wild-type); (B) 0.1 μg each of rho from T83 and T82 (Su78); (C) 0.2 μg of rho from T82.

Experiments correlating rho termination activity with its nascent-RNA-dependent ATP hydrolysis activity. For instance, in 0.12 M KCl, the rho from E. coli B no longer terminates the transcription of T7 DNA, and the T7 RNA made under these conditions also does not activate the rho ATP hydrolysis reaction. Yet under these same ionic conditions, the rate of ATP hydrolysis in the poly(C)-activated reaction is the same as in 0.05 M KCl. The requirements for activation of the ATPase activity of rho by nascent RNA molecules are apparently more stringent than the requirements for activation by poly(C).

Further evidence that the product of the suA gene is rho has come from attempts to isolate rho from one of the strains isolated by Morse and Guertin (6) containing an amber mutation in the suA gene. Although it is not possible to make reliable quantitative estimates of the amount of rho present in crude extracts of cells with any of the current assays, rho factor can be unambiguously identified and quantitatively assayed after chromatography on phosphocellulose. At this stage, there was only 9% as much poly(C)-activated ATPase activity for the rho isolated from 20 g of cells with a suA amber mutation (strain 2055) as was found for the rho isolated from 20 g of cells of an isogenic strain (2034) with the wild-type allele in the suA gene. In both cases, the rho eluted from the phosphocellulose in a single sharp peak in 0.16 M potassium phosphate buffer.

Since the residual level of rho factor in strain 2055 is higher than would be expected for the product of a gene with an amber mutation, caution must be taken in concluding that rho is the product of the suA gene. Mutations in that gene could affect rho indirectly; the suA gene product might be an enzyme that modifies or activates rho. Nonetheless, whether the effect is direct or indirect, one conclusion is clear: rho factor termination activity is reduced in the polarity suppressor strains. This implies that rho is involved in translational polarity.

**DISCUSSION**

From our studies of the mechanism of rho action we propose the following model for how rho causes polar effects. We suggest that termination mediated by rho factor depends on the recognition by rho of a particular sequence or structure of the nascent RNA as it emerges from RNA polymerase. If a ribosome is able to translate that RNA as it emerges from the RNA polymerase, it could interfere with this recognition pro-
Table 2. Correlation of rho termination activity with rho ATPase activity

<table>
<thead>
<tr>
<th>DNA</th>
<th>Rho</th>
<th>(^{32}P) released as</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PP(_i) (pmol)</td>
</tr>
<tr>
<td>T4</td>
<td>—</td>
<td>140</td>
</tr>
<tr>
<td>Wild-type</td>
<td>58</td>
<td>85</td>
</tr>
<tr>
<td>Su78</td>
<td>165</td>
<td></td>
</tr>
<tr>
<td>T7</td>
<td>—</td>
<td>199</td>
</tr>
<tr>
<td>Wild-type</td>
<td>67</td>
<td>68</td>
</tr>
<tr>
<td>Su78</td>
<td>196</td>
<td></td>
</tr>
</tbody>
</table>

The reaction conditions were identical to those of Table 1 except that \([\gamma-^{32}P]ATP\) (3800 cpm/\(\mu\)mol) was used instead of \([\text{H}]CTP\) and the amounts of rho were 0.4 \(\mu\)g per mixture. After incubation for 30 min at 37\(^\circ\), the amounts of \(^{32}P\) released as \(P_i\) and PP, were analyzed as described previously (9). The release of \(P_i\) is corrected for 11 pmol released in the absence of any enzyme components.

cess, thus preventing rho-mediated termination. However, when translation is prematurely terminated at a nonsense codon, the ribosome would leave the RNA and expose a segment of the emerging nascent RNA to rho action.

The involvement of rho in translational polarity would also depend on the presence of rho recognition sites within cistrons. Studies of transcription of the lac operon \(\text{in vitro}\) have indicated that there is a site recognized by rho in the middle of the \(z\) gene, the gene for \(\beta\)-galactosidase (12). One striking characteristic of polar mutants in the \(z\) gene is that the severity of the polar effect is dependent on the position of the mutation in the gene; amber mutants near the beginning of the \(z\) gene give a much stronger polar effect than those near the end of the gene (13). Similar gradients of polar responses have also been found for mutations in genes for the tryptophan operon of \(E.\ coli\) (14). A gradient of polarity could be explained by rho action, if there are several rho termination sites within a gene and if the sites are not all totally effective. In this case there would be more chances for rho to terminate transcription when a ribosome leaves the nascent RNA early in the gene than when it leaves it late in the gene.

It is not known yet whether there are several rho sites within the \(z\) gene, but it is a large gene with at least 3600 base pairs (3) and rho sites could be distributed as frequently as one for every 800 to 1000 nucleotides transcribed (15). The efficiency of rho action in the cell is also not known, but it would depend on the amount present, and the current estimate, which could be low by a factor of one hundred, indicates that there is only one rho molecule for 100 RNA polymerase molecules (16). In contrast, completely effective rho termination \(\text{in vitro}\) requires one rho per RNA polymerase (17).

Since rho must also interact with RNA polymerase to cause termination of RNA synthesis, an alteration of the enzyme could also affect rho action. This could be the way \(N\) gene product in \(\lambda\) acts to cause transcription to continue \(\text{in vivo}\) past sites where rho is known to function \(\text{in vitro}\) (7). This same alteration could also overcome the polar effects of nonsense mutations in bacterial genes transcribed by read-through from \(\lambda\) genes (18, 19).

Kuwano et al. (20) have presented evidence that extracts of cells containing mutations in the \(suA\) gene lack an endonuclease activity that is present in extracts of wild-type cells. Although pure rho is not itself a ribonuclease, it could possibly be a component of the endonuclease responsible for the activity detected by Kuwano et al. Thus, we cannot conclude categorically that rho's function in causing polarity is not due to degradation of nascent RNAs exposed when ribosomes dissociate at a nonsense codon. However, since it is known definitely that rho does terminate transcription \(\text{in vitro}\), we prefer the hypothesis involving this mechanism for its \(\text{in vivo}\) function as well. This hypothesis is also consistent with the observations of Imamoto (21) that \(\text{trp}\) RNA molecules are not synthesized \(\text{in vitro}\) beyond nonsense mutations in the \(\text{trp}\) operon. In order to remove the uncertainty that remains concerning the involvement of rho factor in polarity, it will be necessary to analyze further rho function in cellular metabolism.

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