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Autoregulation of gene expression: *rho*

ROBERTA HABER and SANKAR ADHYA
Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892 USA

Abstract. Autoregulation of the *rho* gene insures a controlled level of a critical gene product independent of cellular changes. We have investigated the autoregulation of *rho,* the gene that encodes the transcription termination factor, *rho.* In a DNA dependent *in vitro* coupled transcription-translation system, *rho* represses its own synthesis, confirming the autoregulatory nature of *rho. srho* is believed to perform its negative regulatory role by modulating transcription termination at an early site in the operon.

Keywords. Autoregulation; *rho* gene; transcription termination.

Introduction

The essential feature of autoregulation is the direct modulation of gene expression by the product of the same gene. Thus, a protein controls the rate of synthesis of additional copies of itself.

Autoregulation is not a new concept in genetics; the phenomenon has been known in prokaryotic and eukaryotic organisms for more than two decades. However, autoregulation is newly appreciated as a mechanism of regulation of genes whose products perform critical cellular functions. Autoregulation provides a mechanism for amplification of gene expression (Kourilsky and Gros, 1974), for regulation of proteins involved in DNA replication (Braun *et al.,* 1985), for buffering the response of genes to environmental changes (McAall and Bloom, 1971; Hagen and Magasanik, 1973), and for maintaining a constant intracellular concentration of a gene product, independent of cell size, cell growth rate and changes in gene copy number (Sompayrac and Maaloe, 1973).

A genetic mechanism which is not autoregulatory may appear so on the surface. For example, a gene or operon whose product(s) participate in the synthesis of the gene's inducer or co-repressor could appear to be an autoregulated system, if the biochemistry were not known. The best way to demonstrate the existence of an autoregulatory system and to discern the molecular mechanism of that control is to duplicate the control system *in vitro.*

The expression of many of the global control genes of *Escherichia coli* appears to be self-regulated, allowing a critical level of the gene product to be maintained in the cell. An example of one such gene is that of the transcription termination protein, *rho.* Ratner first observed that in *rho*$^-$ mutant *E. coli* cells, the defective *rho* protein was present at a higher level than *rho* protein in wild type cells (Ratner, 1976). He suggested that the *rho* gene is autoregulatory and *rho* protein controls its level by modulating transcription termination at the beginning of the *rho* gene. The enhanced expression of the *rho* gene in the mutant cells has been confirmed in *vivo* in two ways: (i) In *rhoo* mutant backgrounds there is enhanced β-galactosidase synthesis from a chromosomal *rho-lacZ* protein fusion (Garges, 1983) and enhanced galactokinase synthesis from a *rho-galK* operon fusion plasmid (Barik *et al.,* 1985).

(ii) The rate of synthesis (Brown *et al.,* 1982) as well as the steady state level (Garges, 1983; Barik *et al.,* 1985) of *rho* mRNA is higher in *rho*$^-$ cells than in *rho*$^+$ cells. These results argue in favor of an autoregulated *rho* gene.

In this paper, we examine autoregulation of *rho* in a coupled transcription-translation system and provide evidence that the *rho* 112 mutant is altered in autoregulation.

Materials and methods

Strains

The list of *E. coli* K-12 strains and plasmids used in this study is presented in table 1.

Table 1. Strains and plasmids used in this study.

Strain	Genotype	Reference
E. coli K12:		
G737	F$^-$ *su*$^-$ *recB21 recC22 sbcB15 Thr1 leuB6 Thi1 lacY1 galK2 ara14 xyl5 mtl1 proA2 his4 argE3 rpsL31 tsx33*	(Garges, 1983)
G736	G737 *rho112**	(Garges, 1983)
Plasmid:		
pBR322	*bla*$^+$ *tet*$^+$	(Sutcliffe, 1979)
pEG25	*bla*$^+$ *rho*$^+$	(Gulletta *et al.* 1985)

**rho* 112 mutation is described in Das *et al.* (1978).

Coupled transcription-translation reactions

S-30 extracts were prepared by Codon Co. of Houston, Texas, USA according to the method of Zubay (1973). Components of the reaction were 20 mM Tris-acetate pH 8·0, 8 mM KOAC, 0·01 mM of all amino acids except methionine, 2 mM adenosine 5'-triphosphate (ATP), 0·5 mM UTP, 0·5 mM GTP, 0·5 mM CTP, 0·2 mg/ml *E. coli* tRNA, 10 µg/ml pyruvate kinase, and 5 mM phosphoenolpyruvate. Each 10 µl reaction contained 0·25 µg cesium chloride banded plasmid DNA and 2·5 µCi [^{35}S]-methionine (Amersham, 600 Ci/mMol). Reactions were carried out at 37° for 40 min, then stopped by addition of trichloroacetic acid (TCA) to a final concentration of 10%. Samples were precipitated, washed with cold acetone and suspended in sample buffer for sodium dodecyl sulphate (SDS)/polyacrylamide gels. 10% polyacrylamide gels were run according to the method of Laemmli (1970).

Results

S-30 extracts for coupled transcription-translation reactions were made from wild type *E. coli* (G737) and from a *rho* 112 mutant strain (G736) as described in 'materials and methods'. These extracts were used to carry out protein synthesis directed by plasmid pEG25. pEG25 is a derivative of pBR322 carrying a 2·9 kbp DNA fragment with *rho*$^+$ and its flanking regions substituted for the HindIII-PvuII region of the

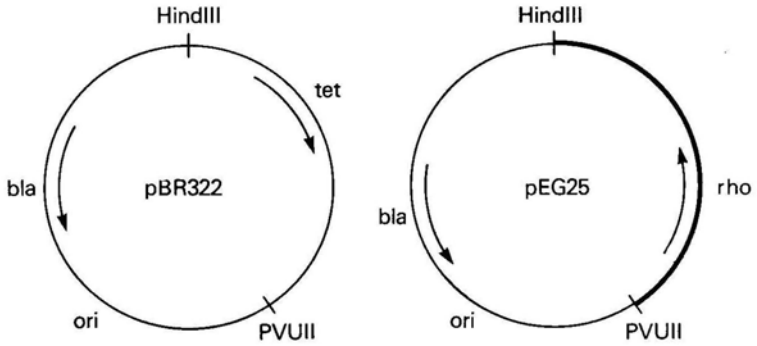
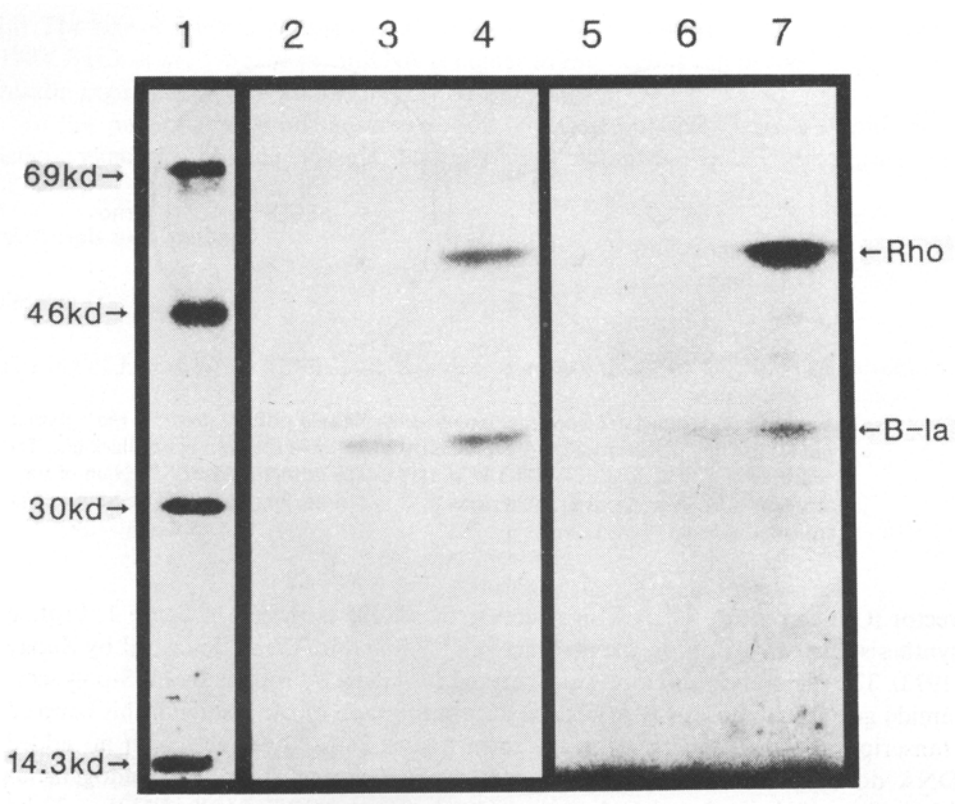


Figure 1. Diagrammatic representation of vector plasmid pBR322 (left) and *rho*$^+$ plasmid pEG25 (right). The bacterial DNA containing the *rho*$^+$ gene is shown by the thick line. The construction is described in Gulletta *et al.* (1985). The arrows represent direction of transcription, *bla,* gene for Ampicillin resistance; *tet,* gene for tetracyclin resistance; *rho,* structural gene for *rho*.

vector (Gulletta *et al.,* 1985). The structure of pEG25 is shown in figure 1. Protein synthesis was carried out in the presence of [^{35}S]-methionine as described by Zubay (1973). The incubation mixture was analyzed for labelled proteins by SDS-polyacrylamide gel electrophoresis (PAGE). An autoradiograph of the results of this coupled transcription-translation reaction is shown (figure 2). S-30 extracts with no added DNA do not show any labelled protein, confirming the absence of endogeneous DNA (figure 2, lanes 2 and 5). In the presence of both pBR322 and pEG25, a 33 kd protein is made in both the *rho*$^+$ S-30 and the *rho* 112 mutant S-30 (figure 2, lanes 3 and 6). The 33 kd band is the precurser of *ß*-lactamase directed by the *bla* gene present on both plasmids. pEG25 directs the synthesis of an additional protein whose mobility corresponds to a molecular weight of 50 kd (figure 2, lanes 4 and 7). We conclude that this protein is *rho* monomer because its estimated size is consistent with the size of *rho* deduced from the DNA sequence of the *rho* gene (Pinkham and Platt, 1983), and because the radioactive band comigrates with purified *rho* in SDS-PAGE (data not shown).

These results demonstrate DNA-dependent synthesis of *rho* protein *in vitro* using crude cell-free extracts made both from *rho*$^+$ and *rho* 112 strains as a source of the components of the transcription-translation reaction.

We have scanned the intensity of various protein bands in the autoradiograms to determine the relative amounts of *rho* and *ß*-lactamase synthesized. The quantitation of the scanning is shown in table 2. The results are expressed assuming the amount of *ß*-lactamase monomers synthesized under the conditions described in 'materials and methods' as unit in each extract. Compared to the synthesis of *ß*-lactamase, the synthesis of *rho* is 7-fold higher in the *rho* 112 extract than in the *rho* $^+$ extract. The differential ability of wild type and mutant extracts is similar to that observed *in vivo* as discussed above. These results show the following: If we assume that protein stability is the same in both extracts during the 40 min labelling period, our results confirm that the difference in *rho* levels between *rho*$^+$ and *rho*$^-$ backgrounds is not at the level of activity, but at the level of synthesis. In addition, assuming that the only critical difference in the macromolecular synthetic machinery



Figured 2. Coupled transcription-translation reactions were carried out and products run on 10% SDS-polyacrylamide gels, as described in 'materials and methods'. Lane 1, molecular weight markers; lanes 2-4, wild-type extracts; lanes 5-7, *rho*112 extract. The reactions shown in lanes 2 and 5 contain no exogeneous DNA; the reactions shown in lanes 3 and 6 contain 0·25 μg of pBR322; the reactions shown in lanes 4 and 7 contain 0·25 μg of pEG25.

Table 2. DNA directed synthesis of proteins*in vitro.*

Extract	Relevant genotype	*B-lactamase*	*rho*
G737	*rho*$^+$	1	1·1
G736	*rho*112	1	7·5

of the two extracts is the presence and absence of active *rho* protein, we conclude that *rho* protein inhibits its own synthesis. The implications of these conclusions are discussed next.

Discussion

Many genes are autoregulated. In an autoregulated system, the product of a gene directly participates in regulating the expression of that gene. The best evidence that a gene is autoregulated is obtained *in vitro.* The demonstration that the *rho* cell-free

extract is capable of increased *de novo* synthesis of *rho* protein indicates that *rho* is autoregulated *in vitro*. Results similar to ours have been obtained independently by Kung *et al.* (1984) and Barik *et al.* (1985) using other *rho*⁻ mutants. These authors have also shown that inactivation of *rho* protein in a wild type extract by addition of anti-*rho* antibody increased plasmid-directed *rho* synthesis, while addition of purified *rho* protein to a *rho*⁻ extract decreased *rho* synthesis to the wild type level.

Rho protein is an essential regulator of transcription in *E. coli* (Das *et al.,* 1976; Adhya and Gottesman, 1978). The ability of *rho* protein to repress *rho* gene expression provides an inefficient mechanism to maintain a constant intracellular level of *rho,* independent of growth rate and other cellular changes. Plasmid-directed *rho* gene expression is higher in the *rho* 112 extract than in the wild-type extract. When the *rho* protein is defective, the cell may need more of it than it would need of wild-type *rho* in order to fulfil its termination requirements. Therefore, an apparent defect in autoregulation may actually save the *rho* 112 mutant strain by providing the cell with higher levels of the inefficiency protein. This ability to compensate for a defective *rho* protein by overproduction illustrates a benefit of autoregulation.

How does *rho* negatively control gene expression? *rho* is a factor in transcription termination. Termination of RNA synthesis in *E. coli* occurs at specific sites on the genome, resulting in functional RNAs of discrete sizes (Adhya and Gottesman, 1978). At many such sites, the protein factor *rho* is required for RNA polymerase to halt activity, thus preventing transcription of promoter distal genes (Roberts, 1969; de *Crombrugghe et al.,* 1973).

Rho protein is a hexamer composed of identical 48 kilodalton subunits which assume a ring-like conformation (Galluppi and Richardson, 1980). Two regions of the *rho* protein have the capacity to bind RNA *in vitro.* The primary binding region of the protein binds to a region of RNA which is approximately 60 bases long. Concurrently, the secondary binding region binds an 8-base region of RNA. The secondary binding is accompanied by hydrolysis of ATP (Richardson and Corey, 1982).

Basically, there are 3 steps for *rho*-dependent termination of transcription (Adhya and Gottesman, 1978; Das *et al.,* 1978; Morgan *et al.,* 1983, 1984). The first event is the *rho*-independent pausing of RNA polymerase at specific termination sites on the DNA template. The second event is the binding of *rho* protein to a long stretch of RNA (approximately 70–90 nucleotides) which has minimal secondary structure. The last step is *rho* binding to the paused RNA polymerase, coupled with ATP hydrolysis and release of polymerase and RNA from the DNA template. The NusA protein of *E. coli,* which has been shown to bind both *rho* and RNA polymerase *in vitro,* may mediate the *rho*-driven separation of RNA polymerase from the template (Schmidt and Chamberlin, 1984).

A number of *rho* mutants have been isolated and demonstrated to have a defect at one of these steps of transcription termination. As we have discussed, these *rho* muta tions are defective in autoregulation and show increased *rho* transcription, strongly arguing that *rho* modulates its own synthesis at the level of transcription termination. In fact, Brown *et al.* (1982) and Matsumoto *et al.* (1986) identified by S 1 nuclease-analysis of *rho* mRNA several intraoperonic termination sites preceding the *rho* coding region. Brown *et al.* (1982) suggested that *rho* may be modulating *rho* mRNA elongation at these sites. We are currently analyzing the sites of *rho* action both genetically and biochemically and determining the stage at which *rho* 112 malfunctions in autoregulation.

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