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Genetics

Evidence that ribosomal protein S10 participates in control of transcription termination

(*nusE* gene/ρ protein/λ *N* gene product/antitermination/translation)

D. I. FRIEDMAN*, A. T. SCHAUER*, M. R. BAUMANN*, L. S. BARON†, AND S. L. ADHYA‡

*Department of Microbiology and Immunology, The University of Michigan, Ann Arbor, Michigan 48109; †Department of Bacterial Immunology, Walter Reed Army Institute of Research, Washington, D.C. 20012; and ‡Laboratory of Molecular Biology, National Cancer Institute, Bethesda, Maryland 20205

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ABSTRACT **We report the isolation of an *Escherichia coli* K-12 strain with a mutation, *nusE71*, that results in a change in ribosomal protein S10. Phage λ fails to grow in hosts carrying the *nusE71* mutation because the λ *N* gene product is not active. The *N* product regulates phage gene expression by altering transcription complexes so that they can overcome termination barriers. This suggests that a ribosomal protein is involved in antitermination of transcription.**

Regulation of operon expression by transcription termination was discovered during study of the early operons of coliphage λ (ref. 1, reviewed in ref. 2). This transcription initiates at two promoters, p_L and p_R, but prematurely terminates at specific sites, t_{R1} and t_{L1} (see Fig. 1). These termination reactions depend on the action of the host ρ protein (1). The λ *N* gene is located between p_L and t_{L1} and thus is expressed even though termination occurs at t_{L1}. The *N* gene product, pN, is an 11,800-dalton protein (3) that acts to relieve transcription termination at sites downstream from p_L and p_R (4–6). Functional studies led to the proposal that transcription complexes initiating at p_L and p_R are modified by pN at specific recognition sites and override subsequent transcription termination barriers (7–9). More recently, mutations have been isolated that define these recognition sites, called nut_L and nut_R. They map, respectively, downstream from p_L (10) and p_R (11–13). Effective pN action also requires participation of host functions. A number of mutants of *Escherichia coli* have been isolated that fail to support the growth of phage λ because pN activity is limited (14, 15). Much of our understanding of the mechanism of pN action derives from structural and functional analysis of these mutants. Some of the involved mutations have been mapped on the *E. coli* chromosome: *nusA* at 68 min (16), *nusB* at 11 min (17), *nusC* at 88 min (unpublished results). The *nusA* gene product is a 69,000-dalton protein identified by its role in a coupled *in vitro* transcription-translation reaction (18). The *nusB* gene codes for a 14,000-dalton protein (M. Strauch and J. Swindle and C. Georgopoulos, personal communications). The *nusC* mutation maps in the *rpoB* gene and results in an alteration of RNA polymerase (unpublished results).

In this paper we report the characterization of another *nus* gene defined by the *nusE71* mutation. Genetic and biochemical studies locate the mutation in the gene encoding ribosomal protein S10.

MATERIALS AND METHODS

Media. TB, the tryptone medium used, was described (16).

Strains. All bacterial strains are derivatives of *E. coli* K-12. K37 is a streptomycin-resistant derivative of W3110 (19) Strains isogenic with K37 are: K95, *nusA1* (16); K450, *nusB5* (17); K554, *nusC60*. K1102 is an *E. coli* K-12 derivative carrying the *nusA* region from *Salmonella typhimurium* (15). K556 and the Hfr derivative K1308 are reported in this study. The recipient strain AB1133 was obtained from E. Adelberg.

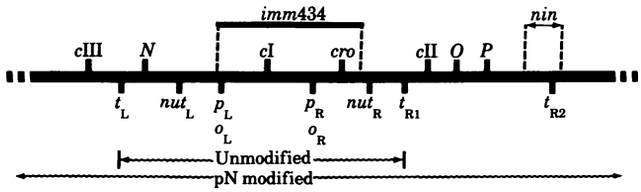

FIG. 1. Map of the early region of phage λ DNA. The wavy lines represent RNA transcripts originating at promoters p_L and p_R. The upper transcript is synthesized in the absence of pN (4–6) and the lower is produced in the presence of pN.

λ*fus*3 (20) was obtained from R. Jaskunas and the λ Charon 4 derivative 4F113 (21, 22) was obtained from M. Nomura. The DNA structures of these transducing phages are shown in Fig. 2. 4F113*imm434nin*$^+$ was constructed from 4F113 for these studies. λ*punA* was isolated on K95 (23). The sources and derivations of all other phages used are as listed in ref. 16.

Preparation and Analysis of Ribosomes. Ribosomes were isolated as described by Isono and Krauss in 0.01 M $MgCl_2$/0.01 M Tris·HCl, pH 7.4 (24). Proteins were extracted from purified ribosomes by adding 2 vol of glacial acetic acid (25). The proteins were then precipitated with acetone and washed (26), vacuum dried, and frozen at −70°C. Two-dimensional electrophoresis was performed according to the method of Howard and Traut (27); 6% acrylamide was used for basic proteins and 8% acrylamide was used for acidic proteins in the first dimension.

RESULTS

Effect of *nusE71* on Growth of λ. The *nusE71* mutation was obtained by using a procedure that selects for host mutants that fail to support the activity of *N* protein (14). The Nus phenotype can be identified by comparing the growth of phage that require pN (*N*-dependent) with the growth of phage that do not require pN (*N*-independent). Only the latter—e.g., λ*nin*,—grow in *nus* mutants under nonpermissive conditions.

As shown in Table 1, at 42°C the *nusE71* mutant fails to support the growth of the *N*-dependent phage λ*imm434*cI^-. [λ*imm434* has the *N* gene of λ and both the p_L and p_R promoters and repressor of phage 434 (28).] However, λcI^-60*nin* as well as λ*imm434nin* grow well under identical conditions. At 32°C the *nusE71* mutant, like *nusA* and *nusB* mutants, displays a different character. *N*-dependent phage (e.g., λcI^- and λ*imm434*cI^-) grow in the *nusE71* host. However, phage that

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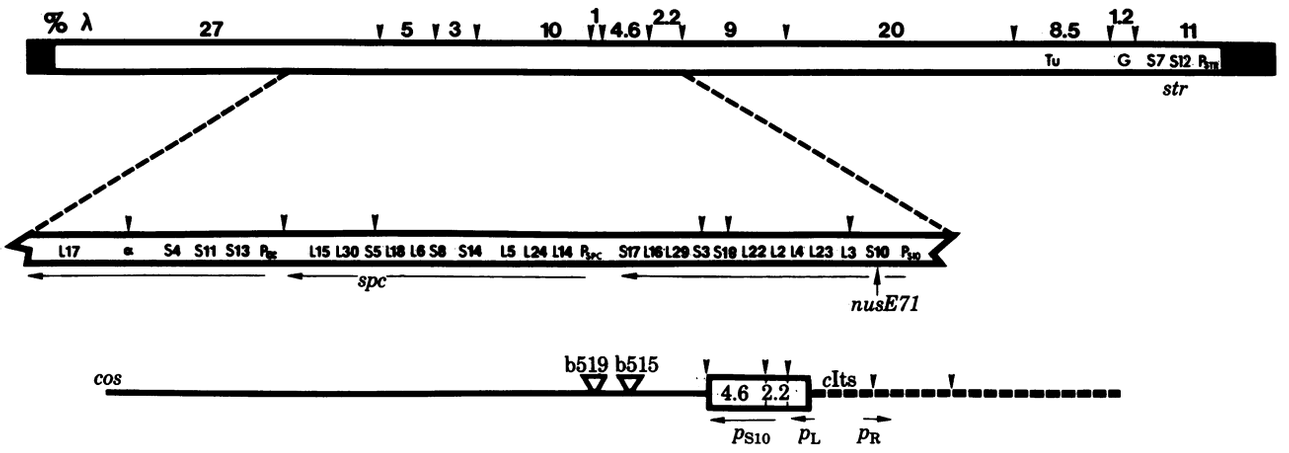

FIG. 2. (*Upper*) Map of DNA of the λ*fus*3 specialized transducing phage. This defective phage carries DNA from the 72-min region of *E. coli* (20); λ DNA is represented by the black regic ..t the ends. The four operons illustrated contain ribosomal protein genes as well as the gene coding for the α subunit of RNA polymerase and the elongation factors Tu and G. The downward arrowheads are *Eco*RI cleavage sites. (*Lower*) Map of DNA of the 4F113*imm*434*nin*$^+$ phage. The parent, 4F113, was constructed by inserting the 2.2% and 4.6% *Eco*RI fragments of the λ*fus*3 phage into Charon 4 *in vitro* (21). Ribosomal proteins L2, L3, L4, L23, and S10 are expressed by this clone. The *imm*434*nin*$^+$ derivative was constructed in this laboratory. ▽ indicates a deletion; the broken line indicates DNA from the λ*imm*434*nin*$^+$ parent; the downward arrowheads are *Eco*RI cleavage sites.

require higher levels of *N* activity, such as λcI$^-$c17 and λ*imm*434c17 (29), fail to grow in the *nusE*$^-$ host.

A mutation, *punA*, that maps within the *N* gene has been shown to permit λ growth in the *nusA1* mutant at 42°C (23). The growth patterns of λ*punA* in the various *nus* mutants as well as the *nus*$^+$ host are also shown in Table 1. λ*punA* grows in the *nus*$^+$ host as well as in the *nusA1* and *nusE71* hosts at 42°C. However, it fails to grow in the *nusB5* or the *nusC60* hosts at 42°C, as well as in a hybrid strain of *E. coli* that carries the *nusA* gene of *Salmonella*. The inability to grow in these hosts suggests that λ*punA* still requires pN activity for growth. Moreover, the failure of growth in the *E. coli* hybrid demonstrates that the altered *N* of *punA* still requires the *E. coli nusA* function to be active.

The *punA* mutation, unlike the *nin* mutation, does not free λ from *N*-dependency, indicating that growth of λ*punA* in *nusA1* or *nusE71* hosts requires the synthesis of an altered pN. DNA sequence analysis has confirmed the genetic mapping of *punA* by showing that it is a single base-pair change near the center of the *N* gene (N. Franklin, personal communication). Because *punA* maps within the structural gene and not in the promoter or translation start region of the *N* gene, it is likely that *nusA1* and *nusE71* do not influence pN synthesis and therefore the inhibition is at the level of pN activity.

Mapping of *nusE71*. Initial mapping of the *nusE71* mutation was done by Hfr × F$^-$ crosses. An Hfr derivative carrying the *nusE71* and *pyr*$^-$ mutations was mated with an F$^-$ *nus*$^+$ spectinomycin-sensitive (*spc*$^-$) strain carrying auxotrophic and fermentative markers (AB1133). Appropriate prototrophic recombinants were selected and tested for acquisition of donor markers. It was found that the nonselected markers, *nusE71* and spectinomycin resistance, were cotransferred at a high frequency.

A more precise location for the *nusE71* mutation was obtained by using specialized λ transducing phages that contain various amounts of genetic material from the *str–spc* region of the *E. coli* chromosome (72 min). Lysogens of the *nusE71* mutant constructed with λ*fus*3 (Fig. 2 *Upper*) are Nus$^+$. This result locates the *nusE71* mutation in the *str–spc* region of the *E. coli* chromosome.

The integration of λ*fus*3 into the chromosome of the *nusE71* host results in lysogens that are *nusE71*/*nusE*$^+$ merodiploids. We conclude that the *nusE*$^+$ allele is dominant because this merodiploid becomes Nus$^+$.

The *nusE* gene was identified as one of the five ribosomal protein genes present in the Charon 4 transducing phage 4F113. This phage contains a cloned fragment from the λ*fus*3 phage that carries the genetic information for ribosomal proteins L2, L3, L4, L23, and S10 (22). The Charon 4 vector from which the 4F113 transducing phage was constructed carries a *nin* deletion and therefore plates on all *nus*$^-$ hosts. A λ*imm*434*nin*$^+$ de-

Table 1. Growth of λ in *nus* mutants

	Bacteria									
	K37 *nus*$^+$		K95 *nusA1*		K450 *nusB5*		K556 *nusE71*		K554 *nusC60*	K1102 *nusA Salmonella*
Phage	32°C	42°C	32°C	42°C	32°C	42°C	32°C	42°C	32°C	32°C
λcI$^-$	+	+	+	−	+	−	+	−	−	−
λ*imm*434cI$^-$	+	+	+	−	+	−	+	−	−	−
λcI$^-$*nin*5	+	+	+	+	+	+	+	+	+	+
λ*imm*434*nin*5	+	+	+	+	+	+	+	+	+	+
4F113*imm*434*nin*$^+$	+	+	+	−	+	−	+	+	−	NT
λ*punA*	+	+	+	+	+	−	+	+	−	−
λcI$^-$c17	+	+	−	−	−	−	−	−	−	−
λ*imm*434c17	+	+	−	−	−	−	−	−	−	−

Bacteria were grown in TB to stationary phase. Lawns were poured on TB plates by using agar overlays. Dilutions of the various phage were spotted on the lawns and the plates were incubated at the indicated temperatures. + means growth; − means no growth; NT, not tested.

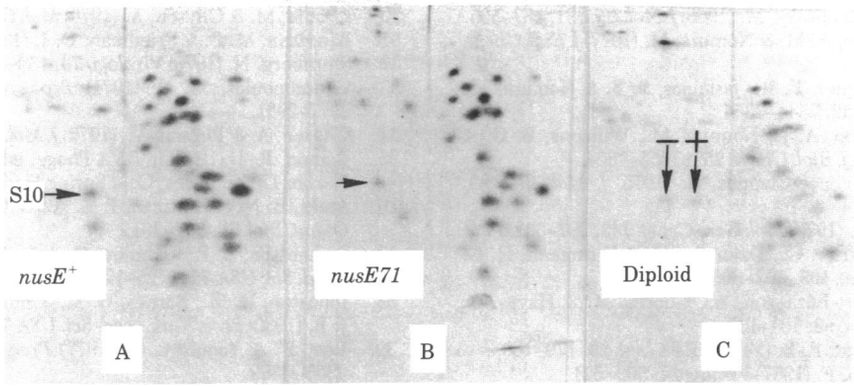

FIG. 3. Two-dimensional gel electrophoresis of ribosomal proteins. Ribosomes from the following strains were analyzed: (*A*) K37 (*nus*$^+$), (*B*) K556 (*nusE71*), (*C*) K1484 (*nusE71/nusE*$^+$). The − and + in *C* indicate the mutant and wild-type forms of the protein, respectively.

rivative of 4F113 was constructed (Fig. 2 *Lower*). As shown in Table 1, the 4F113 *imm434nin*$^+$ phage forms plaques on the *nusE71* host at 42°C, but not on the *nusA* or *nusB* mutants at nonpermissive temperatures. Moreover, lysogens of the *nusE71* host constructed with 4F113*imm434nin*$^+$ become Nus$^+$.

***nusE71* Alters Ribosomal Protein S10.** The ribosomal proteins from the *nusE71* mutant, K556, were analyzed by using two-dimensional gel electrophoresis. A comparison of the ribosomal proteins isolated from K556 and the parental strain K37 revealed an alteration in ribosomal protein S10. As shown in Fig. 3, the S10 protein from the *nusE71* mutant is shifted to the more acidic side of the gel. Analysis of the proteins from a *nusE71/nusE*$^+$ diploid strain (K1484) shows two spots in the region of S10, corresponding to the two different S10 proteins observed for the haploid mutant and wild-type strains. Because these proteins were isolated from purified ribosomes, the fact that both forms of the S10 protein can be observed demonstrates that either can be placed on the ribosomes. *In vitro* mixed extracts from haploid *nus*$^+$ and *nusE71* strains showed a pattern identical to that of the diploid (data not shown).

DISCUSSION

We have presented evidence that a mutation (*nusE71*) in the gene encoding ribosomal protein S10 results in an interference with the activity of the λ *N* gene product. This product, pN, modifies transcription complexes so that they are capable of overcoming termination barriers in *cis* with the λ early promoters (7–9). The *nusE71* mutation is one representative of a class of mutations that exhibit a similar phenotype but map at a number of sites on the *E. coli* chromosome (16, 17). The *nusC* mutation has been located in the *rpoB* gene, which encodes the β subunit of RNA polymerase. In addition to *nusC*, other mutations that map in the region of *rpoB* influence the activity of pN (30–33). The location of a *nus* mutation in a gene encoding a ribosomal protein suggests a role for at least one ribosomal protein in the action of pN.

The existence of two different mutations that map within the *N* gene supports the idea of specific interactions for pN. The first, *mar*$^-$, inhibits growth of λ in hosts that carry a mutation, *ron*$^-$, that maps in the *rpoB* gene (34), suggesting a functional interaction between pN and RNA polymerase. The second, *punA*$^-$, permits growth of λ on either *nusA1* or *nusE71* mutants, implying an interaction of pN with host proteins other than RNA polymerase.

We propose the following working model for pN activity. The basic postulate is that pN forms a complex with RNA polymerase and a set of host proteins to form a termination-resistant complex. Because the *nusE* protein is a ribosomal protein, one or more of the host factors are either ribosomal proteins or conceivably the complete ribosomes. In the first case S10 participates in the pN antitermination complex as a subunit in a situation somewhat analogous to participation of ribosomal protein S1 in Qβ phage replicase (35). In the second case, the role of pN could be to tightly associate RNA polymerase with the first trailing ribosome, preventing subsequent dissociation of this ribosome at nonsense codons.

A coupling of transcription and translation has previously been postulated, and it has been suggested that nonsense codons may disrupt this coupling to trigger transcription termination (8, 36–40).

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