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Identification of the *nus*B Gene Product of *Escherichia coli*

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Summary. *Escherichia coli nus*B mutants fail to support the activity of a phage λ gene product, pN, which regulates phage gene expression by influencing transcription termination. We report the identification of the *nus*B protein on SDS-polyacrylamide gels as a 14,500 dalton protein.

Introduction

Temporal expression of coliphage λ gene products occurs primarily by a process of transcription termination and antitermination. This subject has recently been reviewed by Herskowitz and Hagen (1980) and the following summarizes details on transciption termination relevant to our study. In the case of early gene products this regulation is controlled by the product of the λ *N* gene, pN. Transcription of early genes initiates at two promoters, p_L and p_R, and terminates at termination signals t_{L-1} and t_{R-1}. Details of this process are shown in Fig. 1. The *N* gene lies between p_L and t_{L-1} and thus is expressed early. In the presence of pN, transcription initiating at either P_L or P_R proceeds through a variety of termination barriers. It has been postulated that pN acts near the early promoters (Friedman et al. 1973) at sites called *nut* (Salstrom and Szybalski 1978) to modify transcription complexes so that they can overcome termination barriers.

A series of mutants of *Escherichia coli* have been isolated that define a set of host factors necessary for pN activity. These mutants are calles *nus* for *N*-*u*tilization *s*ubstance (Friedman 1971) or in some cases *gro*N (Georgopoulos 1971). The following is a brief summary of the various *nus* mutants. The *nus*A gene maps at minute 68 (Friedman and Baron 1974) and codes for a 69,000 dalton protein, L factor, which is involved in stimulating a coupled in vitro transcription-translation reaction (Greenblatt et al. 1980; Kung et al. 1975). The *nus*E gene codes for ribosomal protein S10 (Friedman et al. 1981). A variety of studies implicate the β and σ subunits of RNA polymerase in pN activity (Pironio and Ghysen 1970; Georgopoulos 1971; Baumann and Friedman 1976; Sternberg 1976; Nakamura et al. 1979). A fourth locus, *nus*B (Friedman et al. 1976) or *gro*NB (Georgopoulos et al. 1980) has been identified. In this communication, we report the identification of the *nus*B gene product as a 14,500 dalton protein. This identification was made possible by the isolation of a λnusB$^+$ transducing phage and various λnusB$^-$ derivatives.

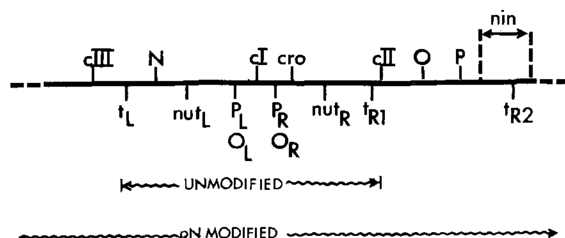

Fig. 1. The early control region of λ. Early promoters, terminators as well as the patterns of unmodified and pN modified transcription are shown. (See text for details)

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Materials and Methods

Bacterial and Phage Strains. All *nus* mutants used in these studies are derivatives of K37, a *str*^{R} derivative of W3102 (Friedman et al. 1973). K95 carries the *nus*A-1 mutation and K450 carries the *nus*B-5 mutation (Friedman et al. 1976). Strains 159 and the 159 (λ) lysogen, which contains an *ind*$^-$ prophage, are *uvr*A$^-$ and were obtained from M. Ptashne (Ptashne 1967). The minicell producing strain DS410/pK280 is *min*A *min*B *str*^{R} *thi*$^-$ and contains the pKB280 plasmid which carries the λcI$^+$ gene fused to a *lac* promoter (Reeve 1978; Backman and Ptashne 1978). λcI857 and λcI857*nus*B$^+$ were previously described (Friedman et al. 1976).

Labelling of Proteins. Methods for the growth, UV irradiation and infection of bacteria were as described previously by Epp and Pearson (1976). Minicells were prepared and labelled using the procedures of Reeve (Reeve 1979).

Polyacrylamide Gel Electrophoresis. SDS-Polyacrylamide gel electrophoresis was performed using a modification of the Laemmli technique (Laemmli 1970) to produce a 14–20% polyacrylamide gradient in the separating gel and a 5% stacking gel. Between 10 and 20 µl of each sample was loaded on the gels.

Analysis of DNA. Phage DNA was isolated using the method of Davis (Davis et al. 1980). Restriction enzyme cleavage of DNA was accomplished using standard procedures and the resulting fragments were analyzed using 0.8% agarose gels (Helling et al. 1974).

Results

$\lambda nusB^+$ and Derivative Transducing Phage

The λnusB$^+$ transducing phage employed in these studies was previously described (Friedman et al. 1976) and the following is a brief description of some salient features. The transducing phage was obtained by inducing a λcI857 lysogen that contained

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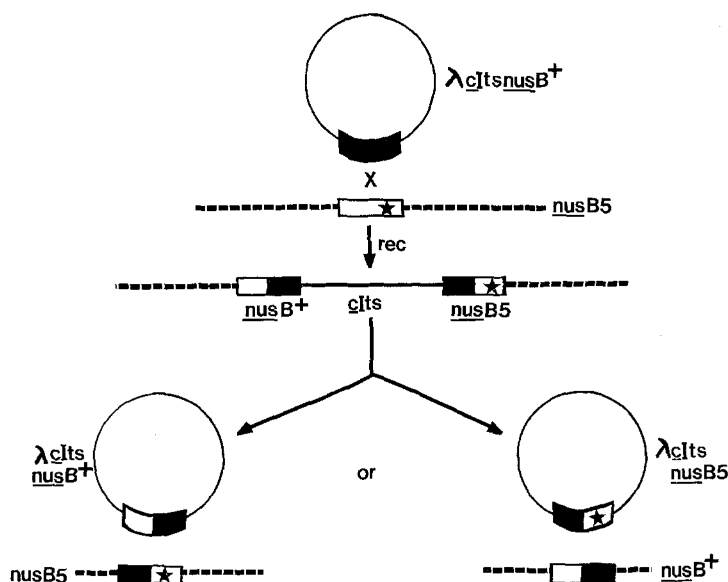

Fig. 2. A derivative of λ*nus*B$^+$ that carries the *nus*B-5 strain was constructed in a two-step procedure by M. Gottesman (personal communication). First, strain K450, which carries the *nus*B-5 mutation, was lysogenized with the *nus*B phage. The recombination occurred between the bacterial genes on the phage and the bacterial chromosome resulting in the phage genome being flanked by a duplication of bacterial genes including *nus*B$^+$ and *nus*B-5. Second, the λ*nus*B-5 phage was obtained by inducing this lysogen. The prophage will excise by homologous recombination and, depending where the recombination occurs, either a *nus*B$^+$ or a *nus*B-5 transducing phage will be generated. The *nus*B-5 phage was identified by screening for phage that form plaques on a *nus*B$^+$ host but not on a *nus*B$^-$ host

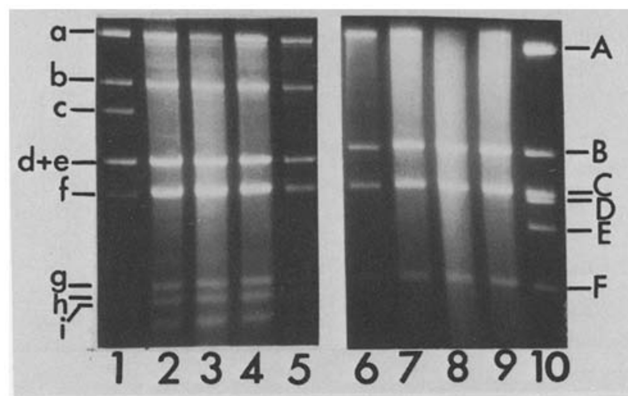

Fig. 3. Restriction fragment patterns of λ*nus*B$^+$ and derivatives. Lanes 1 through 5 are *Ava*I digestion patterns. Lanes 6 through 10 are *Eco*R1 digestion patterns. Lanes: (1) λ$^+$, (2) λd12cb, (3) λd12ct, (4) λ*nus*B-5, (5) λ*nus*B$^+$, (6) λ*nus*B$^+$, (7) λ*nus*B-5, (8) λd12ct, (9) λd12cb, (10) λ$^+$. Capital letters indicate positions of fragments produced by *Eco*R1 digestion of λ$^+$ DNA. Lower case letters indicate positions of fragments produced by *Ava*I digestion of λ$^+$ DNA (see Fig. 4). *Ava*I fragments d and e comigrate on these gels

a λ prophage at an unusual site, in the *tsx* gene at minute 10. Since this gene maps close to the *nus*B gene, excision of the prophage by illegitimate recombination (Franklin 1980) should result in the formation of λ*nus*B$^+$ transducing phage. Such transducing phage were identified in a lysate produced by thermo-induction of the prophage located in *tsx* as plaque formers on a *nus*B$^-$ host. Since *E. coli nus*B-5 lysogens constructed with the λ*nus*B$^+$ transducing phage exhibit the Nus$^+$ phenotype, it was concluded that the transducing phage has the bacterial promoter necessary for *nus*B expression.

In order to use the *nus*B$^+$ transducing phage to identify the *nus*B protein, we isolated derivatives of the transducing phage that fail to express the *nus*B$^+$ phenotype. The *nus*B-5 mutation (Friedman et al. 1976) was placed on the transducing phage according to the procedure shown in Fig. 2 (M. Gottesman, personal communication). Additional λ*nus*B$^-$ derivatives were isolated using a procedure which enriches for phage with genomic deletions (Parkinson and Huskey 1971).

The various *nus*B transducing phage were analyzed by restriction enzyme cleavage and gel electrophoresis (see Fig. 3). Analysis of the *Eco*R1 and *Ava*I restriction patterns show that in these transducing phage there is a substitution of bacterial genes

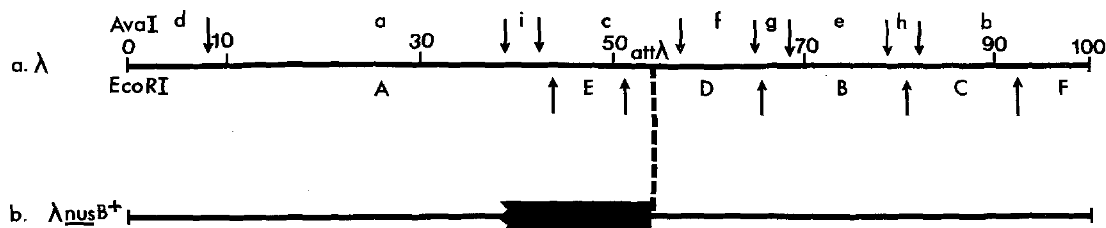

Fig. 4a, b. Location of the bacterial DNA sequences on λ*nus*B$^+$ and its derivatives. **a** *Eco*R1 and *Ava*I restriction sites on wild type λ DNA. The numbers refer to the % distance from the left end of the DNA with 100% equal to the length of λ DNA. The arrows above the line indicate the positions of the *Ava*I restriction sites. The lower case letters indicate the fragments produced by *Ava*I digestion. The arrows below the line indicate the *Eco*R1 restriction sites and the capital letters represent the fragments produced by *Eco*R1 digestion. **b** The position of the bacterial DNA sequences on the *nus*B$^+$ transducing phage as determined by its *Ava*I and *Eco*R1 restriction patterns (see Fig. 3). The shaded region represents the bacterial DNA

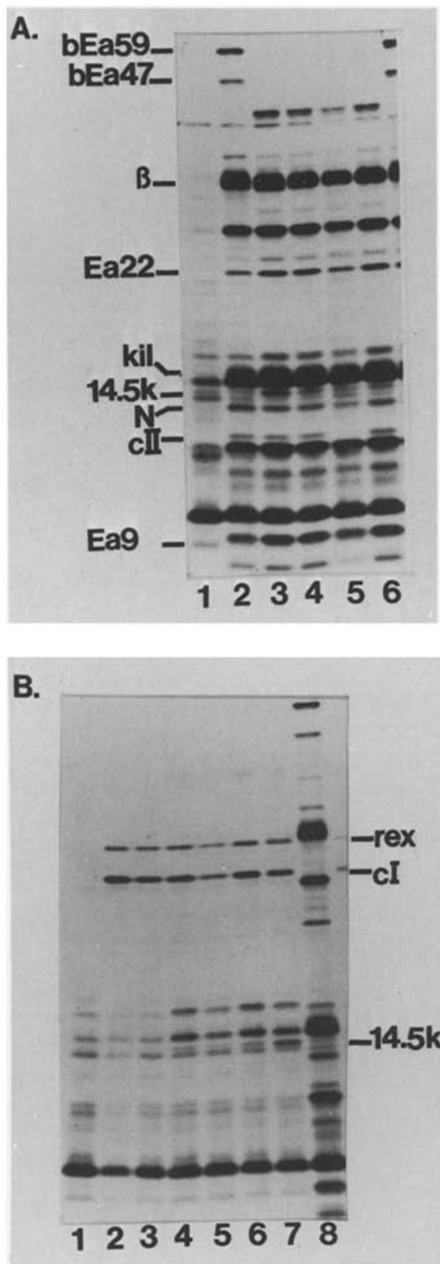

Fig. 5A, B. Autoradiograms of SDS polyacrylamide slab gel electrophoresis of U.V.-irradiated *E. coli* cells infected with various phages and labelled with 75 µci/ml ^{35}S-methionine. **A** *E. coli* strain 159 host cells labelled from 0 to 10 minutes after infection. Lanes: (1) uninfected, (2) λcI857, (3) λnusB$^+$, (4) λnusB-5, (5) λd12ct, (6) λd12cb. **B** *E. coli* strain 159 (λ) labelled from *10* to *25* minutes after infection. Lanes: (1) uninfected, (2) λcI857, (3) λcI857, (4) λd12cb, (5) λd12ct, (6) λnusB-5, (7) λnusB$^+$, (8) λcI857 in 159. The location of some of the λ proteins are indicated for reference. The 14.5 k protein is the 14,500 dalton *nus*B protein (see text). bEa59 and bEa47 are proteins coded for by genes in the *b*2 region of λ. These proteins are not synthesized by the λnusB$^+$ phage and its derivatives

for the phage genes to the left of the *att* site (see Fig. 4). This conclusion is supported by the observation that the *nus*B transducing phage do not direct the synthesis of *b*2 proteins (see Fig. 5), those proteins encoded by the region of the λ genome to the left of *att* (Hendrix 1971). Although the *nus*B$^-$ derivatives λd12ct and λd12cb were isolated as putative deletion mutants, examination of the restriction fragments reveals no obvious differences in the genomes of λnusB$^+$ and the two putative deletion mutants.

Identification of the nusB Protein

The *nus*B protein was identified by comparing the proteins synthesized by the λnusB$^+$ and the *nus*$^-$ derivative phages. Phage encoded proteins were selectively labelled with ^{35}S-methionine by using two different methods to reduce labelling of host encoded proteins; infection of U.V.-irradiated bacteria and infection of minicells. Both methods gave the same results and for brevity we will focus our discussion on the results obtained using U.V.-irradiation.

Proteins isolated from cells infected following U.V.-irradiation were separated on SDS 14–20% polyacrylamide gels and analyzed by autoradiography. Figure 5 shows two such autoradiograms. We observe one difference between the λnusB$^+$ and λnusB$^-$ infections. A more intense band corresponding to a molecular weight of 14,500 daltons is observed in the track from the λnusB$^+$ infection. Since this is the only difference in the protein patterns observed that can be related to the *nus* phenotypes of the various λnusB derivatives, we conclude that the 14,500 dalton protein is the product of the *nus*B gene.

We note the appearance of a weakly labelled band at the 14,500 dalton point in the cases of infection by λnusB$^-$ phages. This could reflect one of two situations: (1) The *nus*B$^-$ phages direct synthesis of a low level of *nus*B protein, a level not sufficient to produce the Nus$^+$ phenotype. (2) The transducing phage encodes a second protein having a molecular weight of 14,500. The fact that this low level labelling is also observed in the case of infections of minicells (data not shown) demonstrates that this protein cannot be a host protein specifically induced by a λnusB infection.

Discussion

We have demonstrated that the *nus*B gene encodes for a 14,500 dalton protein. Similar experiments have led to the same conclusion by Swindle et al. (1981). This identification should be an important step in determining the regulation of *nus*B gene expression as well as the role of the protein in the physiology of the cell. As outlined in the Introduction, previously reported experiments have defined functional roles for other *nus* proteins. The *nus*A gene product is active in stimulating protein synthesis in a coupled transcription-translation system, *nus*E encodes for a ribosomal protein and a variety of studies have shown that both the β and σ subunits of RNA polymerase are involved in pN activity.

Based on these studies the following model has been proposed to explain pN effects on transcription termination (Friedman et al. 1981). There are four elements involved: (1) RNA polymerase, (2) the series of host *nus* proteins, (3) the N-recognition site, *nut* (Salstrom and Szybalski 1978) and (4) the termination sequence and termination protein, Rho (Roberts 1968). We postulate that the *nus* functions along with pN, form a tight complex with RNA polymerase that is resistant to the action of termination factors such as Rho. Since one *nus* protein is ribosomal protein S10 and a suppressor of *nus*A has been identified as a mutation in the gene encoding ribosomal protein L11 (personal communication, D. Ward and M. Gottesman), we have suggested that one possible candidate for the Nus complex is the ribosome itself. Accordingly, the role of pN might be to couple the leading ribosome to RNA polymerase. Such a role for *nus*

factors suggest that the *nus*B protein could be involved in transcription or translation. Proof for such a role awaits further studies following from the identification of the protein.

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