Regulation by N Gene Protein of Phage Lambda of Anthranilate Synthetase Synthesis In Vivo

(N protein assay/temperature-sensitive N mutant protein/N\(^{\text{A}}\) and N\(^{\text{B}}\) specificity/\(\lambda\) repressor)

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ABSTRACT The N protein of bacteriophage lambda is a positive regulator of early \(\lambda\) gene expression. In a \(\lambda trp\) transducing phage, \(\lambda trp46\)\(\text{Nam}\), the synthesis of \(trp\) enzymes in vivo is also dependent on the presence of active \(N\) protein. The DNA of this phage has been used in a protein-synthesizing system in vitro to develop a biochemical assay for the activity of the \(N\) protein. From the following observations it appears that it is \(N\) protein itself that stimulates \(trp\) enzyme synthesis in vitro. An activity that stimulates \(trp\) enzyme synthesis can be made in vitro by \(\lambda N^+\) DNA but not by \(\lambda \text{Nam}^\text{DNAm}\) DNA. This activity can also be detected in extracts of induced \(N^+\) lysogens but not of \(\lambda \text{Nam}^\text{lysogens}\). Furthermore, the activity is temperature sensitive in similar extracts of \(\lambda Nts\) lysogens. No such activity is detectable in extracts of induced lysogens of \(\lambda \text{imm}^{11}\). This phage makes all \(\lambda\)-coded gene products outside the immunity region, but lacks an \(N\) protein activity able to substitute for \(N^+\) protein in vivo. Our experiments also show that the action of \(N\) protein is inhibited by the \(cl\) repressor protein in vivo as it is in vitro.

The synthesis of specific proteins in microorganisms can be regulated by the selective activation of transcription of their genomes (1). In coliphage lambda, genetic expression is controlled primarily at the level of transcription by a sequentially acting set of \(\lambda\)-encoded regulatory proteins, the products of genes \(cl\), \(N\), and \(Q\) (2). The \(cl\) repressor protein is a negative regulator acting to maintain the prophage state by blocking transcription of other \(\lambda\) genes (3). When repressor is inactivated, \(\lambda\) mRNA is transcribed and translated into \(N\) protein, which activates delayed-early transcription required for the synthesis of proteins concerned with \(\lambda\)-specific recombination and replication. In addition, the \(N\) protein stimulates the synthesis of the \(Q\) protein, a positive regulator of late, morphogenetic gene transcription (4).

Although considerable information has been obtained on the regulatory properties of \(N\) protein from genetic experiments, it seemed to us that the understanding of the molecular details of \(N\) action requires a system capable of exhibiting in vitro the same regulatory properties as \(N\) protein does in vivo. To this end we have developed an assay for the activity of \(N\) protein in vitro based on the \(N\)-dependent synthesis of anthranilate synthetase from \(\lambda trp\) DNA. The system is derived from the DNA-dependent enzyme-synthesizing system developed by Zubay to study the regulation of the \(lac\) operon in vitro (5).

The \(\lambda trp\) phage used here, \(\lambda trp46\)\(\text{Nam}\), was constructed by Franklin as a \(\lambda trp\) transducing phage in which a portion of genes to the left of \(N\) is substituted by DNA from the \(trp\) operon of \(Escherichia\) coli (6). In this particular \(\lambda trp\) phage, the \(trpD\) and \(trpE\) genes, coding for the anthranilate synthetase–phosphoribosyl anthranilate transferase complex, replace part of the \(cIII-int\) operon of \(\lambda\), while the \(trp\) promoter and operator are deleted (Fig. 1). As a result, the synthesis of anthranilate synthetase in vivo is no longer regulated by the \(trp\) repressor, but is fully dependent on control by the \(N\) protein. Using the DNA of this phage, we show that the \(N\) protein is required for anthranilate synthetase synthesis in vitro. By all the criteria applied, the genetic regulation of \(trp\) enzyme synthesis using this phage is maintained in vitro. Therefore, this system allows the quantitative assay of the \(N\) protein activity. A similar assay for the \(N\) protein, based on the \(N\)-dependent synthesis of \(\alpha\) endolysin in vitro from \(\lambda \text{Nam}^\text{DNA}\), has been developed independently by Greenblatt (7).

MATERIALS AND METHODS

Bacterial and Phage Strains. A derivative of \(E.\) coli K-12, strain Q13 (\(\lambda\) sup-\(\text{rns}\) \(\text{ppn}^+\)) (8), cured of \(\lambda\), was mutagenized with diethylosulfate to lac\(-\)\(\text{Z}\), and transduced by P1 grown on W3101 \(\text{trpDch}9778\) (9) to \(\text{trpDch}\). The lac\(-\)\(\text{trp}D^-\) strain allows the simultaneous detection of low amounts of \(\beta\)-galactosidase and anthranilate synthetase made in vitro. In all experiments, the S-30 extracts in the protein-synthesizing reaction mixtures were derived from uninfected cultures.

\[ \begin{array}{cccccc}
\text{imm}^{11} & \text{int} & \text{red} & \text{cII} & \text{N} & \text{cI} & \text{fed} \\
\hline
\lambda^+ & \text{P}_{\lambda} & \text{P}_{\text{red}} & \text{P}_{\text{int}} & \text{P}_{\text{cII}} & \text{P}_{\text{N}} & \text{P}_{\text{fed}} \\
\lambda trp46^{\text{cIII}^+} & \text{P}_{\lambda \text{trp46}} & \text{P}_{\text{cIII}^+} & \text{P}_{\text{Nam}} & \text{P}_{\text{cIa}} & \text{P}_{\text{fed}} \\
\end{array} \]

**FIG. 1.** Genetic maps of the portion of the genome of interest in phages \(\lambda^+\) and \(\lambda trp46\). Gene \(cl\) codes for \(\lambda\) repressor protein that prevents initiation of transcription to the left at \(\text{P}_{\lambda}\) and to the right at \(\text{P}_{\text{red}}\). The \(N\) gene product is a positive regulator required for normal transcription to the left, from \(\text{cIII}\) to \(\text{int}\), and to the right, beyond gene \(\text{fed}\). The \(\text{fed}\) gene product can prevent synthesis of repressor and turn off the synthesis of \(\lambda\) exonuclease (the product of \(\text{reda}\)). The \(\text{int}\) gene product is required for recombination between the \(\lambda\) genome and the host chromosome during integration and excision of the prophage. The region of nonhomology between \(\text{imm}^{11}\) and \(\lambda\) is designated \(\text{imm}^{11}\). In transducing phage \(\lambda trp46\), genes \(D\) and \(E\) of the \(\text{trp}\) operon of \(E.\) coli code for the two proteins of the anthranilate synthetase–phosphoribosyl anthranilate transferase complex. The \(\lambda trp46\) phage used here also carries two nonsense mutations in \(N\), a temperature-sensitive mutation in \(cl\), and an absolute defective mutation in \(\text{fed}\).
of this strain. S-100 extracts tested for N activity were prepared from induced lysogens of the same strain. Trp transducing phage λtrp46Nam7am7am3c1s2s3fd1Sam7, referred to as λtrp46Nam in the text, carries trp genes D and E but lacks the trp operator and promoter such that anthranilate synthetase expression is dependent on N function in vivo (6). λ trp 50 Nam is similar to λ trp 46 Nam but carries an intact trp operator and promoter, so anthranilate synthetase expression is regulated by the trp repressor, in vivo (6). These phages, as well as λlel2sfd1, were gifts from N. Franklin. The fed mutation, apparently equivalent to σro or tof, eliminates a "turn-off" product that is a delayed inhibitor of the N operon (6). λNa8cfs1sU32, λplac5c1ts857Sam7, λimm1c1ts5, λNam7am3c1ts857Sam7, and λlelts857 were kindly provided by M. Konrad, R. Schlief, W. Dove, W. Penrose, and H. Murialdo, respectively.

In Vitro Protein-Synthesizing System. The methods described by Zubay et al. (5) for examining the DNA-dependent synthesis of β-galactosidase were used with the following modifications. Magnesium sulfate (2 mM) and thymidine (10 μg/ml) were added to the growth medium. After growth to 5 × 10^5/ml, cells were not frozen but used immediately for making S-30 cell-free extracts. Phenylmethylsulfonylfluoride (dissolved in dimethylsulfoxide at 100 mM) was added to lysing buffer II (5) at 0.1 mM. The preincubation reaction contained each of the 20 amino acids at a final concentration of 6.8 μM. S-30 extracts were dialyzed for 4 hr with a rapid dialyzer (10). Cyclic AMP (0.25 mM) and isoprpylthiogalactoside (0.25 mM) were included in the synthesizing reaction only when λplacDNA was used as a template. Transfer RNA, pyridoxine-HCl, triphosphopyridine nucleotide, flavin adenine dinucleotide, and p-aminobenzoic acid were omitted from the reaction. Omission of these vitamins and cofactors facilitates the fluorometric detection of anthranilate. The concentration of tryptophan in the reaction was reduced to 60 μM. Polylethylene glycol was added to a final concentration of 10 mg/ml, as prescribed by Pouwels and Van Rotterdam (11). In spite of these modifications, the system synthesizes 22 × 10^{-4} units/ml of β-galactosidase reproducibly. Phage DNA (500 μg/ml) was stored at 4° in Tris-HCl pH 8.0 (10 mM). Samples of the synthesizing reaction (100 μl, containing 650 μg of S-30 protein) were incubated at the temperatures indicated in the legends to the figures for 60 min, chilled, and centrifuged (10,000 × g for 5 min). The supernatant was assayed for enzyme activity.

Enzyme Assays. β-Galactosidase was assayed as described (5). Anthranilate synthetase was assayed fluorometrically following the production of anthranilate from chorismate and glutamine (12). The concentration of chorismic acid was reduced to 0.02 mM. One unit of anthranilate synthetase is the amount required to form 0.1 μmol of anthranilate in 20 min at 37°. The activity of anthranilate synthetase measured requires formation of the anthranilate synthetase–phosphoribosylanthranilate transferase complex composed of the trpE and trpD proteins. Since the trpE protein is present in the S-30 extracts, these anthranilate synthetase assays measure the synthesis of the product of the trpD gene. Any conversion of chorismate to anthranilate by trpE product alone, due to the presence of ammonium ions in the synthesizing reaction, is negligible, as shown by anthranilate synthetase activity in a control containing λplac5 DNA. The activity of phosphoribosylanthranilate transferase, which requires trpD protein alone, was assayed as described (13). Both activities were linear with time for at least 10 min, and were linearly dependent on the amount of protein-synthesizing mixture added. The anthranilate synthetase activity required chorismate, and the phosphoribosylanthranilate transferase activity required phosphoribosyl pyrophosphate. Assays of enzymes synthesized in independent reactions were reproducible to within 12%.

Preparation of Crude S-100 Extracts for Assays of N Activity. Cultures (100 ml) of the appropriate temperature-inducible lysogen were grown at 30° to 5 × 10^6 cells per ml. Cultures were induced by being heated quickly to 43° (in an 80° bath). After the cultures were incubated for 11 min at 42°, chilled rapidly to 35° (in an ice bath), and incubated for 15 min at 35°, each was poured onto enough frozen broth to reduce the temperature to 0°, and then washed once in 25 ml of Tris-acetate pH 8.2 (10 mM)–magnesium acetate (14 mM)–potassium acetate (60 mM)–dithiothreitol (1 mM), and suspended in 1 ml of this buffer supplemented with phenylmethylsulfonylfluoride (1 mM) and polyethylene glycol (10 mg/ml). Cells were lysed by sonication; the lysate was centrifuged (100,000 × g for 90 min). The supernatant can be stored for up to 1 week at either 0° or -30° before N activity is assayed. Protein concentrations in these S-100 extracts ranged from 16 to 20 mg/ml.

RESULTS

Stimulation of Phosphoribosyl Anthranilate Transferase Synthesis by λN+DNA. The first experiments were designed to determine if N protein activity could be observed in vitro. This test is similar to an earlier experiment of Franklin, who showed that infection of cells with λtrp46Nam phage leads to nondetectable synthesis of anthranilate synthetase unless these cells are coinfected with λ phage capable of making functional N protein (6). Table 1 shows the result of the analogous experiment done in vitro. When λtrp46Nam DNA is used as a template for synthesis of phosphoribosylanthranilate transferase, a low but appreciable synthesis is observed. Inclusion of λNam DNA with λtrp46Nam DNA resulted in a 30% reduction of enzyme activity synthesized. On the other hand, when λ+ DNA was added, there was a 3-fold stimulation over the amount observed with λNam DNA, and a 2-fold increase over that with λtrp46Nam DNA alone. The low synthesis from λtrp46Nam DNA alone may be caused by the read-through characteristic of in vitro protein-synthesizing systems (14). The reduction of synthesis observed when λNam DNA was added is presumably due to the high DNA

| Table 1. Synthesis of stimulatory activity of anthranilate synthetase by λ+DNA |
|----------------------------------|-----------------|-----------------|
| Template DNA*                   | Phosphoribosyl anthranilate transferase activity (units/ml) × 10^{-4} |
| λtrp46Nam                       | 15              |
| λtrp46Nam + λNam                | 10              |
| λtrp46Nam + λ+                  | 30              |

* Each reaction mixture contained 40 μg/ml of λtrp46Nam DNA. Both λNam DNA and λ+ DNA were present at 80 μg/ml. Synthesis was performed at 37° for 60 min.
Fed- lysogens

Activity indicates that the product(s) of anthranilate synthetase synthesis in vitro fed+ the maximum similar to our understanding from linearly dependent simulation of the synthesis of anthranilate synthetase, while extracts from AN+ obtained when extracts crude protein-synthesizing the in N. Our further evidence of the synthesis of anthranilate synthetase, while extracts of ANam+ induced lysogens, Fig. 3 shows the results of this experiment in which synthesis proceeded for 60 min at various temperature-sensitive in vitro. Fig. 3 shows the results of this experiment in which synthesis proceeded for 60 min at various

concentration used (120 μg/ml total). The stimulation of enzyme synthesis by AN+ DNA could be due to the synthesis of active N protein itself or of another λ gene product dependent for its synthesis upon the presence of N protein. Our further evidence, presented below, suggests that it is the N protein itself that is stimulating trpD enzyme synthesis in the in vitro system.

Assay for Stimulatory Activity of Anthranilate Synthetase Made In Vivo. The preceding experiment suggested that this protein-synthesizing system was responding to N activity made in vitro. If so, such activity should also be detectable in crude extracts of induced lysogens. Fig. 2 shows the results obtained when various amounts of S-100 extracts of induced AN+ and ANam lysogens were added to the protein-synthesizing reactions containing λtrp46Nam DNA. Addition of extracts from induced AN+ lysogens caused a 6-fold stimulation of the synthesis of anthranilate synthetase, while addition of extracts from induced ANam lysogens did not. This stimulation of anthranilate synthetase synthesis was roughly linearly dependent on the amount of AN+ extract added, under our experimental conditions. Further addition of extract from AN+ induced lysogens caused an inhibition in the amount of anthranilate synthetase synthesized (data not shown). Similar results were obtained when AN+fed+ or AN+fed− lysogens were used as a source of AN+ S-100 extract, although maximum stimulation was about 2-fold lower with the fed+ extract than with the fed− extract. These results indicate that the product(s) of AN+ that stimulate anthranilate synthetase synthesis in vitro can be detected in crude extracts of induced lysogens.

Temperature Sensitivity of Anthranilate Synthetase Stimulating Activity in Extracts of Induced Nts Lysogens. Since N mutations have pleiotropic effects on most other λ-coded functions, it was possible that the observed anthranilate synthetase stimulation in extracts of N+ lysogens could be due to some other λ-coded protein whose synthesis is dependent on N protein, rather than the N protein itself. To test this possibility, we performed experiments using extracts from a lysogen with a temperature-sensitive mutation in gene N. The cultures were induced and grown at the permissive temperature to allow synthesis of N protein and any other N-dependent proteins. Extracts from AN+ts lysogens were tested to determine whether or not the stimulatory activity was temperature sensitive in vitro. Fig. 3 shows the results of this experiment in which synthesis proceeded for 60 min at various

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**Fig. 2.** Stimulation of anthranilate synthetase synthesis from λtrp46Nam DNA. Various amounts of S-100 extracts of induced lysogens of AN+clts and ANamclts were added to reaction mixtures containing λtrp46Nam DNA (18 μg/ml) or λplac5 DNA (40 μg/ml). Protein-synthesizing mixtures were incubated at 37° for 60 min and then assayed for anthranilate synthetase activity. The arrow indicates the anthranilate synthetase activity observed in reactions containing λplac5 DNA only, and corresponds to the background value observed with no DNA added.

**Fig. 3.** Temperature-dependent synthesis of anthranilate synthetase and β-galactosidase from λtrp46Nam DNA stimulated by either N+ or Nts S-100 extracts. Protein-synthesizing reaction mixtures containing either λtrp46Nam or λplac5 DNA at the concentrations given in Fig. 2 were incubated at the temperatures indicated for 2 min before addition of S-100 extracts of induced AN+ or λNts lysogens to a final protein concentration of 1.2 mg/ml. This amount of S-100 extract lies within the linear range of the activity stimulating anthranilate synthetase synthesis. Samples were incubated at these temperatures for 60 min, chilled, centrifuged, and assayed for anthranilate synthetase and β-galactosidase activity. (ΟΟΟ) Anthranilate synthetase activity using λtrp46Nam DNA and S-100 extract from a AN+clts lysogen. (ΟΟΟΟΟ) Anthranilate synthetase activity using λtrp46Nam DNA and S-100 extract from a AN+clts lysogen. (ΟΟΟΟΟΟ) β-Galactosidase activity, using λplac5 DNA, with no S-100 extract added. The inset shows the ratio of anthranilate synthetase activity stimulated by Nts extracts relative to N+ extracts as a function of temperature. All activities shown are corrected for background values as follows. Anthranilate synthetase activity with λtrp46Nam DNA and no S-100 extract added gave 13.5 ± 1.5 × 10−4 units/ml of protein-synthesizing reaction mixture, at all temperatures; 100% represents 66.5 × 10−4 units/ml after this background is subtracted. β-Galactosidase activity with λtrp46Nam DNA and no added S-100 extract gave 0.05 A405 unit after incubation for 2 hr at 28° with o-nitrophenylgalactoside; 100% represents 1.65 A405 unit after subtraction of this background.
temperatures. The S-100 extract derived from λNts8 lysogens showed maximum ability to stimulate anthranilate synthetase synthesis at 34°C; this ability decreased to half-maximal activity at 35.8°C. In contrast, the ability of added S-100 extracts from λN+ lysogens to stimulate anthranilate synthetase synthesis was reproducibly less sensitive to the temperature of synthesis, remaining at 100% at 35.8°C and declining to half-maximal activity at 39°C. As a control to assess the overall capacity of the system to synthesize enzyme at these temperatures, we examined the temperature dependence of the synthesis of β-galactosidase from λlac5 DNA in these protein-synthesizing reaction mixtures. This synthesis showed a lower sensitivity to temperature of incubation when compared with the N+-dependent synthesis of anthranilate synthetase. The inset of Fig. 3 shows the ratio of anthranilate synthetase activity stimulated by the Nts extract to that stimulated by the N+ extract at different temperatures. This figure illustrates that the synthesis of anthranilate synthetase is temperature sensitive when stimulated by Nts protein in vitro.

**Regulation of Anthranilate Synthetase Synthesis In Vitro.** Anthranilate synthetase synthesis from λtrp46Nam phage is regulated in vivo by N protein from λ, but not by a presumed equivalent N protein from the closely related hybrid phage, λimm21 (6), in keeping with the inability of λimm21 to provide a substitute for the activity of Nλ in regulating λ transcription in vivo (4). Table 2 shows the result of an experiment that tests the specificity of N protein in vitro. Extracts of λN+-induced lysogens but not of λimm21-induced lysogens stimulate anthranilate synthetase synthesis using λtrp46Nam DNA.

The functioning of the N protein is regulated by the ci repressor in vivo; repressor blocks positive regulation by N protein even when N product is provided in trans (6, 15). Table 2 shows that neither N+ extract alone, nor Nλ extract with purified λ repressor, affects anthranilate synthetase synthesis from λtrp50Nam DNA, in which both trp operator and promoter are present. In contrast, addition of λ repressor abolishes the stimulation from λtrp46Nam DNA observed when Nλ extract alone is added. These experiments indicate that regulation in vitro parallels that observed in vivo. It is worth noting that there was no difference in the maximum amounts of anthranilate synthetase synthesized from trp repressor-regulated λtrp50Nam DNA and from N-regulated λtrp46Nam DNA, even though all our extracts are derived from trpR+ strains. A similar lack of repression by the trpR+ repressor has been reported by Pouwels and Van Rotterdam (11). We have also examined the effect of cAMP on N activity, since cAMP seems to regulate λ repressor synthesis in vivo (16, 17), as well as regulate the induction of catabolite-repressible operons (5, 18) and the synthesis of chloramphenicol acetyl transferase, both in vivo and in vitro (18, 20). Addition of cAMP to 0.5 mM had no significant effect on anthranilate synthetase synthesis.

**DISCUSSION**

Our experiments indicate that it is possible to synthesize active N protein in vitro, and that the N-dependent stimulation of anthranilate synthetase synthesis from λtrp46Nam DNA can be used as a functional assay for the N protein. Extracts of λN+ lysogens stimulate anthranilate synthetase synthesis, and those of λNam lysogens do not. Since N muta-

### Table 2. Specific control of anthranilate synthetase synthesis by λN protein and ci repressor

<table>
<thead>
<tr>
<th>DNA template*</th>
<th>Additions†</th>
<th>Anthranilate synthetase activity (%)‡</th>
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<td>λtrp46Nam</td>
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<td>Nλ+ extract + λ repressor (10 µg/ml)</td>
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<tr>
<td>λtrp46Nam</td>
<td>Nλ+ extract + cAMP (0.5 mM)</td>
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</tr>
<tr>
<td>λlac5</td>
<td>Nλ+ extract</td>
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* Concentrations of DNA in the protein-synthesizing mixtures were as follows: λtrp46Nam DNA, 18 µg/ml; λtrp50Nam DNA, 50 µg/ml; λlac5 DNA, 40 µg/ml.
† S-100 extracts were added to a final concentration in the reaction mixture of 1.2 mg/ml. Synthesis was done at 37°C for 60 min. Purified λ ci repressor was added before addition of S-30 extracts. The molar ratio of ci repressor monomer to λtrp DNA was 200 for λtrp50Nam and 600 for λtrp46Nam DNA.
‡ 100% represents 42 × 10⁴ units of anthranilate synthetase per ml of protein-synthesizing reaction.

- , No addition.

Tions have pleiotropic effects, it could be argued that the differential stimulation of anthranilate synthetase synthesis is due not to the N protein itself, but to some other N-dependent λ-gene product. That N protein itself is responsible can be concluded from the observation that extracts made from lysogens of λ with a temperature-sensitive mutation in gene N are temperature sensitive with respect to stimulation of anthranilate synthetase synthesis in vitro. This conclusion is independently supported by our observation that anthranilate synthetase synthesis is not stimulated by extracts of induced λimm21 lysogens. Besides containing a hypothetical Nλ product unable to supplant the Nλ product in vivo (6), these extracts also contain any other potential activator of trp enzyme synthesis coded by the λ genes outside imm21. The failure of λimm21 extracts to stimulate anthranilate synthetase synthesis strongly suggests that it is the Nλ protein itself that is responsible for stimulating anthranilate synthetase synthesis. In contrast with λNam mutants, λN+ phase is capable of normal replication after induction. Therefore, one might argue that the failure of Nam extracts to stimulate anthranilate synthetase synthesis is due to the lower number of gene copies present in Nam extracts relative to N+ extracts. However, gene dosage itself cannot account for this differential stimulation, since both λNλ and λN+ replicate after induction, yet Nλ extracts fail to stimulate anthranilate synthetase synthesis, as do N+ extracts at high temperature. Our final proof that regulation by N protein in this system mimics that found in vivo is the demonstration that added ci repressor blocks the N-activation of trp enzyme synthesis.
The $N$ protein is currently thought to act by antagonizing the $\rho$ transcription termination factor of $E. coli$, which acts at the $t_{14}$ site (21). This antitermination activity allows the extension of mRNA beyond $t_{14}$ through $cIII$ to $int$ (22). Repression by the $\lambda$ repressor presumably inhibits $N$ action because the 3'-end of the nascent RNA at $t_{14}$ is absent and therefore unavailable for $N$-dependent extension to $int$. In this connection, the inability of $N^{N1}$ protein to substitute for $N$ may be due primarily to the different nucleotide sequences on the $\lambda$ and $21$ DNAs (and mRNAs) in the $t_{14}$ region. At present our experiments show that regulation of $trp$ enzyme synthesis from $\lambda trp4$Nam is subject to control by $N$ protein and $cI$ repressor in vivo, as shown by Franklin in vivo. The advantage of the in vitro system is that it provides a biochemical assay for the $N$ gene product that may prove useful in studying the factors regulating the synthesis of $N$ protein, as well as the detailed mechanism of the action of $N$ protein itself. In addition, it may be possible to extend this assay to the other genetic regulators known to control $trp$ enzyme synthesis in this phage.

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