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Suppression of Transcription Termination by Phage Lambda

Douglas F. Ward and Max. E. Gottesman

One of the most important advances in our understanding of gene regulation in prokaryotes came with the proposal of the "operon" concept by Jacob and Monod (*1*). This concept proposed that regulation of gene expression was achieved by controlling the frequency of initiation of transcription. However, while many genes are in fact controlled in this manner, it is becoming apparent that the transcription of very many other genes is regulated by an alternative mechanism—regulation by control of transcription termination (*2, 3*). Much insight into this process has been achieved by investigations into the mechanism of action of the phage lambda *N* gene product. This protein positively controls lambda development by preventing transcription termination (*4–8*).

Transcription Termination

Transcription initiated at a promoter must stop somewhere. By the operon theory, transcription of every gene of an operon is equal; termination of transcription is assumed to occur at the end of the operon. However, transcription termination can occur within an operon. Polar mutations are characterized by their capacity to inactivate both the cistron in which they are located and the cistrons promoter-distal to the site of the mutation (*9*). Polarity is caused by the action of transcription termination sites located within the operon which prevent transcription of distal genes (*2*). These intraoperonic terminator sites are normally nonfunctional but become active as a result of the polar mutation. Most mutations causing polarity are nonsense mutations that cause termination of translation; the lack of translation activates the transcription termination sites. Thus, terminator sites are not always active; their activity can be prevented or controlled.

Discovery of the "attenuator" structure (*3, 10*) proved that controlling transcription termination had biological significance as a means of gene regulation in wild-type operons. The attenuator is a transcription termination site typically located between the promoter and the

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first structural gene of an operon. Since termination at this site is incomplete, a fraction of the RNA polymerase molecules initiating at the promoter is able to escape termination and continue transcribing into the structural genes of the operon. The transcription of the operon has thus been "attenuated." The efficiency of transcription termination at an attenuator is not fixed; the *trp* attenuator, for example, is affected by the cellular level of charged tryptophanyl transfer RNA ($tRNA^{Trp}$) (*11*). Attenuators have been found in many of the amino acid biosynthetic operons (*12*) and in the *rplJL-rpoBC* transcription unit (*13*), which encodes ribosomal proteins and subunits of RNA polymerase.

Summary. The bacteriophage lambda *N* gene product positively controls development by preventing termination of transcription at terminator sites critical to the sequential expression of phage genes. Many host transcription factors, including RNA polymerase, are involved in *N* gene action. Recent findings have shown that ribosomal proteins are also involved. The current understanding of how the *N* protein affects transcription termination is reviewed, and a possible model and current problems are discussed.

The most exploited system for studying transcription termination is bacteriophage lambda. Regulation of the development of lambda is positively controlled by the product of the *N* gene, which acts by suppressing transcription termination. In the following sections we describe how *N* is able to prevent the activity of transcription termination sites. Because of recent developments, our previously complete understanding of this system now appears only fragmentary. It has also become clear that control of transcription termination is not a simple event—many more factors are involved than was previously thought.

Bacteriophage Lambda

After *Escherichia coli* is infected by bacteriophage lambda, the immediate early phage genes *N* and *cro* are expressed (*14*) (Fig. 1a). The appearance of the *N* product (p*N*) then enables expression of the phage delayed early genes (Fig. 1b) which encode functions involved in replication, recombination, and control of the later stages of lambda development. At this stage lambda development can diverge and follow two mutually exclusive pathways. In the lytic pathway, expression of the late genes is turned on (Fig. 1c), and proteins appear that are required for viral DNA packaging and virion maturation. This is followed by activation of the lysis functions that cause host cell rupture and release of progeny. In the alternative pathway, the lysogenic response, activation of the late genes does not occur. Instead, the lambda genome is inserted into the host chromosome and expression of the lambda genes is turned off by the action of the *cI* repressor; the integrated phage DNA, referred to as a "prophage," becomes dormant and is replicated passively as part of the host genome.

Expression of the lambda delayed early genes is negatively controlled by the *cI* repressor (*1*) and positively controlled by p*N* (*14*). With many other phages, positive control is mediated by an activation of new promoters (*15*); indeed, this mechanism is also used by lambda to initiate the lysogenic pathway (*16*). If p*N* acted in this manner, then positive control of delayed early gene expression by p*N* should prevail over the negative control by *cI* repressor (Fig. 2). This is not the case; expression of delayed early genes requires not only the presence of *N* product but also the absence of the *cI* repressor (*4*). Since repressor inhibits the two lambda promoters, P_L and P_R, which control early gene expression (*17*), it appears that p*N* allows transcription from P_L and P_R to proceed past stop signals located between the immediate early and delayed early genes.

The essential prediction of this model is that a single polycistronic messenger RNA will encode both the *N* gene and the delayed early genes under P_L control. This has been confirmed (*6*).

How p*N* might function to prevent termination in the P_L and P_R operons emerged from studies on factors involved in transcription termination. Using an in vitro transcription system consisting of purified RNA polymerase and lambda DNA, Roberts isolated an *E. coli* protein, called Rho, which caused termination of transcription at specific sites (*8*). In the presence of Rho protein, transcripts produced from the P_L and P_R promoters corresponded to those produced in vivo in the absence of *N* function. These transcripts, 12*S* and 9*S* in size for P_L and P_R, respectively, encode the immediate early *N* and *cro* genes.

It can therefore be concluded that *N* action is consistent with the model proposed in Fig. 2c. In the absence of p*N*, that is, early in infection before p*N* has been made, transcription from P_L and P_R is terminated at transcription termination sites t_{L1} and t_{R1} by the action of the host Rho termination protein. Expression of the delayed early genes is blocked. The *N* product prevents Rho-mediated termination at t_{L1} and t_{R1}, enabling transcription to proceed beyond the terminator sites and into the delayed early genes.

Specificity of *N* Action

Transcription termination sites can be divided into those dependent on Rho factor and those active in the absence of Rho. p*N* is able to overcome termination (antiterminate) at both classes of sites (*7*), suggesting that p*N* does not suppress termination by inactivating Rho. At least one terminator that is resistant to p*N* has been identified in lambda (*7*). Its role may be to prevent p*N*-modified transcription extending into the late gene region in the antisense direction.

Bacterial terminator sites are also suppressed by p*N*. However, this suppression only occurs when the bacterial DNA is transcribed from the lambda P_L or P_R promoters (*18, 19*). Since p*N* is a diffusible protein some *cis*-acting element must control the ability of p*N* to antiterminate transcription (*18, 20*). This element is termed the *N utilization*, or *nut*, site. The location of the *nut* site has been defined genetically for the leftward P_L operon (*18*). Mutants in *nutL* were selected by their predicted characteristics (*21*); such mutants will prevent *N* action on leftward transcription from P_L and will therefore result in loss of expression of genes distal to the first terminator, t_{L1}; the *N* gene will be expressed normally since it lies before the t_{L1} terminator; expression of the rightward P_R operon will not be affected, and the mutant phages will be plaque forming since there are no vital genes in the P_L operon distal to *N*.

These *nutL* mutations do not lie in the P_L promoter (*21*), an indication that recognition of p*N* involves sequences other than at the promoter itself. The DNA sequence analysis (*22*) has shown that the *nutL*44 and *nutL*63 mutations are centered in a region of hyphenated dyad symmetry, whose transcript might form a stem and loop structure (Fig. 3). Comparison with the DNA sequence from the

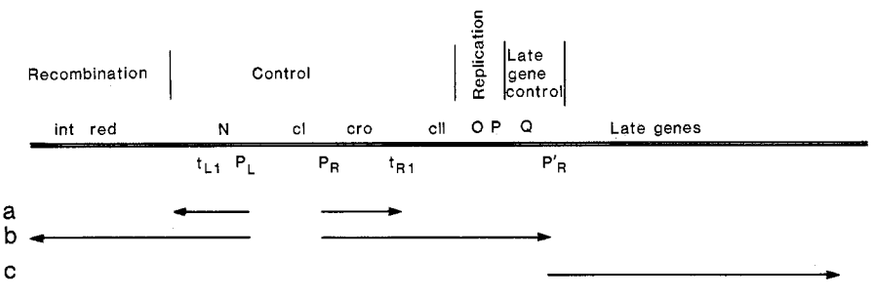

Fig. 1. Genetic map of bacteriophage lambda. In the genetic map of lambda, genes are clustered according to function. The control region contains the genes involved in regulation of phage development. The *cI* and *cro* genes encode repressors of the P_L and P_R promoters. These promoters direct transcription early in phage infection. (a) Immediate early transcription is confined to the *N* and *cro* genes by the action of the t_{L1} and t_{R1} terminator sites. (b) Delayed early transcription includes genes involved in recombination, replication, and late gene control. (c) Late transcription is directed from the P'_R promoter under the control of the *Q* gene product and includes functions for virion packaging, maturation, and cell lysis.

P_R operon (*23*) showed the presence of an almost identical sequence (16 out of 17 bases) in the same orientation with respect to the direction of transcription and preceding the t_{R1} terminator. If this is the *nutR* site, then it is interesting that unlike *nutL*, which lies close to the P_L promoter, the putative *nutR* site is separated from P_R by about 250 base pairs including the entire *cro* gene. It remains to be established what elements of the *nut* structures are required for p*N* recognition or whether the difference between *nutL* and *nutR* (Fig. 3) is biologically significant. Furthermore, there is no evidence to indicate whether the DNA of the *nut* site or its RNA transcript is the recognition element.

The finding that the p*N* recognition (*nut*) sites are distinct from P_L and P_R raises the question as to whether the lambda promoters play a necessary role in p*N*-mediated antitermination of transcription. This problem was resolved (*24*) by constructing a plasmid in which the lambda *nutR* and t_{R1} terminator sites were inserted between the *E. coli gal* promoter and genes coding for resistance to the antibiotic tetracycline (Fig. 4). Strains carrying this plasmid, but lacking *N* function, are unable to grow in the presence of tetracycline since transcription from the *gal* promoter terminates at t_{R1}, preventing expression of the tetracycline resistance genes. However, when p*N* was supplied *in trans*, the t_{R1} terminator was suppressed and the strain became resistant to tetracycline. Thus, p*N* acts in this system to antiterminate transcription from P_{gal}; without the inserted lambda *nut* site, transcription from P_{gal} is not responsive to p*N* function. It was concluded that the *nut* sites are necessary and sufficient for p*N* activity.

Numerous phages that closely resemble lambda in their physiology and in the organization of their genome have been isolated. Of these, two (P22 and 21) have been shown to possess a gene whose properties are equivalent to the *N* gene (*25*). However, these phages will not complement a lambda N^- mutant. We assume that these other *N*-like proteins recognize a *nut* site other than that of lambda. Hybrids between phage 21 and lambda have been constructed by recombination between the homologous regions of these phages (*26*). Such hybrids contain the control region of phage 21, including its *N*-like gene. They retain the *N* specificity of phage 21, indicating that they also contain the putative *nut* sites of phage 21. Similarly, hybrids of P22 and lambda also include both the *N*-like gene and the corresponding *nut* sites of P22 (*25*).

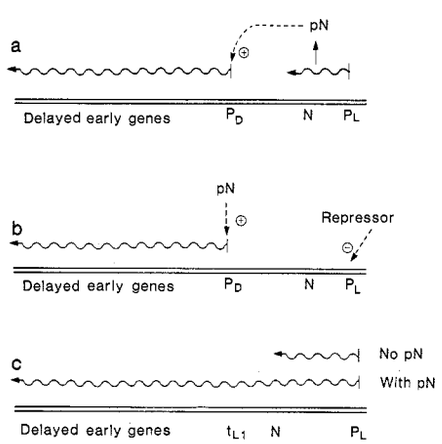

Fig. 2. *N* protein does not activate new promoters. One model suggests that p*N* acts as a positive activator of new promoters that control delayed early gene expression, for P_D (a). If this model is correct then p*N* supplied *in trans* should activate the putative P_D promoter even when the P_L promoter is repressed by the *cI* repressor (b). This does not occur. Thus delayed early gene expression is directed from the P_L promoter (and, similarly, the P_R operon is directed from P_R). The mechanism of *N* action is consistent with the model (c) in which p*N* acts to allow transcription to pass through termination signals (for example, t_{L1}) into the delayed early genes.

Comparison of the preliminary DNA and amino acid sequence of P22 gene 24 (the *N*-like gene) with that for lambda reveals no obvious similarity (*27*). While the *nutL* sequence for P22 has not been defined genetically, a sequence bearing a remarkable similarity to lambda *nutL* has been found in the expected location (*28*). If this is the P22 *nutL* site, we will have to explain how two apparently dissimilar proteins can fulfill the same function and recognize closely related nucleic acid sequences.

Involvement of Host Factors in *N* Function

RNA polymerase. The ability of p*N* to prevent termination at both Rho-dependent and Rho-independent terminators (*7*) indicates that p*N* must act at a point common to both types of termination reactions. Such a point is RNA polymerase itself. This notion is supported by the isolation of several RNA polymerase mutations that prevent p*N* activity.

The *ron* mutations, which lie in the RNA polymerase beta subunit (*rpoB*), restrict the growth of lambda variants carrying *mar* mutations in the *N* gene (*29*). Similarly, the GroN mutant strain bears an *rpoB* mutation that prevents lambda growth (*30*). RNA polymerase isolated from GroN cells showed altered in vitro properties, namely, increased sensitivity to salt and rifampicin. Transcription of the *N* gene is normal in the GroN mutant strain; it is p*N* activity that is deficient. Compensatory mutations in the *N* gene which restored *N* function (*30*) were isolated; one such mutant simultaneously lost *N* activity in a wild-type host. Such patterns of compensatory mutations are consistent with a protein-protein interaction between p*N* and RNA polymerase. However, conclusive evidence that p*N* binds to RNA polymerase has not been forthcoming. Furthermore, it has not been possible to reproduce the prevention of termination effect of p*N* in a purified transcription reaction (*31*).

Rho. Termination of transcription at Rho-dependent terminators is dependent on the interaction of Rho protein with ATP, RNA, and RNA polymerase (*32*). Isolation of compensatory *rpoB* and *rho* mutations suggests that the RNA polymerase target site for Rho is the beta subunit (*33*). With respect to lambda *N*

function, two classes of *rho* mutations exist. The first class (*34*) suppresses transcription termination and thereby enables the growth of lambda even in the absence of *N* function. The second class, exemplified by the mutation *rhoHDF026*, interferes with lambda growth by restricting *N* function (*35*). This class is only partially Rho-defective; transcription termination at Rho-dependent sites still occurs with moderate efficiency. One possible explanation of this class is that Rho and p*N* (or some complex involving p*N*) could compete for binding to the polymerase beta subunit; the *rhoHDF026* mutant protein may compete more favorably than wild-type Rho.

The nus *mutants*. In spite of the suggestive evidence that the target for p*N* is RNA polymerase, the inability to reproduce the antitermination effect of p*N* in a purified transcription system indicates that this reaction must be more complex than was first thought. This should not be surprising since the process of transcription termination is also complex. The coupling of transcription to translation, as shown in transcription attenuation and in polarity, suggests that the regulation of termination involves components other than RNA polymerase and Rho. It is possible that the target of p*N* action could be one or more of these components, for example, ribosomes or factors linking translation to transcription.

This perspective is supported by the isolation of mutations in a series of genes whose products are involved in p*N* activity. These are the *nus* mutants. None of these mutations affect subunits of RNA polymerase. Instead, they define gene products whose role in transcription termination is revealed for the first time.

The *nusA* gene

Induction of a lambda prophage results in the death of the host cell. Under certain conditions, this killing is dependent on the activity of the *N* gene product. As a result of selecting for cell survival, it is simple to isolate *E. coli* mutants which prevent p*N* activity. One such mutant defines the *nusA* gene (*36*). The *nusA1* mutation is temperature sensitive in that growth of lambda is severely restricted at 42°C, but less so at 32°C. There are two types of lambda variants able to grow on the *nusA1* mutant host: either those in which phage growth is independent of *N* function, such as *nin* or *byp* which delete or bypass terminators critical to phage development (*37*); or those mutants with alterations in the *N* gene, for example, λ*punA* (*38*).

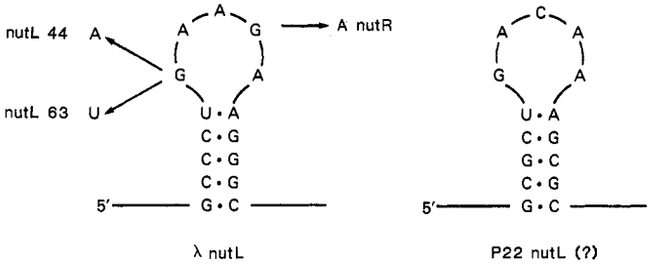

Fig. 3. Structure of the *N* utilization sites. The DNA sequence of the lambda *nutL* site displays a hyphenated dyad symmetry. It is possible for the RNA transcript to fold into the stem and loop structure depicted at right. Sequence analysis of two *nutL* mutants shows the site of the mutation to be in the loop. The single base difference between *nutL* and the putative *nutR* site is also located in the loop. Analysis of the region inferred to contain the phage P22 *nutL* site shows a sequence with strong homology to the lambda *nut* sites (*27*). Its location and structure suggest that it is the P22 *nutL* site, but this assignment has not yet been proved.

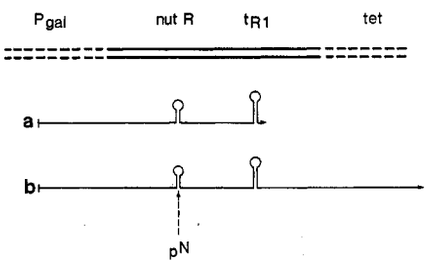

Fig. 4. The *nut* sites are necessary and sufficient for p*N* activity. Insertion of the lambda *nutR* and t_{R1} terminator sites between the *E. coli gal* promoter and the tetracycline resistance determinant rules out promoter specificity in p*N*-mediated antitermination of transcription. (a) Transcription initiated at the *gal* promoter terminates at the t_{R1} terminator. (b) When p*N* is supplied *in trans*, the action of the t_{R1} terminator is suppressed allowing expression of tetracycline resistance.

The *punA* mutation is consistent with an interaction between p*N* and the *nusA* gene product (NusA). Biochemical evidence supports such an interaction. In a series of experiments, Greenblatt and his co-workers have sought to define the *E. coli* proteins that bind to *N* protein in vitro by the use of affinity chromatography. Two host proteins were found to bind specifically to p*N*—one, 25 kilodaltons and the other, 69 kilodaltons (*39*). In extracts from *nusA1* mutants, both proteins still bind to p*N*, but the 69-kilodalton protein has an altered isoelectric point. The binding of the mutant protein to p*N* is more heat labile than that of the wild-type protein, consistent with the known temperature sensitivity of the *nusA1* mutation with respect to lambda *N* function in vivo. These observations suggest that the 69-kilodalton protein is the product of the *nusA* gene. The identity of the 25-kilodalton protein is not known.

Greenblatt has carried his affinity chromatography approach a step further by examining which *E. coli* proteins are able to bind to NusA (*40*). Among those proteins displaying affinity for NusA is the RNA polymerase core enzyme. Holoenzyme does not bind to NusA and, in fact, core enzyme can be eluted from its complex with NusA by the addition of sigma subunit. Genetic support for an interaction between NusA and RNA polymerase comes from the isolation of secondary mutations in the polymerase beta subunit that enhance the defectiveness of the *nusA1* mutation (*38, 41*).

The above studies raise the question of how the transcript specificity of p*N* is imposed by the *nut* sites. Since neither the binding of p*N* to NusA nor the interaction between NusA and RNA polymerase core enzyme requires lambda DNA or RNA, the *nut* sites must be involved in some other step in antitermination by p*N*.

Recently, NusA has been shown to have transcription termination activity in an in vitro transcription reaction with lambda DNA as template (*42*). At least some of the lambda terminators activated by NusA appear to be distinct from the Rho-dependent terminators. In the presence of Rho, NusA had no effect on transcription suggesting that the NusA-dependent terminators do not lie between the promoter and the Rho-dependent sites. NusA is identical to L factor, which is required for optimal expression of β-galactosidase (the product of the *lacZ* gene) in an in vitro transcription-translation system (*43*). In this system the role of L factor was thought to be to prevent premature transcription termination within the *lacZ* gene. This apparent contradiction has not been resolved.

In spite of the pronounced effect of NusA on transcription termination in vitro, the *nusA1* mutant shows relatively minor physiological alteration in vivo. That polarity resulting from some, but

not all, polar mutations is reduced is consistent with the transcription termination activity of NusA (*44*).

The most marked in vivo effects of the *nusA1* mutation occur in combination with *rho* mutations (*44*). In particular, relief of polarity by *rho112*, a *rho* mutant with considerable residual termination activity, is greatly enhanced by the addition of *nusA1*. The interaction between *rho* and *nusA1* mutations is underlined by the growth behavior of strains carrying these mutations. Both *rho112* and *rho15* are temperature sensitive for growth, while *nusA1* is temperature sensitive for lambda growth. The doubly mutant strain, *rho*$^-$ *nusA*$^-$, grows at 42°C and allows the replication of phage lambda. This situation, where two mutations each suppress a phenotype of the other, is not common in genetics. Further investigation is needed.

The rarity of *nusA* mutations suggests that the range of alterations in NusA tolerable by *E. coli* may be extremely limited. The possibility that NusA may be an essential cell component is consistent with the finding that NusA is regulated similarly to that of other essential *E. coli* proteins required for transcription and translation (*45*).

nusB Mutants

The *nusB* mutants were also identified by their resistance to lambda *N* function (*46*). They form the most frequent class of such mutants. Like *nusA1*, they severely restrict the growth of lambda at 42°C. Many, but not all, of these mutants also restrict the activity of the *N*-like product of lambda-21 hybrids.

An additional class of *nusB* mutations, such as *nusB101*, has recently been isolated whose phenotype is the suppression of the *nusA1* mutation (*47*). The double mutant, *nusB101 nusA1*, permits the growth of lambda at 42°C but not lambda-21 hybrids. By itself, *nusB101* has no obvious phenotype; both lambda and lambda-21 hybrids grow at low and high temperatures.

The *nusB101* mutation does not circumvent the requirement for *nusA*. When the *nusA* region of *E. coli* is replaced by the same region from *Salmonella typhimurium*, the resulting hybrid strain blocks lambda *N* activity. The addition of the *nusB101* mutation to the hybrid strain does not restore *N* function.

The *nusB* mutants also show some effects on *E. coli*. The *nusB5* mutant, which is almost identical in phenotype to *nusA1*, also partially suppresses the polarity of certain nonsense mutations (*44*). Some of the *nusB* mutants are defective for cell growth at low temperature (*46*); the nature of this lethality is not known.

Ribosomal Mutations Affect *N* Activity

The regulation by translation of transcription termination in *E. coli* operons raises the possibility that p*N* may suppress termination by interacting with ribosomes rather than with transcriptional factors (*48, 49*). This idea is supported by the recent demonstration that two ribosomal proteins, S10 and L11, are involved in *N* action.

The *nusE* mutation, which lies in gene *rpsJ* encoding protein S10, produces a shift in the isoelectric point of S10 (*49*). Like the previously isolated *nus* mutants, *nusE* prevents p*N* activity at 42°C, but not at 32°C. Ribosomal protein L11 has been implicated in p*N* activity by the isolation of a mutation in *rplK* (L11), which restores *N* function in a *nusA1* host at 42°C (*50*). The role of L11 in transcription termination and *N* activity is unclear. A conditional lethal mutant lacking any antigenically detectable L11 protein still shows mutational polarity. In addition, the activity of p*N* is normal. This suggests that the role of L11 in transcription termination or *N* action is indirect. Many mutations in L11 show a relaxed phenotype (*51*); *rel* mutants increase translational error frequency (*52*). We propose that the rate of translation may also be increased in *rel* mutants, which might suppress transcription termination (see model below).

The involvement of two ribosomal proteins, S10 and L11, in the action of p*N* suggests that p*N* interacts with ribosomes to suppress transcription termination. However, these ribosomal proteins may influence termination as extraribosomal, soluble factors. A precedent for this notion may be found in the inclusion of ribosomal protein S1 in the RNA phage Qβ replicase (*53*). Indeed, a lambda transducing phage carrying the wild-type S10 gene forms plaques on *nusE* hosts at 42°C (*49*), suggesting that S10 might act as a ribosome independent factor. Alternatively, the S10 protein synthesized by the infecting transducing phage might exchange with S10 on preformed ribosomes.

Attempts to resolve this question biochemically are as yet inconclusive. A crude transcription system, responsive to p*N*, has been developed with the use of the supernatant fraction from *E. coli* extracts (*54*) that were centrifuged at 100,000*g*. Although this fraction is, in principle, free of ribosomes, some contamination might account for the action of p*N* in this system.

Model for *N* Action

The expression of an operon in *E. coli* proceeds by a series of discrete steps. The RNA polymerase holoenzyme binds to a promoter and initiates transcription. Its sigma subunit is then released, and the transcript is elongated until a transcription termination sequence is recognized, and transcript termination occurs. Recognition of a transcription termination sequence involves many factors not all of which are clearly understood. Formation of an RNA stem-loop structure based on the dyad symmetry at the terminator is apparently essential for termination. Some terminators also require protein factors, such as Rho and NusA. Transcription termination can be suppressed by translation, suggesting that the close proximity of a ribosome to RNA polymerase interferes with the termination reaction. The ribosome may block termination by distorting the secondary structure of the RNA at the terminator, as in the case of attenuation, or by blocking the access of Rho, as appears to be the case in polarity.

The proximity of RNA polymerase and the first ribosome on the transcript might be achieved passively, by a high rate of ribosome movement, or actively, by protein cofactors. The demonstration that NusA binds to RNA polymerase core enzyme is consistent with its serving as a cofactor. NusA, attaching to RNA polymerase after release of sigma, could form a bridge between RNA polymerase and a ribosome, possibly connecting the two at the ribosomal proteins S10 or L11 (or both). NusB may promote the formation of the bridge, or it may participate in the complex. Stability of the RNA polymerase–NusA–ribosome complex would be dependent on active translation. If a polypeptide chain terminating codon is reached, ribosome detachment from the transcript and from the complex occurs. Without ribosomes, the RNA polymerase–NusA complex will terminate at the appropriate terminator.

The mechanism of antitermination by p*N* can be explained within the framework of this model. *N* product interacts with NusA, and possibly other components of the bridge, to stabilize the polymerase–NusA–ribosome complex. The stability of this complex must be independent of translation. Thus chloramphenicol, which blocks translation by immobilizing ribosomes, does not affect

p*N* function (*55*). Similarly, the p*N*-modified complex does not disintegrate into its components at nonsense codons, and consequently polypeptide chain termination will not result in transcription termination. Thus p*N* will suppress polarity caused by amber or ochre mutations and, in a similar fashion, termination at attenuator sites.

The role of the *nut* sequence in p*N*-mediated antitermination of transcription may be to serve as the point at which the polymerase–NusA–ribosome complex is assembled. There is a potential translation initiation region about 20 base pairs before *nutL*. At *nutR*, the termination codon of the *cro* gene is very close to the *nutR* sequence. Therefore, at both *nutL* and *nutR*, the access of ribosomes to transcribing RNA polymerase is possible.

Conclusion

The role of the *N* gene protein as the positive regulator of phage lambda development is accomplished by preventing transcription termination. Implicated in the action of p*N* is the *E. coli* protein NusA, to which p*N* has been shown to bind, RNA polymerase, termination factor Rho, NusB, and ribosomal proteins S10 and L11. The studies in this article on the lambda *N* function make it clear that transcription termination in vivo is more complex than previously realized and involves the interaction of many components.

Two further thoughts might be mentioned. First, in addition to the *nus* mutants described in this article there are many *nus* mutants still uncharacterized (*56*); and second, lambda possesses a second positive control gene *Q* which regulates late gene expression (*57*). The *Q* gene product also acts as a transcription antitermination factor (*58*). The *nus* mutants are still proficient for *Q* function. A search for host mutants defective for *Q* action has not been started.

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