

MicroReview

Transcription antitermination: the λ paradigm updated

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Summary

Coliphage λ employs systems of transcription termination and antitermination to regulate gene expression. Early gene expression is regulated by the phage-encoded N protein working with a series of *Escherichia coli* proteins, Nus, at RNA sites, NUT, to modify RNA polymerase to a termination-resistant form. Expression of λ late genes is regulated by the phage-encoded Q antitermination protein. Q, which appears to use only one host factor, acts at a DNA site, *qut*, to modify RNA polymerase to a termination-resistant form. This review focuses on recent studies which show that: (i) N can mediate antitermination *in vitro*, independent of Nus proteins. (ii) Early genes in another lambdoid phage HK022 are also regulated by antitermination, where only an RNA signal appears necessary and sufficient to create a termination-resistant RNA polymerase. (iii) A part of the *qut* signal appears to be read from the non-template DNA strand. (iv) A host-encoded inhibitor of N antitermination appears to act through the NUT site as well as with the α subunit of RNA polymerase, and is antagonized by NusB protein.

Introduction

The strategy of controlling gene expression by modulating transcription termination was discovered in coliphage λ (Roberts, 1969). Roberts proposed that the N gene product of λ , which was known to be required for the synthesis of nearly all λ transcripts, acts to permit transcripts initiating at the early λ promoters to override Rho-dependent

termination signals. This proposal was based on his studies identifying Rho-dependent termination signals in λ as well as on a wealth of well-characterized mutants and variants of λ . Following this seminal proposal, *in vivo* and *in vitro* studies contributed to a more sophisticated understanding of N-mediated transcription antitermination. Moreover, additional regulatory systems based on transcription termination were identified in λ (Roberts, 1992), and related phages (Franklin, 1985) as well as in bacteria (Landick and Turnbough, 1992) and eukaryotes (Spencer and Groudine, 1990; Jones and Peterlin, 1994). A second class of transcription terminators, Rho- or factor-independent, has also been identified (Yager and Hippel, 1987). N-modified transcription also overrides this type of terminator (Gottesman *et al.*, 1980; DeVito and Das, 1994).

Studies on the λ transcription-antitermination systems continue to serve as primary sources for new insights into the process of transcription elongation, in eukaryotes as well as prokaryotes (Das, 1993; Greenblatt *et al.*, 1993). Therefore, this review will focus on termination and antitermination regulatory systems in the lambdoid phages. Over the last few years there have been a number of excellent reviews on the topic of transcription antitermination, focusing on the well-characterized N and Q systems of λ (reviews are listed before each section in the *Prologue*). We refer the reader to those reviews for detailed discussions of N and Q as well as references to the older literature. We will limit our discussion to an abbreviated summary of the material that has been previously reviewed and focus on the more recent findings hoping to update the readers on material with which they may not be familiar.

PROLOGUE

The N system

(Reviewed in: Das, 1992; Das, 1993; Roberts, 1993; Greenblatt *et al.*, 1993; Greenblatt, 1992; Friedman, 1988; Friedman and Gottesman, 1983.)

Early-gene transcription of the λ genome originates at two promoters, pL (leftward) and pR (rightward), proceeding in opposite directions (Fig. 1). Transcription on the left passes through the N gene and, on the right the *cro* gene, before encountering Rho-dependent terminators tL1 and tR1 respectively. Approximately 50% of the transcripts initiating at pR terminate at tR1, with the remainder

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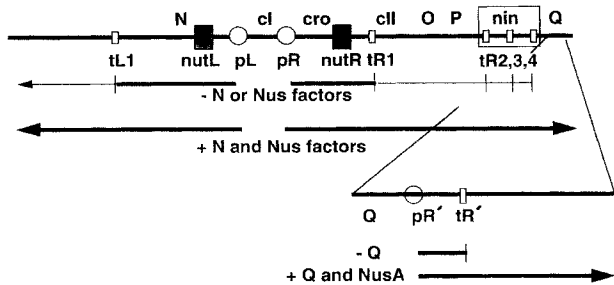


Fig. 1. Map of the regulatory region of λ detailing transcription patterns. To orient the reader, a representative collection of genes are shown at the top of the figure. The N and Q genes are discussed in the text. The *nin* region (Court and Sato, 1969) is defined by a 2.8 kb deletion (Fiandt *et al.*, 1971) that removes three regions of transcription termination (Cheng *et al.*, 1995). The following lists the activities of the products of genes not discussed in detail in the text: repressors, *cl* and *cro*; transcription activator, *cII*; replication, *O* and *P*. The position of regulatory signals are shown below the listing of genes: small open boxes, terminators; large filled boxes, *nut* sites; open circles, promoters. Transcription patterns in the presence and absence of the N and Q antitermination proteins as well as associated Nus factors are shown beneath the map of the regulatory region.

continuing through O and P and terminating in the collection of Rho-dependent and factor-independent terminators in the *nin* region. In the presence of N, transcription transcends these termination barriers and, consequently, more distal genes are expressed. In fact, if λ is integrated into the bacterial chromosome, modified transcription can extend for over tens of kilobases into the bacterial chromosome, overriding a wide range of both Rho-dependent and Rho-independent terminators.

In addition to N, a number of host proteins, Nus, are required for N-mediated antitermination. A role for these proteins was, in most cases, first deduced from genetic experiments with mutants that were unable to support N action. Identification of the affected genes and demonstration that they were recessive showed that the mutations affect a required activity and lead to the identification of the products. *In vitro* studies confirmed that these products were required for N action. Some of the Nus factors had previously been unidentified while others are proteins of known function. NusA, a 56 kDa essential protein, was subsequently shown to affect transcriptional pausing, termination, and antitermination. NusB, a 14 kDa protein essential for cell growth only at low temperatures, may be involved in translation as well as transcription. The *nusE71* mutation, which defined the NusE product, is an allele of *rpsJ*, encoding ribosomal protein S10. NusD was identified as the termination factor Rho. NusG, first identified through a *nusG* mutation that suppressed the effects of the *nusA1* and *nusE71* mutations, is a required factor for the N antitermination *in vitro* as well as an enhancer of termination factor Rho. The N and Nus proteins function as a complex modifying RNA polymerase to a termination-resistant form.

The N–Nus complex is assembled at sites, NUT, located downstream of the pL and pR promoters (see Figs 1 and 2). Transcription initiates at pR and traverses the *cro* gene before reaching the *nut* sequence. The NUT signals, read from the RNA, have been divided into two components, BOXes A and B, with an intervening spacer region (Fig. 2). The *boxA* sequence is conserved not only amongst lambdoid phages but also within the anti-terminator elements of the *rnn* operons. The BOX-B sequence forms a stem–loop structure in the RNA. *In vitro* studies suggest that as soon as NUT RNA is synthesized, N and Nus factors assemble into a complex and associate with RNA polymerase (Fig. 2). These studies also suggest that the assembled complex remains associated with the RNA polymerase as it continues transcription. The NUT RNA may be part of the complex. In this way, the polymerase is modified and retains antitermination activity as it transcribes kilobases of DNA. Both *in vivo* and *in vitro* evidence indicate that host factor NusB, in complex with NusE, binds to BOX-A, and N protein binds to the loop of BOX-B.

All lambdoid phages have mechanisms for converting transcription complexes initiating at early promoters to termination-resistant forms. With one exception (to be discussed below), those studied have systems analogous to the N system of λ . The two other characterized N systems are those of phages 21 and P22. In those cases, N genes and *nut* sites are located at positions on these phage genomes that are analogous to the positions of their λ homologues. Despite these similarities, products of the N genes of different lambdoid phages are specific for their cognate NUT sites under physiological conditions. The arginine-rich amino terminus of N recognizes the BOX-B region of NUT. A consensus *boxA* sequence was deduced from a comparison of *boxA* sequences and shown to be more effective than that found in the λ *nut* region. Both *in vivo* and *in vitro* studies suggest that NusB and possibly S10 (NusE) interact with BOX-A, with the consensus BOX-A being the most effective binder.

The Q system

(Reviewed in: Roberts, 1992.)

N-modified transcription from pR passes through tR1 and the Rho-independent (tR2) and Rho-dependent (tR3 and tR4) barriers in the *nin* region before extending into the Q gene (Fig. 1). The Q gene product is the primary factor in a second λ antitermination system allowing transcription initiating at the pR' promoter, immediately beyond Q, to extend downstream to include the lytic genes. Q-mediated antitermination, like that of N, requires a specific recognition signal, termed *qut*. However, Q, unlike N, does not bind to the RNA but binds to DNA in the region between the –10 and –35 promoter elements and interacts with

phages tested, only those with the *nut* region of λ are excluded.

NEW INFORMATION

Minimal requirements for N-mediated antitermination

Earlier studies using *in vitro* systems defined the several components of N-mediated antitermination (reviewed in: Das, 1992; 1993; Greenblatt, 1992; Greenblatt *et al.*, 1993). Studies characterizing antitermination through one terminator revealed a requirement for only N and NusA (Whalen *et al.*, 1988). These authors suggest that the added factors provide processivity and stability to the antiterminating transcription complex. Consistent with this argument, persistent antitermination through multiple terminators required, in addition, NusB, S10 (NusE), and NusG (Mason *et al.*, 1992a). More recently, De Vito and Das (1994) demonstrated efficient antitermination over short distances with N alone, but only if N was supplied at high concentrations. They also showed that persistent antitermination through a number of terminators over longer distances required, in addition to N, NusA, S10 (NusE), and NusG. Strikingly, NusB was not required for reasonable levels of persistent antitermination, although it was required for maximally effective levels. These results suggest that the essential component directing antitermination is N and that NusB is the most dispensable for persistent antitermination *in vitro*.

Studies by Chattopadhyay *et al.* (1995) employing mobility-shift- and RNase-protection experiments provided evidence that N binds to the 5' leading side of the stem and loop portion of BOX-B. In addition, these studies demonstrated that some changes in the sequence of the BOX-B loop interfere with N binding and antitermination, while other changes do not affect N binding but do interfere with the minimal N–NusA antitermination reaction. These observations led Chattopadhyay *et al.* (1995) to propose that through an association of both N and NusA with BOX-B, NusA helps to bring N in contact with RNA polymerase to create the antitermination complex. At higher N concentrations, the requirement for NusA is eliminated (DeVito and Das, 1994).

We emphasize that although the N system *in vivo* requires a number of proteins in addition to N, N in conjunction with a BOX-B stem and loop structure is sufficient to convert RNA polymerase to a termination-resistant form. If antitermination can be made so simple, obvious questions raised by this work are why *in vivo* is the N system complicated by so many factors, and is a complicated system necessary? We will argue that the added factors permit multiple levels of regulation through N-mediated antitermination and review newly identified components of the N–Nus system that offer the possibilities of additional levels

of regulation. We will also review studies characterizing other antitermination systems of lambdoid phages that suggest that simpler systems also exist.

Regulation of λ N gene expression

The focus of studies on N has primarily been directed toward understanding its role as an antitermination protein with little effort directed toward understanding the regulation of N expression. Classically, it had been shown that N, the first gene transcribed from pL, is controlled by repressor and Cro proteins, both of which bind to operator sites, and respectively shut off or turn down transcription from pR and pL (Ptashne, 1992). N levels are also controlled post-translationally by the Lon protease, which rapidly degrades the N protein (Gottesman *et al.*, 1981).

Recent studies have shown that N can be further controlled at the level of translation by the RNA endonuclease RNase III (Kameyama *et al.*, 1991). The AUG start of N is located 223 nucleotides (nt) from the start of transcription. This long RNA leader contains the NUT-L site, encompassing nt 34–64. Between NUT-L and the AUG start is a large base-paired stem-loop structure that abuts the N ribosome-binding site and inhibits initiation of translation. RNase III cleaves the structure, opening the ribosome-binding site to allow more efficient translation of N. Since RNase III has a regulatory role in *E. coli* (Court, 1993), it seems likely that λ , through N expression, has added this regulatory scheme to the already large panoply of regulatory mechanisms operating on transcription from its early promoters.

HK022 Nun protein

Building on the earlier observations that HK022 Nun protein acted at λ NUT sites to terminate transcription from early λ promoters, Baron and Weisberg (1992) showed that both the *boxA* and *boxB* components of the λ *nut* sequences are required for maximal Nun-mediated transcription termination. However, results of this mutational analysis also suggested that although there is significant overlap in the recognition of N and Nun, the recognition sequences in *nut* are not identical. Using a suppressor tRNA located immediately downstream from the λ *nutL* sequence as the reporter, Sloan and Weisberg (1993) showed that, *in vivo*, Nun reduced the level of biologically active suppressor tRNA and that most of the RNA terminated in a region about 100 nt downstream of *nutL*.

Also, similar to N, Nun action requires host Nus proteins. Mutations in *Escherichia coli* *nus* genes that cause a failure in N-mediated antitermination also affect Nun-mediated termination (Robert *et al.*, 1987; Robledo *et al.*, 1990). Moreover, other mutations in these *nus* genes have been isolated that reduce Nun action but not N action (Robledo

et al., 1991). Selected as inhibitors of Nun termination at NUT-R, surprisingly, the latter mutations have no effect on Nun termination at NUT-L. Identification of a host mutation, *rpoC100*, that interferes with Nun-directed termination suggests a role for the β' subunit of RNA polymerase in transcription termination (Robledo *et al.*, 1991).

In vitro experiments show that, as does N, Nun functions efficiently in the presence of all of the purified Nus factors, but in this case transcription arrest is the end result (Hung and Gottesman, 1995). Moreover, Nun also functions in a minimal system with BOX-B, independently of Nus factors.

Chimeric proteins expressed from hybrid genes created by *in vitro* splicing of N and *nun* genes have been used to relate function and structure of Nun (K. Henthorn and D. Friedman, in preparation). The proteins expressed from these genes were tested for termination and/or antitermination activity. These experiments, that show that the RNA-binding domains of N and Nun are exchangeable, are consistent with the RNA-recognition element being located in the amino-terminal portion and a transcription-regulating element being located in the carboxy portion of Nun. This assignment is consistent with previous studies showing that the RNA-binding domain of N is in the amino-terminal region of that protein (Lazinski *et al.*, 1989; Franklin, 1993). One chimera, having the amino 3/4 N protein and the carboxy 1/4 Nun protein, exhibits the unusual property of blocking antitermination when *rpoC* is wild type, and promoting antitermination with the *rpoC100* mutant allele that normally interferes with Nun termination. This observation strengthens the argument that interactions with the β' subunit may play an important role in determining the elongation mode of RNA polymerase.

Antitermination in HK022

HK022 early-gene organization is similar to that of λ , and also like λ , HK022 requires transcription antitermination for expression of its *Q* gene. However, we remind the reader that the *nun* gene, while occupying a position on the HK022 genome analogous to the position of *N* on the λ genome, does not appear to function as an antitermination protein (Robert *et al.*, 1987). Although there is no evidence that HK022 has an antitermination function analogous to λ N, there are sites downstream of the early promoters that have similar sequences and are required for transcription antitermination. These common sites, composed of two sequences called *box1* and *box2*, have dyad symmetry and the potential to form a stem and loop structure in the RNA (Oberto *et al.*, 1993). These sequences, however, appear otherwise unrelated to the *boxA* and *boxB* sequences of the *nut* regions of λ and other lambdoid phages. More surprisingly, an HK022-encoded protein does not appear to be necessary when a termination-resistant transcription complex is

formed by RNA polymerase transcribing the *box1* and *box2* region of HK022.

If the only contribution of HK022 to this transcription antitermination reaction is the RNA sequence, is there then an N-like factor expressed by the host that acts at or near BOX 1 and BOX 2? Weisberg and co-workers (Clerget *et al.*, 1995) addressed this question by looking for *E. coli* mutants that fail to support this antitermination. The only mutations identified were in *rpoC*. No evidence for Nus-like or N-like factors was uncovered. One explanation for these results is that a protein is not required for antitermination from the early promoters of HK022, but rather it is the interaction of RNA sequences with RNA polymerase that produces the termination-resistant complex. If the RNA always assumes the same structure after transcription, then it is possible that transcription from the HK022 early promoters always antiterminates, without the delay required for N systems. This raises an interesting question related to N-mediated antitermination. Is the NUT RNA the ultimate determinant for antitermination, with the various proteins, including N, only playing supporting regulatory roles?

Q function

Most of our knowledge of Q action derives from *in vitro* studies from the Roberts laboratory. They have focused their efforts on the *Q* genes of λ and its relative, phage 82. Their studies indicate that RNA polymerase binds at pR' and proceeds to pause sites located, relative to the transcription start, at +16 or 17 in λ (Grayhack *et al.*, 1985) and +25 in 82 (Goliger and Roberts, 1989). Polymerases, paused at these sites, are the substrate for Q. Mutational analysis and DNA footprinting studies indicate that the λ Q recognition site, *qut*, may span a segment from within the pR' promoter to +9 relative to the transcription start (Yarnell and Roberts, 1992). For this reason *qut* and Q action cannot be separated from the pR' promoter. This is unlike N, where unrelated promoters function effectively in N-mediated antitermination providing that the *nut* site is promoter distal and placed upstream of any terminators (De Crombrughe *et al.*, 1979).

λ pR' DNA templates, mutant at positions +2 and +6, reduce RNA polymerase pausing and prevent Q binding to the antitermination complex. Heteroduplex DNA containing these same changes but only in the non-template (non-transcribed) strand exert the same effect, whereas heteroduplexes with these changes only in the template (transcribed) strand have little, if any, effect on pausing or Q function (Ring and Roberts, 1994). We emphasize the point that this signal is recognized in the non-transcribed DNA strand and not the RNA or the transcribed DNA strand. Since RNA polymerase incorporating the 16th nt would cause the DNA region including +2 and

+6 to be melted and thus become part of the transcription bubble, the non-transcribed strand could be free to interact with RNA polymerase to cause pausing, a mechanism which is suggested by heteroduplex analysis as well as *in vivo*- and *in vitro*-footprint studies. Pausing of polymerase at +16 is not sufficient to foster Q interaction, since a polymerase can be paused artificially at +16 on DNA templates, mutant at +2 or +6, and there is no Q binding (Yarnell and Roberts, 1992). Thus, either Q itself recognizes these positions in the non-transcribed strand or Q recognizes a form of RNA polymerase that is altered by an interaction with nucleotides in the nontranscribed strand at these positions to facilitate pausing and interaction with Q.

Q is distinguishable from N in another important way; it is an example of an antitermination protein that acts without an array of other proteins (Barik and Das, 1990), requiring only one additional protein, NusA (Grayhack *et al.*, 1985). Moreover, that requirement does not appear to be absolute (Goliger and Roberts, 1989). Thus, Q protein, with a *qut* site, is sufficient to cause antitermination, and NusA only stimulates the effect. Studies with an *E. coli* carrying a *nusA* gene inactivated by a deletion-insertion, discussed in detail below, confirms that Q-mediated antitermination is effective without NusA (C. Zheng, D. L. Court, and D. I. Friedman, unpublished). Importantly, λ grows in this host, while a λ Q^- derivative fails to grow, indicating a requirement for Q action even in the absence of any NusA. In some ways, the Q system resembles the minimal N system, where high concentrations of N are sufficient for antitermination in the absence of Nus factors. The difference, however, is that the Q system is more processive than the minimal N system. Perhaps the basis for this difference resides in two elements that may be specific for Q, the paused RNA polymerase intermediate at the *qut* site, and the involvement of the nontemplate DNA strand.

The inhibitor

One focus of this review has been the complexity of the N antitermination system which is influenced by a large number of host proteins. This stands in distinct contrast to the simpler Q system that is modulated by, perhaps, one additional protein, and the even simpler early antitermination system of HK022 that apparently requires only an RNA sequence. This complexity takes an added turn when we now examine the possibility of an additional factor that inhibits N action. The hypothesis that a factor exists that acts at BOX-A to inhibit N-mediated antitermination (Patterson *et al.*, 1994) emerged from two lines of apparently contradictory evidence. First, it was found that point mutations in *boxA* (*boxA5* or *boxA16*) inactivate the *nut* site (Olson *et al.*, 1984; Robledo *et al.*, 1990). Second, it was found that deletions that included *boxA* appear to have little

effect on antitermination through Rho-dependent and Rho-independent termination signals (Patterson *et al.*, 1994; Peltz *et al.*, 1985; Zuber *et al.*, 1987). Moreover, *nut* sites with those deletions direct antitermination independently of NusB, while still requiring N and the other Nus factors. These paradoxical observations can be explained by postulating that an inhibitory molecule recognizes BOX-A and that NusB competitively blocks the action of the inhibitor in wild-type situations (Fig. 2). Moreover, as a corollary, it was postulated that a *nut* region with the BOXA-5 mutation is a preferred target for the inhibitor, explaining why a deletion of *boxA* is less detrimental to N-mediated antitermination than is the *boxA5* mutation. Although this proposed role for NusB action was based more on deduction than direct evidence, subsequent studies have lent significant credence to the model.

We discuss four results supporting the model and its corollary. First, high level expression of transcripts from a plasmid containing a *nut* region with the *boxA5* mutation suppressed the *nusB5* mutation for growth of λ (Patterson *et al.*, 1994). It was argued that excess *boxA5* transcripts selectively titrate a diffusible inhibitor, lowering its functional levels, and thereby freeing other NUT sites on λ transcripts from inhibitor and reducing the requirement for NusB. Second, the *nusB101* mutation suppresses the N antitermination defects of mutations in the *nusA* and *E* genes in a *boxA*-dependent manner (Court *et al.*, 1995). As described above, in a *nus+* host, the *boxA* region of *nutR* can be deleted without preventing antitermination, but the suppressor activity of *nusB101* is not observed if the *boxA* sequence is deleted. Third, NusB and S10 are required for the *in vitro*-antitermination reaction controlled by the BOX-A sequence of the leader region of the *rrn* operons of *E. coli* (Squires *et al.*, 1993) and bind specifically to the consensus BOX-A RNA sequence from an *rrn* leader region (Nodwell and Greenblatt, 1993). Fourth, DeVito and Das (1994) argue that the dispensability of NusB for persistent *in vitro* antitermination in a purified system is consistent with its role as an antagonist of an inhibitory function; in the *in vitro* reaction where there appears to be no inhibitor, NusB is not needed for effective antitermination.

Role of the α subunit of RNA polymerase

Several transcription factors in *E. coli* (e.g., Crp, Fnr, IHF, and OmpR–EnvZ, as well as the phage P2 Ogr protein) exert their action through contact with the α subunit of RNA polymerase (reviewed in: Ishihama, 1992; Russo and Silhavy, 1992; Busby and Ebright, 1994). These interactions have been identified by mutations in *rpoA*, the gene encoding the α subunit. Different point mutations located in different positions of the carboxy-terminal domain (CTD) of *rpoA* show specificity by blocking the

action of specific transcription factors. This suggests that different activators contact different amino acid patches of the carboxy domain of α . A variant α deleted for the entire carboxy terminus has a general defect for all of the transcription factors tested.

A newly identified *rpoA* mutation, *D305E*, suppresses the effects of *nus* mutations (*nusA1*, *nusB5*, and *nusE71*) on N action (A. Schauer, S.-W. Cheng, L. St. Pierre, D. Alessi, D. Hidayetoglu, D. Court, and D. I. Friedman, submitted). The *rpoAD305E* mutation also changes an amino acid in the carboxy terminus of α . Initially, it was assumed that *rpoAD305E* might enhance an interaction of one of the components of the N–Nus complex with RNA polymerase, in this way compensating for an ineffective interaction of the mutant Nus factors. Surprisingly, a truncated α , missing the CTD, exhibits suppression very similar to that by the *D305E* point mutation. If the mechanism of suppression by the point and deletion mutations are similar, then it is difficult to conceive how this would occur by an enhanced interaction favouring antitermination. Rather, under Nus⁻ conditions, the carboxy terminus of α appears inhibitory for N-mediated antitermination.

How then do the *rpoA* mutations suppress the effect of *nus* mutations? Taking into consideration what we know about the α subunit and the N system, our attention is directed toward two types of sites on the λ genome. The first are the early λ promoters that are located upstream of the *nut* sites. An argument favouring these promoters as the responsible sites can be made on the basis of the *in vivo* studies showing that the wide array of contacts α makes with proteins or DNA that play important roles in stimulating transcription, are at promoters. In fact, Giladi *et al.* (1992) have shown *in vitro* that α plays a regulatory role at the pL promoter. Perhaps an interaction at the early λ promoters mediated through α , altering RNA polymerase *per se* or the rate of initiation, ultimately reduces the effectiveness of N–Nus complex formation at the *nut* site. Accordingly, the α mutations might interfere with the interaction at the promoter and thus facilitate complex formation downstream at the *nut* site. This facilitated formation of N antitermination complexes would allow suppression of mutations that reduce N antitermination.

The second are NUT RNAs, the sites of N–Nus–RNA polymerase complex assembly. In the previous section, we discussed a putative inhibitor of N-mediated antitermination. Perhaps this inhibitor, positioned by BOX-A RNA, also interacts with *rpoA* (Fig. 2B). Deletion of the carboxy terminus of α or deletion of the *boxA* region prevent interaction of the inhibitor with α (Fig 2C). This model provides a unifying explanation of how α and the inhibitor might be involved in N-mediated antitermination. An obvious candidate for the inhibitor is the α CTD itself. This seems unlikely for two reasons. First, the fact that inhibitor activity can be titrated (Patterson *et al.*, 1994) means that the inhibitor

does not act as a component of RNA polymerase. Second, overproduction of α has little effect on λ growth *in vivo* (L. St. Pierre, D. Court and D. Friedman, unpublished).

Recent work suggests that the α subunit may also contact the nascent RNA strand through its carboxy terminus during elongation (Liu and Hanna, 1995). *In vitro* studies, in which a modified nucleotide capable of photocrosslinking was placed at specific positions in the newly transcribed RNA, show that following photoactivation, residues as far as 44 nt from the 3' end of the nascent RNA are crosslinked to the α subunit. Interestingly, when NusA is added to the transcription reaction, NusA becomes cross-linked to RNA and the crosslinking of RNA to α is not observed.

Nus factors

NusA

A factor that plays a role in transcriptional pausing, termination, and antitermination, NusA, is essential for *E. coli*, as shown by the identification of a number of conditional lethal mutations, including amber, ts, and cs (Craven and Friedman, 1991; Nakamura *et al.*, 1986; Nakamura and Uchida, 1983). However, *E. coli* can tolerate the absence of NusA providing it also has low Rho activity (Zheng and Friedman, 1994). This was shown by the construction of an *E. coli* with a 533 bp deletion of *nusA* substituted with a *cat* cassette (Δ *nusA533*) in a strain that also carries *rho* mutations that substantially reduce Rho termination activity.

Results of a number of studies on Rho and NusA action provide an explanation for the viability of this double mutant. NusA protein reduces the elongation rate of RNA polymerase (Yager and Hippel, 1987), coupling transcription and translation (Landick and Turnbough, 1992; Das and Wolska, 1984; Kung *et al.*, 1975). Thus, translation of genes with internal Rho-dependent terminators prevents premature transcription termination by Rho (Ruteshouser and Richardson, 1989). To terminate transcription, Rho must access, unencumbered, RNA some 50–100 nt upstream of the site of termination and translocate to the paused polymerase. Bound ribosomes may either block the binding of Rho or its access to the downstream paused polymerase. Nonsense mutations uncouple translation from transcription, allowing unimpeded access of Rho to the paused polymerase leading to transcription termination (Adhya *et al.*, 1976; Platt and Richardson, 1992; Platt, 1994). It has been proposed (Zheng and Friedman, 1994) that in the absence of NusA, as in the strain carrying Δ *nusA533*, RNA polymerase elongates more rapidly causing a general uncoupling of transcription and translation (Das and Wolska, 1984). This allows access by Rho to the paused polymerase, leading to premature transcription termination throughout the genome. This premature termination is lethal to the cell. However, when Rho

activity is reduced by the compensating *rho* mutation, premature termination is reduced and the cell can survive.

NusB and NusE

The functions NusB and S10 (NusE) appear to play roles in both transcription and translation. These two proteins interact with each other to form a complex (Mason *et al.*, 1992b) that binds to RNA containing the consensus BOX-A sequence (Nodwell and Greenblatt, 1993). Although such an interaction has not been demonstrated for the BOX-A sequence in the λ *nut* region, biological studies indicate that NusB interacts with the λ BOX-A sequence (Friedman *et al.*, 1990; Patterson *et al.*, 1994; Court *et al.*, 1995). It has been proposed that the interaction of NusB with BOX-A can facilitate S10 interaction with RNA polymerase during N antitermination (Patterson *et al.*, 1994) (Fig. 2A). However, it is clear that both *in vivo* and *in vitro* S10 remains essential for N-mediated antitermination reactions when *boxA* is deleted or NusB is not provided and thus can interact in the N-antitermination complex independently of NusB (Patterson *et al.*, 1994; DeVito and Das, 1994) (Fig. 2B).

S10 is undoubtedly a translation factor because it is a protein component of the 30S ribosome subunit (Nomura and Morgan, 1977). Although NusB is not an integral ribosomal protein, it does influence translation, as shown by the observation that *nusB* mutants have slowed translation rates relative to wild type (Taura *et al.*, 1992), and is associated with ribosomes following high-salt wash (Das and Wolska, 1984). NusB null mutants have the interesting property of being cold sensitive for growth (Taura *et al.*, 1992; Georgopoulos *et al.*, 1980). At elevated temperatures, these *nusB* null mutants grow, but, as discussed above, exhibit reduced translation rates and a Nus⁻ phenotype (Taura *et al.*, 1992). The nature of the cold-sensitivity defect is not yet understood (Court *et al.*, 1995). It is interesting to speculate that, as in the case of λ in the absence of NusB, the proposed inhibitory function discussed in an earlier section, may prevent growth at low temperature; i.e. the inhibitor could cause the cold-sensitive phenotype.

NusG and NusD (Rho)

NusG is an essential function in *E. coli* (Sullivan and Gottesman, 1992) and is a component of the transcription-elongation machinery (Li *et al.*, 1992). The gene was first identified by mutations that suppressed the N-antitermination defect caused by the *nusA1* mutation (Sullivan *et al.*, 1992). *In vivo* studies showed that NusG is required for efficient Rho-mediated termination (Sullivan and Gottesman, 1992). Experiments with simple transcription systems confirm the *in vivo* observations that Rho-mediated transcription termination is enhanced by NusG and also

showed that there is a proximal shift toward the promoter in the position of Rho-terminated transcripts (Li *et al.*, 1993; Nehrke *et al.*, 1993).

During transcription elongation by RNA polymerase, the Rho hexamer binds to the free RNA, and NusG binds polymerase. Although each individually binds weakly, together they stabilize one another (Li *et al.*, 1993) and thereby form a more stable and effective termination complex (Nehrke and Platt, 1994). In fact, some Rho-dependent termination sites are only active *in vitro* in the presence of Rho and NusG (Burns and Richardson, 1995).

In vivo studies have not demonstrated a requirement for NusG in N-mediated antitermination (Sullivan and Gottesman, 1992), although purified NusG was subsequently shown to enhance elongation in a pure N-dependent transcription-antitermination assay (Mason *et al.*, 1992a). NusG expressed from a multicopy plasmid can rescue the NusD026 (because of a *rho* mutation) defect on N-antitermination (Sullivan *et al.*, 1992; Das *et al.*, 1983) suggesting that the NusD mutant lacks an efficient interaction with NusG in the N-antitermination complex. Indeed, an affinity-chromatography study showed that at higher temperatures where the wild type binds effectively, the Rho026 protein binds poorly to NusG (Li *et al.*, 1993). We suggest that Rho is an integral component of the antitermination complex (Fig. 2) and that the Rho026 protein inhibits antitermination by compromising an important interaction of Rho and NusG within the complex.

EPILOGUE

Although the new information reviewed here has not revolutionized our thinking about N-mediated transcription antitermination, it has contributed to a more refined understanding. It has focused attention on the β' subunit of polymerase as being a potentially major player in determining the fate of the transcription complex as well as the role an RNA site might play. Perhaps the most unexpected finding is the evidence suggesting a previously undescribed player, a diffusible inhibitor that antagonizes N-mediated antitermination. The inhibitor itself has not been identified, however, the circumstantial evidence from a number of experiments provides strong support for assuming its existence. The anti-sigma factors (Duncan and Losick, 1993; Ohnishi *et al.*, 1992) offer another example where an inhibitor is part of a regulatory scheme. Obviously, proof of this idea awaits the genetic and functional identification of the inhibitor.

What role could such an inhibitory function play? Perhaps it serves with NusB as an important cellular component of the antitermination regulatory scheme, when conditions are either less favourable for the lytic pathway or more favourable for the lysogenic pathway. By interfering with

N-mediated antitermination, expression of genes encoding lytic functions located downstream of the transcription terminators would be decreased.

The characterization of the antitermination mechanism in the early operons of HK022 adds a new twist to any consideration of the role of antitermination early in phage infection. If only the HK022 RNA site is required for antitermination, then it is difficult to understand what advantage is offered to the rest of the lambdoid phages in having a special protein for antitermination. We emphasize our contention that the complex of proteins required for processive transcription antitermination by N plausibly accommodate multiple pathways for regulating λ gene expression at an early step, immediately following the first initiation event. It follows from this argument that the antitermination system in the early operons of phage HK022 is more primitive than that of other lambdoid phages, at least if possible regulatory mechanisms are considered.

We can offer several different, but not mutually exclusive, ways by which the addition of a protein component to an RNA signal for antitermination might provide further levels of regulation: (i) control of the efficiency of translation of any of the antitermination proteins, (ii) control of the rate of the degradation of the protein(s), and (iii) the competition between a protein inhibitor and antitermination factors for an RNA site. All three of these possible modes of regulation, at least under some conditions, appear to be available to influence λ N-mediated antitermination.

The major question waiting to be answered is how the addition of N and Nus factors alters RNA polymerase so that it disregards both Rho-dependent and intrinsic termination signals.

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