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Biochemistry

Transcription termination and late control in phage lambda

(*in vitro* transcription/*Q* gene/phage $\phi 80$)

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ABSTRACT **A transcription termination site occurs between the promoter for late gene expression of bacteriophage lambda and the late genes themselves. It is proposed that the lambda *Q* gene product controls late gene expression by overcoming this termination barrier.**

The messenger RNA of the *Escherichia coli* phage λ is synthesized by the bacterial host enzyme RNA polymerase (EC 2.7.7.6; nucleosidetriphosphate:RNA nucleotidyltransferase) (1, 2). This RNA appears at levels adequate for phage growth only if two controlling proteins are synthesized, those encoded by λ genes *N* and *Q* (3). I have suggested previously (4, 5) that the *N* gene protein functions by overcoming transcription termination barriers, a proposal based partly on the observation that two termination sites that are active *in vitro* in the presence of the transcription termination factor rho are close to sites at which the *N* gene protein is known to function *in vivo*. This hypothesis has received substantial experimental support (6–9).

Here I provide evidence suggesting that the λ *Q* gene protein, the activator of late gene expression, functions by the same mechanism. One *in vitro* transcript of λ DNA produced by RNA polymerase in the presence or absence of rho factor is a small RNA species 200 nucleotides in length, the λ 6S RNA (10). I show that this RNA originates near the site at which λ late gene expression is initiated *in vivo*. Furthermore, I show that DNA of the related phage $\phi 80$ is a template for *in vitro* synthesis of a small RNA which arises from the corresponding region of its genome. The existence of these terminators preceding the late genes suggests that the λ *Q* gene product and the comparable protein from $\phi 80$ function by preventing transcription termination.

MATERIALS AND METHODS

Bacterial and Phage Strains. λ *c*I857 *S7* (designated λ^+ in the figures and text) was obtained from M. Ptashne. The phage $\phi 80i^{\lambda}(Q_{80})$ is named hy5 in ref. 11; it was obtained from E. Signer via W. Gilbert. Phage $\phi 80i^{\lambda}(Q_{\lambda})$ is a recombinant of $\phi 80i^{\lambda}(Q_{80})$ and λ which acquired the *QSR* region of λ and was obtained from P. Chadwick. Each phage was checked for presence of the appropriate site of action of *Q* gene product by a spot test for induction of late functions as described in ref. 13. Heteroduplex analysis of the two phages (kindly performed by J. Wolfson) revealed the expected nonhomology in the rightmost 10% of the molecule which includes the *QSR* region, and complete homology elsewhere; similar heteroduplexes are described in ref. 1. λdbio30-7 *nin*5 is described in refs. 14 and 11. Bacterial strains MSO, 1147, 5061, 1118, 1153, 509, 1142, 1107, and 766 were obtained from E. Signer and are described in ref. 13. All λ phages (except λdbio30-7 *nin*5) carried the *C*I857 repressor; they were grown by heat induction and purified by differential centrifugation and CsCl density centrifugation.

DNA Preparation. Template DNA was prepared by phenol extraction, sometimes in the presence of 0.2% sodium dodecyl sulfate, and was dialyzed into 0.01 M Tris-acetate pH 7.9 (at 4°), 0.001 M EDTA. DNA of λdbio30-7 *nin*5 was a gift of P. Chadwick. Separated strands were prepared as described (15). Bacterial DNA was prepared according to ref. 16.

Enzymes. *E. coli* RNA polymerase, prepared according to the procedure of ref. 17, was a gift of C. Hering. rho-Factor was prepared as described (4).

RNA Synthesis *In Vitro*. The standard reaction contained in 0.10 ml: 0.02 M Tris-acetate pH 7.9 (at 4°); 0.1 mM dithiothreitol; 0.1 mM EDTA; 4.0 mM Mg acetate; 0.10 M KCl; 0.15 mM ATP, GTP, and CTP; 0.05 mM UTP; 1.5–10 μg of RNA polymerase; and 5–20 μg of DNA. One of the nucleotides (usually UTP) was labeled in the α-position with ^{32}P (obtained from New England Nuclear). After an incubation of 20 min at 37°, the reaction was stopped by addition of 200 μl of 2× SSC (0.15 M NaCl, 0.015 M Na citrate) and 200 μl of phenol. *E. coli* tRNA (40–80 μg) was added as carrier, the mixture was shaken briefly on a Vortex mixer and centrifuged to separate the phases, and the aqueous phase was precipitated with 2 volumes of 95% ethanol. The RNA was collected by centrifugation; dissolved in 0.30 ml of 0.01 M Tris-acetate pH 7.9, 0.30 M KCl, 0.01 M Mg acetate, and 0.5% sodium dodecyl sulfate; precipitated again with ethanol; dissolved in 0.30 ml of the above solution; precipitated with ethanol; dried with a stream of nitrogen; and dissolved in gel sample buffer.

Electrophoresis. Electrophoresis through polyacrylamide/agarose tube gels was performed as described in ref. 18, except that the acrylamide concentration was 2.4%. For the preparation of purified 6S RNA used in the experiment described in Fig. 3, a 3.6% polyacrylamide gel containing no agarose was used. Tube gels were cut into 1.1-mm transverse slices and RNA was eluted from the slices into 2× SSC containing 0.5% sodium dodecyl sulfate by gentle shaking overnight at 37°. Eluted RNA was used directly for hybridization. An apparatus modified from that described by Studier (19) was used for electrophoresis in slab gels composed of acrylamide and agarose as described above. These were dried under reduced pressure (19) and subjected to contact autoradiography.

Hybridization. Hybridization to separated strands was performed as described (4), using a saturating concentration of DNA. Hybridization to bacterial DNA was performed

Abbreviation: SSC, 0.15 M NaCl, 0.015 M Na citrate.

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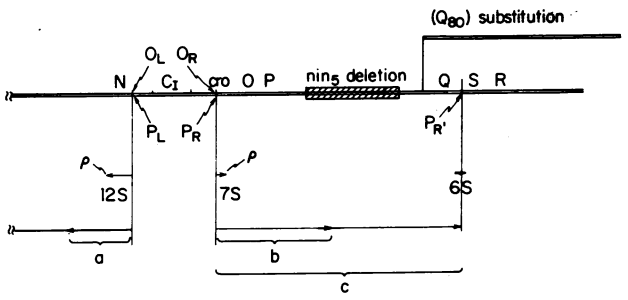

FIG. 1. A partial genetic and physical map of the right portion of the λ chromosome and a model of *in vitro* transcription from λ DNA. The double lines represent phage DNA and the single lines represent RNA molecules synthesized *in vitro* from the corresponding regions of λ DNA. Distances are approximately to scale, according to refs. 11 and 12. RNA species *a*, *b*, and *c* are drawn according to lengths assigned as described in the legend to Fig. 6, and the additional assumption is made that species *c* and the 6S RNA terminate at the same site. The right end of the map corresponds to the physical end of the linear DNA obtained from phage particles; during phage growth, however, the DNA is circular or concatenated, so that the late genes on the left half of the DNA are continuous with genes *S* and *R* and are in the same unit of transcription.

similarly, after heat denaturation of the DNA at 100° for 10 min.

RESULTS

***In Vitro* Transcripts of λ DNA in the Presence and Absence of rho Factor.** Fig. 1 shows a partial genetic and physical map of phage λ which includes the regulatory genes *N* and *Q* and some of the regions that require the *N* and *Q* gene products for their expression. When λ infects a bacterial cell, or when phage repressor is inactivated in a lysogen, RNA synthesis initiates at promoters P_L and P_R yielding, respectively, mRNA for the *N* and *cro* (or *tof*) genes (1, 22). The *N* gene product acts at sites to the left of gene *N* and to the right of gene *cro* (8, 23) to allow the appearance of mRNA and the expression of genes in the regions beyond *N* and *cro*. In the absence of functional *N* product there is little gene expression and much less RNA appears. The mRNA for these regions beyond genes *N* and *cro* arises from extension of RNA molecules initiated at the early promoters P_R and P_L (6). Among the genes in the P_R operon dependent upon the *N* function for their expression is gene *Q*. The *Q* gene protein acts at a site between genes *Q* and *S* to provide expression of all late genes to the right of this site (13) and to allow the appearance of mRNA for these genes (1).

The general pattern of transcription of λ DNA *in vitro* in the presence and absence of rho factor is illustrated in Fig. 2. RNA synthesized in the presence of rho includes a 12S RNA from P_L which may correspond to mRNA for the *N* gene, and a 7S RNA from P_R which may correspond to mRNA for the *cro* gene (4). In the absence of rho factor, RNA polymerase that initiates at P_L and P_R extends into the regions beyond genes *N* and *cro*, producing the larger RNA species that are discussed below.

A Transcript from the λ Late Promoter. A third promoter (named $P_{R'}$) that is active *in vitro* yields a "6S" RNA (10), which is about 200 nucleotides in length. Unlike synthesis of the 7S and 12S species from promoters P_L and P_R, which terminates only in the presence of rho factor *in vitro*, synthesis of the 6S RNA terminates in the absence of rho factor (Fig. 2 and ref. 10). This small RNA arises from the vicinity of the *Q* gene, and its direction of synthesis is rightward, as is that

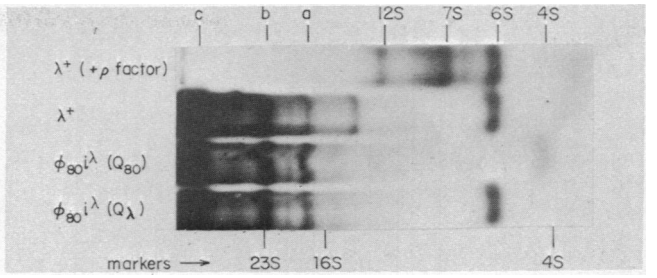

FIG. 2. Gel resolution of *in vitro* transcripts of λ^+, $\phi 80 i^\lambda(Q_{80})$, and $\phi 80 i^\lambda(Q_\lambda)$ DNAs. Synthesis was performed in the standard reaction, except that 0.4 μg of rho factor was added to one of the incubations. The samples were processed and subjected to electrophoresis in a slab gel as described in *Materials and Methods*. ^{14}C-Labeled *E. coli* stable RNA was electrophoresed in adjacent slots of the slab to provide molecular weight markers. The 4S "oop" RNA (20) is synthesized from all three templates under these conditions, but it is present in much smaller amounts than the λ 6S and φ80 4S RNAs and is not visible here. The "oop" RNA, containing 81 nucleotides, nearly coelectrophoreses with the φ80 4S RNA, so that the latter is also approximately 80 nucleotides in length.

of the late messenger RNA (20). It has been suggested that promoter $P_{R'}$, which yields the 6S RNA *in vitro*, serves as the promoter of late transcription *in vivo* (20). Here I provide support for this suggestion by demonstrating that the 6S RNA arises from the genetically defined site of initiation of late gene expression.

Herskowitz and Signer (13) found that a site required for λ late gene expression is located between the *Q* gene itself and gene *S*, the first gene of what appears to be a single operon comprising the entire late region. Deletions that remove gene *Q* and portions of the prophage genome to the left of it do not prevent expression of prophage late genes when *Q* protein is provided by a superinfecting phage. However, all deletions that extend from the left through gene *Q* and into gene *S* or further to the right prevent expression of any late genes in the prophage; thus, a site between genes *Q* and *S* is required for *Q* protein-dependent expression of late genes. There is independent evidence that initiation of transcription of the late genes occurs in this region (2). I have examined the extent of hybridization of purified radioactive 6S RNA (synthesized *in vitro*) to DNA from the set of prophage deletions used by Herskowitz and Signer: Fig. 3 reveals that 6S RNA anneals only to DNA containing the late site. I infer that promoter $P_{R'}$ is located between genes *Q* and *S* and that it is probably the site at which late gene transcription is initiated *in vivo*.

A Possible Transcript from the φ80 Late Promoter. Bacteriophage φ80 has a gene that corresponds in function to the λ *Q* gene, and its product acts at a region in the φ80 genome equivalent to the *QSR* region of λ (25–27). Each gene product recognizes a nucleotide sequence specific to its own genome, since neither can elicit late gene expression from the other phage. Among numerous recombinant phages that are hybrids of λ and φ80, there exist phages in which the *QSR* region of one is combined with the remaining late genes of the other; these phages are viable probably because they possess both the *Q* (or φ80 *Q*-like) gene and its compatible site of action. Here I utilize two λ/φ80 hybrid phages that differ only in the *QSR* region to demonstrate that a small RNA distinct from the λ 6S RNA originates from the "*QSR*" region of φ80. Fig. 2 presents a gel analysis of *in vitro* transcripts of DNA from these two phages. DNA of $\phi 80 i^\lambda(Q_\lambda)$ is a template for synthesis of the 6S RNA (and

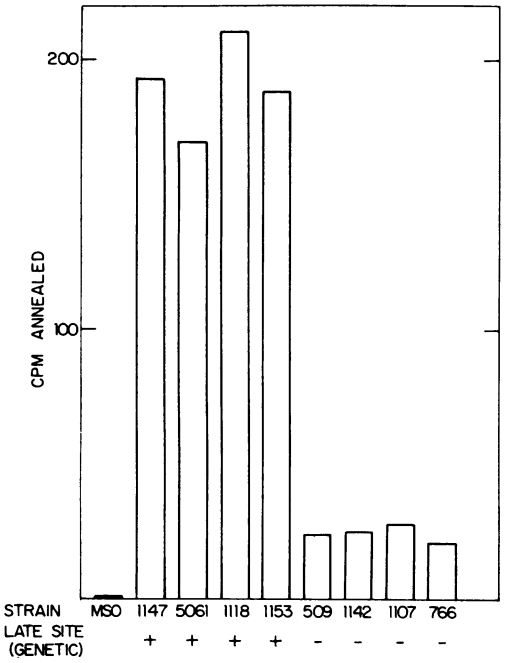

FIG. 3. Annealing of purified λ 6S RNA to bacterial DNA carrying λ prophage deletions. The eight numbered strains constitute an ordered set of deletions with endpoints in gene *Q* (1147 and 5061), either in gene *Q* or between genes *Q* and *S* (1118 and 1153), in gene *S* (509), in gene *S* or gene *R* or between them (1142), and beyond the beginning of gene *R* (1107 and 766) (ref. 13). Strain MSO has no prophage genes. 6S RNA was synthesized *in vitro* at a specific activity of 2.5 μCi/nmol of nucleotide and purified as described in *Materials and Methods;* 1370 cpm of 6S RNA (estimated to be 85% pure) was annealed to 40 μg of each DNA, an amount which provides a concentration approaching saturation. Hybridization to 20 μg of DNA produced a similar result with values approximately 25% lower. The nine DNA preparations were equivalent in their ability to anneal radioactive *E. coli* ribosomal RNA.

produces a pattern of synthesis essentially identical to that from λ^+ DNA), whereas DNA of $\phi 80 i^{\lambda}(Q_{80})$ yields no 6S RNA. Instead, the latter DNA is template for synthesis of an RNA about 80 nucleotides in length (named the $\phi 80$ 4S RNA) which is not synthesized from the template with the λ Q gene. (The $\phi 80$ 4S RNA is distinct from the λ 4S "oop" RNA; see the legend to Fig. 2.)

The data presented in Fig. 4 demonstrate that the direction of synthesis of the $\phi 80$ 4S RNA is the same as that of phage late gene expression *in vivo*. RNA transcribed *in vitro* from $\phi 80 i^{\lambda}(Q_{\lambda})$ and $\phi 80 i^{\lambda}(Q_{80})$ DNA was resolved by electrophoresis on tube gels and the eluted 6S and 4S RNA peaks were annealed to *r*-strand DNA of both phages. Figure 4c reveals that each RNA anneals to the *r* strand of its template DNA, which implies that each is synthesized toward the right on the genetic map. Neither RNA anneals to *r*-strand DNA of the other phage. Thus the absence of each RNA in transcripts of the other DNA is not due merely to an accidental difference in promoter function *in vitro*, but must represent a difference in nucleotide sequences present in the DNA.

In addition to the 6S and 4S RNAs from λ^+ and $\phi 80$ DNA, a comparable but distinct small RNA is synthesized *in vitro* from DNA of the *Salmonella* phage P22, which also has a late control function analogous to the λ *Q* gene product (unpublished experiments with S. Hilliker and D. Botstein). The association of a promoter closely followed by a terminator with the site of initiation of late gene expression in three

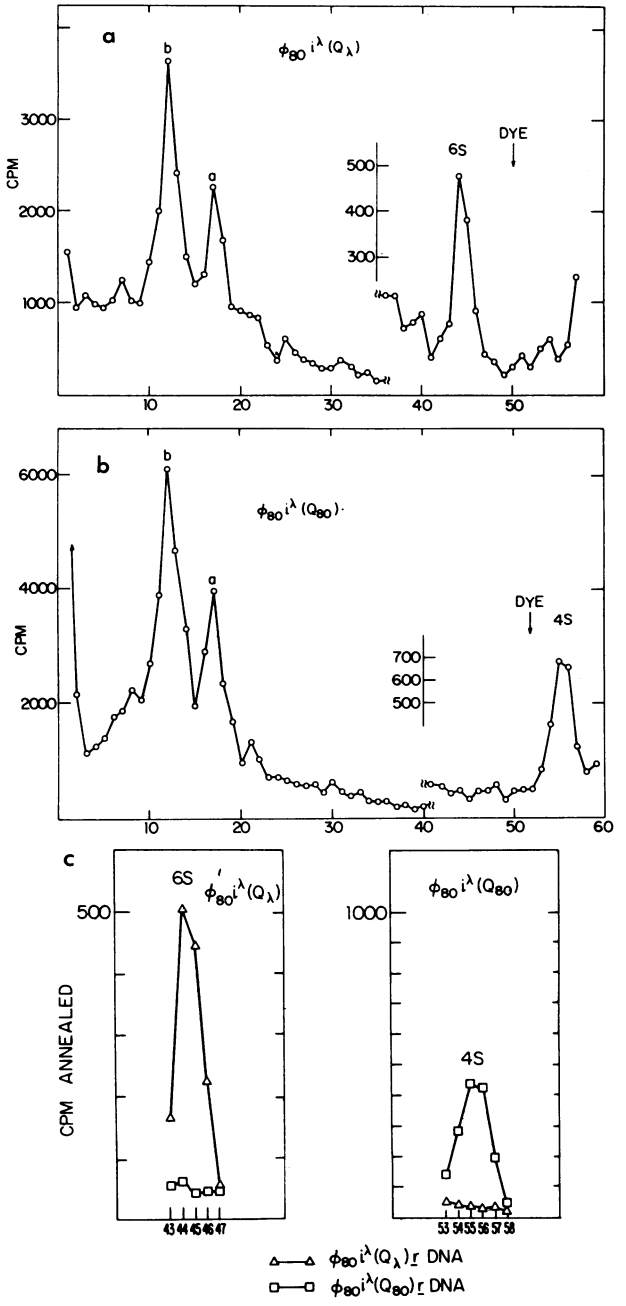

FIG. 4. Origin of $\phi 80$ 4S RNA and λ 6S RNA. RNA was synthesized in a standard reaction, except that the four nucleotides were present at 0.10 mM. After gel electrophoresis followed by elution of RNA from each gel slice, radioactivity of aliquots was determined directly or after annealing to the indicated separated strands of DNA. (panel a) Total RNA synthesized from $\phi 80 i^{\lambda}(Q_{\lambda})$ DNA; (panel b) total RNA synthesized from $\phi 80 i^{\lambda}(Q_{80})$ DNA; (panel c) 6S RNA from $\phi 80 i^{\lambda}(Q_{\lambda})$ template and 4S RNA from $\phi 80 i^{\lambda}(Q_{80})$ template annealed to *r*-strand DNA of $\phi 80 i^{\lambda}(Q_{\lambda})$ (△) and $\phi 80 i^{\lambda}(Q_{80})$ (□).

lambdoid phages suggests that this configuration is involved in the mechanism of late gene control.

Longer Transcripts Synthesized in the Absence of rho Factor. At least three RNA species larger than 16S ribosomal RNA are synthesized from λ^+ or $\phi 80 i^{\lambda}(Q_{\lambda})$ DNA by purified RNA polymerase in the absence of rho factor. The species *a*, *b*, and *c* are discernible in the gel analysis of Fig. 2 and are well resolved in the gel presented in Fig. 5. I estimate their molecular weights to be respectively 6×10^5, 1.0

$\times 10^6$, and 2.3×10^6. Species *a* anneals to the *l* strand and species *b* and *c* anneal to the *r* strand of λ^+ or $\phi 80 i^\lambda(Q_\lambda)$ DNA (data not shown).

Species *b* and *c* probably both originate from the early promoter P_R and constitute RNA that would be terminated in the presence of rho factor to produce the 7S RNA. These two molecules could arise from a single promoter if the first termination site were only partially efficient. The assignment of both RNAs to P_R is supported by their absence in synthesis from DNA of the phage λdbio30-7 *nin*5, which is deleted for approximately 5.4% of the λ genome in the region between P_R and the $\lambda/\phi 80$ crossover (see Fig. 1). RNA synthesized *in vitro* from DNA of this phage includes neither species *b* nor species *c*, but contains instead an RNA of molecular weight 1.7×10^6. This RNA anneals to *r*-strand DNA (data not shown), as do species *b* and *c*. A reasonable interpretation of this change is that the termination site that produces species *b* is deleted, so that all RNA proceeds to the termination site that normally yields species *c*; the difference in molecular weight between *c* and the 1.7×10^6 MW transcript from λdbio30-7 *nin*5 is that expected from the length of the deletion (within experimental error). It is consistent with the assignment of both species to P_R that *c* is synthesized from both λ and $\phi 80 i^\lambda(Q_\lambda)$ DNA. It is further consistent that species *b* is synthesized from both $\phi 80 i^\lambda(Q_\lambda)$ and $\phi 80 i^\lambda(Q_{80})$ DNA, whereas the latter DNA does not yield species *c*. Instead, DNA of $\phi 80 i^\lambda(Q_{80})$ produces the somewhat longer RNA named *c'* in Fig. 5. Since a transcript of molecular weight 2.3×10^6 initiated at P_R would terminate in the region of nonhomology of these two DNAs, this difference is reasonable. The termination sites that produce *c* and *c'* might be those that produce the 6S RNA from promoter $P_{R'}$ and the 4S RNA from the comparable promoter of the $\phi 80$ hybrid. None of these data exclude the possibility that *b* and *c* are initiated at a promoter other than P_R in the region between P_R and the $\lambda/\phi 80$ crossover before gene *Q*, but it is most likely that these major transcripts originate from the known dominant rightward promoter.

The *l*-strand specific RNA species *a* is synthesized from DNA of λ and both $\lambda/\phi 80$ hybrids, but it does not appear in synthesis from λdbio30-7 *nin*5 DNA. Since the latter phage does not contain P_L or the region to the left of P_L (11, 14), but does contain all other λ DNA (except the 5.4% deletion) that is common to the other three phages, RNA species *a* almost certainly arises from P_L. It is possible that the termination site that produces species *a* is only partially efficient *in vitro*, since some *l*-strand specific RNA of higher molecular weight is synthesized (data not shown).

DISCUSSION

There is evidence that at least some of these terminators that are active *in vitro* also function *in vivo*. RNA species comparable in size and site of origin to those synthesized from P_R and P_L in the presence of rho factor have been detected in induced cells, although there is no evidence from nucleotide sequence analysis that they terminate at identical sites (22, 23). Two *r*-strand specific transcripts that could correspond to the *in vitro* species *b* and *c* have been observed in induced cells (22). A separate indication that transcription termination by purified RNA polymerase *in vitro* can be authentic is the occurrence of a small *l*-strand specific λ RNA (the "oop" RNA) both *in vivo* and in the transcript by purified RNA polymerase (26).

Some transcription passes all of these terminators *in vivo*, however. RNA synthesis initiated at early promoter P_R in the presence of the *N* gene protein proceeds through the terminators that produce the 7S RNA and RNA species *b* and *c*, ultimately extending into the late genes (27). If $P_{R'}$ is the promoter for late gene expression, the terminator that produces the 6S RNA must be passed during synthesis of the bulk of late mRNA. Messenger RNA synthesis initiated at P_L in the presence of *N* gene protein proceeds well beyond the terminator responsible for species *a*. The terminators that yield the 12S, 7S, and 6S RNAs and species *b* show a striking correlation with the four known sites of action of the regulatory proteins *N* and *Q*. Since the accumulation of mRNA *in vivo* requires these regulators, a reasonable hypothesis is that both regulators function by overcoming the barriers imposed by the termination sites.

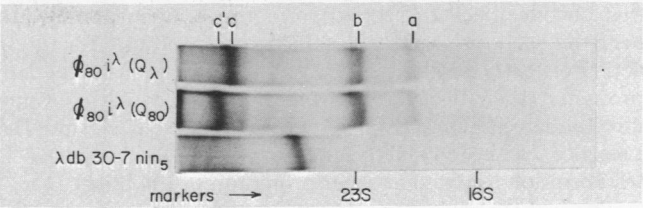

FIG. 5. Gel resolution of high molecular weight, *in vitro* transcripts of $\phi 80 i^\lambda(Q_\lambda)$, $\phi 80 i^\lambda(Q_{80})$, and λdbio30-7 *nin*5 DNAs. The procedures are described in the legend to Fig. 2, except that the duration of electrophoresis was approximately doubled. The molecular weight assignments of species *a* and *b* given in the text were made by comparison with the mobilities of 23S and 16S ribosomal RNA (21); species *c* comigrates with an RNA synthesized *in vitro* from phage T7 DNA known to have a molecular weight of 2.3×10^6 (18), and it is assumed also to have this molecular weight.

The proposal that the λ *N* protein prevents transcription termination is strongly supported by the discovery by Richardson *et al.* (9) that rho factor is altered in a bacterial strain containing the polarity suppressor mutation *SuA*. Adhya *et al.* (7), Franklin (8), and Segawa and Imamoto (28) have shown that the *N* function relieves nonsense (and other) polarity in regions expressed under its direction, and they have argued that such polarity is due to transcription termination. Since this polarity is also relieved by loss of the rho transcription termination function, it is most likely that the *N* protein operates in both polarity relief and stimulation of λ gene expression by preventing termination.

I present evidence here that λ promoter $P_{R'}$, which is closely followed by a (rho-independent) terminator, is located at the genetically defined site required for *Q*-protein dependent expression of λ late genes. Since this is also close to the site at which initiation of mRNA synthesis for the late genes occurs (2), it seems very likely that $P_{R'}$ is the late promoter. If this is true, the *Q* protein is not required for initiation of late transcription by RNA polymerase; it may function instead by preventing termination at the site that follows the late promoter. This model also applies to the *Q*-like system of $\phi 80$ (and *Salmonella* phage P22), which appears to have a comparable set of promoter and terminator associated with its system of late control. The possibility that the λ *Q* gene product affects transcription termination has been considered by others (20).

The proposal that the *N* and *Q* gene products operate independently in the same genome by the same basic mechanism is consistent with the fact that the *N* gene protein affects transcription only from specific promoters. Thus phages λ and λi^{21} possess *N* (and *N*-like) functions of different specificity, each of which allows continued gene expression only for transcription from its own promoters (3). The sites

that encode specific recognition of the *N* gene protein are near the early promoters (8, 29). If the specificity for interaction of the *Q* product is located (by analogy) near the late promoter, then transcription initiated at $P_{R'}$ will continue into the late region only if *Q* protein is present, despite the presence in the cell of *N* protein which similarly affects transcription initiated at other promoters of the phage.

The fact that only some terminators appear to require the rho factor *in vitro* must be reconciled with this model. Either there exist two fundamentally different mechanisms of termination, both of which are countered by the regulatory proteins, or both types of termination in fact require rho factor *in vivo*. In the latter possibility, the rho-independent terminators might be sites at which the RNA polymerase falters and frequently stops *in vitro* but which it would pass under the conditions of *in vivo* synthesis if the rho factor did not act. Rosenberg *et al.* (30) have found that the "rho-independent" terminator which produces the λ "oop" RNA is only partially efficient *in vitro*, and that its efficiency is considerably increased by rho factor; this finding directly supports the possibility that rho is also involved in termination at the sites that function in its absence *in vitro*. The rho-independent terminator responsible for species *b*, which is only partially efficient *in vitro*, might be much more efficient *in vivo* in the presence of rho factor. This terminator, which is removed by the *nin*5 deletion, could thus be the site between genes *P* and *Q* at which the *N* gene protein must act to allow expression of gene *Q* (27). The ability of the *nin*5 phage to grow in the absence of *N* function is then explained by the absence of this termination barrier and the known leakiness of the terminator that yields the 7S RNA (27).

Bertrand *et al.* (31) have found in the *E. coli* tryptophan operon a structure strikingly similar to that proposed here for the λ late messenger RNA: preceding the first gene there is a transcription terminator at which the frequency of termination appears to be controlled by the conditions of cell growth. In a further case, a set of small transcripts synthesized by RNA polymerase *in vitro* from the *l* strand of DNA of λ, P22, and probably φ80 may be involved in control of phage repressor synthesis by the c_{II} and c_{III} gene products (in λ) in a manner analogous to that suggested here for the λ *Q* gene protein (unpublished experiments with S. Hilliker and D. Botstein). It appears that antagonism of transcription termination by specific protein effectors may be a common mechanism of positive control.

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