JOURNAL OF BACTERIOLOGY, Oct. 1986, p. 1–5
0021-9193/86/100001-05$02.00/0
Copyright © 1986, American Society for Microbiology
Vol. 168, No. 1

MINIREVIEW

Antitermination Mechanisms in rRNA Operons of *Escherichia coli*

EDWARD A. MORGAN
Department of Experimental Biology, Roswell Park Memorial Institute, Buffalo, New York 14263

In this review I assess the available information about antitermination mechanisms in rRNA operons (*rrn* operons) of *Escherichia coli*. The reader is referred to reviews on *rrn* operon structure (18) and lambda antitermination (6) for references supporting statements on these topics.

Why *rrn* operons have antitermination mechanisms. The idea that *rrn* operons might have antitermination mechanisms evolved from studies of transcriptional polarity in operons that code for translated mRNAs. Transcriptional polarity is known to be caused by premature transcription termination resulting from the uncoupling of transcription and translation when translation is interrupted by premature nonsense codons or by other mechanisms (1). The essence of coupling is that ribosomes translating an mRNA concurrent with mRNA synthesis act, in a way not yet clear, to prevent transcription termination at termination sites within protein-coding regions. Termination at sites in protein-coding regions has been found to be largely or solely Rho dependent in all cases examined. Presumably, Rho-dependent termination signals are frequent in sequences that have not specifically evolved as termination signals.

The phenomenon of polarity demonstrates that coupling is required for efficient end-to-end transcription of mRNA. However, 6,000-base-pair *rrn* operons are transcribed end-to-end without detectable transcription termination despite the fact that coupling during transcription of *rrn* operons is unlikely because *rrn* transcripts are not translated. These facts led to early experiments to demonstrate that antitermination mechanisms in *rrn* operons compensate for the inability of coupling to reduce premature termination (17).

***rrn* operon structure.** The promoter-leader regions of the seven *E. coli rrn* operons each consists of two highly conserved tandem promoters (p_1 and p_2) separated by 109 to 119 base pairs, followed by a highly conserved, transcribed but untranslated leader region that is 171 to 173 base pairs in length from the start of p_2 transcripts to the start of the 16S rRNA gene. Between the 16S and 23S genes is a region of 350 to 450 base pairs (the spacer region) containing precursor-specific sequences and one or two tRNA genes. The 23S rRNA genes are followed by 5S rRNA genes and, in some cases, one or two tRNA genes. As discussed below, both the leader and spacer regions are suspected of being involved with antitermination.

Evidence for *rrn* antitermination. Early evidence for *rrn* antitermination was obtained when insertions of Tn*9*, Tn*10*, and IS*1* were observed to cause far less transcriptional polarity in *rrn* operons than in protein-coding operons (4, 17, 26). These experiments indicated that transcription termination was reduced in *rrn* operons. It was suggested that modifications of RNA polymerase to reduce termination could be responsible for the low polarity of these insertions, but it was also pointed out that the frequent transcription of *rrn* operons, structural properties of *rrn* transcripts, or association of rRNA with ribosomal proteins could also cause reduced termination within *rrn* operons.

To determine whether modifications of RNA polymerase are responsible for reduced termination in *rrn* operons, *rrn* promoter-leader regions and other types of promoters were fused to protein-coding genes preceded by termination signals (in the order promoter-*rrn* leader region-termination signal-reporter gene). The termination signals used in early experiments were insertions of Tn*9*, IS*1* (11, 12), and a noncoding segment of 16S rRNA (2, 14). Termination caused by each of these insertions has a large Rho-dependent component. Enzyme measurements were then used to demonstrate that these insertions cause less efficient termination when transcription is from the intact *rrnC* promoter-leader region (p_1p_2-leader region) or the *rrnG*p_2-leader region than from the *ara*, *lac*, or *tac* promoters. For example, it was shown that insertions of IS*1* causing greater than 90% termination when transcription was from the *ara* or *lac* promoters caused less than 25% termination when transcription was from the *rrnC* promoter-leader region (11, 12) and that the 16S ribosomal-DNA segment caused a greater than 95% reduction in enzyme synthesis when transcription was from the *lac* or *tac* promoters, but only a 40 to 50% reduction when transcription was from the *rrnG*p_2-leader region (2, 14). Recent studies with tandem termination regions suggest that not all RNA polymerase molecules become resistant to termination when transcribing through *rrn* leader regions but that RNA polymerase molecules which do become resistant terminate very inefficiently and can retain their resistance to termination while reading through more than one termination region (B. Albrechtsen and C. Squires, personal communication).

To localize further the regions responsible for antitermination, the *rrnC* and *rrnG* promoter and leader regions were separated and tested for antitermination activity by using operon fusion assay systems similar to those described above. Transcription through the *rrnC* and *rrnG* leader regions from the *lac* or *tac* promoters caused RNA polymerase to become as resistant to termination as when transcription was from intact *rrn* promoter-leader regions (12, 14). Promoter-proximal 67-base-pair regions of the *rrnC* and *rrnG* leader regions cause antitermination equal to antitermination caused by the intact leader region (14; W. Holben and E. Morgan, unpublished data). It has also been shown that translation or inversion of the *rrnC* and *rrnG* leader regions abolishes antitermination (14; Holben and Morgan, unpublished data). Other evidence for *rrn* antitermination has more recently been obtained with different terminators, and measurements of termination have been made by using intact *rrn* operons or different operon fusion systems (see below). These findings are in good agreement with the conclusion that *rrn* leader regions specify antitermination.

While it is clear that *rrn* leader regions specify a physiologically important component of *rrn* antitermination, operon fusion assay methods have also demonstrated that transcription from the *rrnC*p_1p_2 promoter region in the absence of the leader region can, for certain termination regions, be terminated less efficiently than transcription from the *lac* promoter (12). This observation is similar to the finding that the termination efficiency of a Rho-independent terminator also significantly varied when different nonribosomal promoters were compared by using an operon fusion assay system (13). It is not yet clear whether termination efficiency is dependent on the promoters examined or whether these observations reflect problems inherent in the use of operon fusions (see below).

Terminator specificity. The experiments described above show that *rrn* antitermination mechanisms reduce termination at the poorly characterized Rho-dependent terminators caused by insertions of Tn*9*, IS*1*, and a 16S ribosomal-DNA segment. These poorly characterized terminators were used in these preliminary experiments because the insertions were thought to cause Rho-dependent termination of the type causing polarity in protein-coding operons and because *rrn* antitermination mechanisms were thought to be designed to reduce termination at precisely these types of terminators.

There are recent studies examining termination at well-characterized Rho-dependent terminators by measuring enzyme synthesis from a reporter gene located downstream of these terminators. By these methods the *trp t'* Rho-dependent terminator was shown to be 95% efficient when transcription is from the *rrnG*p_2 promoter, but only 65% efficient when transcription is from the p_2-leader region (C. Squires, personal communication). Similarly, antitermination conferred by the *rrnB* leader region was shown to reduce termination from 75 to 25% at the lambda t_{R1} Rho-dependent terminator (H. de Boer, personal communication). Other preliminary observations have also shown that *rrnA* antitermination functions reduce termination at the lambda t_{R1} and t_{R3} Rho-dependent terminators (M. Cashel and M. Gottesman, personal communications).

The effect of *rrn* antitermination mechanisms on termination at Rho-independent terminators has also been examined by measuring enzyme synthesis from reporter genes located downstream of terminators. The *rrnB* antitermination functions reduce the efficiency of termination at the lipoprotein terminator from 91 to 51% (H. de Boer, personal communication). The *rrnG* leader region antitermination functions reduce the efficiency of termination at the *trp t* terminator from 80 to 25%, but have no effect on termination at the *rpoC* terminator, which is 98% efficient (C. Squires, personal communication). Surprisingly, the *rrnB* antitermination functions also reduce the efficiency of termination at the intact *rrnB*t_1t_2 terminator region from 91 to 63% (H. de Boer, personal communication). Other preliminary evidence indicates that the *rrnA* antitermination functions reduce termination at the *rrnB*t_1 terminator and at the Rho-independent terminator in IS*2* (M. Cashel, personal communication).

Although recent evidence described above indicates that *rrn* terminators terminate about as efficiently as other known Rho-independent terminators and respond to *rrn* antitermination similarly to other Rho-independent terminators, *rrn* terminators have been extensively studied because it is possible that *rrn* terminators might have special properties that make them unusually efficient or immune to *rrn* antitermination mechanisms. The DNA sequences of five different *rrn* terminators (15, 18) have been determined, and the in vivo and in vitro efficiencies of several terminators have been measured (9, 21, 22, 28, 30; H. de Boer, personal communication; C. Sigmund and E. Morgan, unpublished data). The sequence properties and efficiencies of these terminators are similar to the sequence properties and efficiencies of other known terminators (15, 18–20; see above). Although certain primary sequence and secondary structural features are shared by some, but not all, *rrn* terminators (15, 18) and these features may reflect special termination properties, these features may also simply be fortuitous similarities or be nonfunctional sequences that have a common evolutionary origin. In summary, the available evidence does not clearly demonstrate that *rrn* terminators are unusually efficient or immune to *rrn* antitermination mechanisms.

It has been determined that 80 to 90% of RNA polymerase molecules read through the *rrnD* terminator in vivo when RNA polymerase is modified by the lambda *N* antitermination protein in the lambda *nutL* antitermination region (A. J. Podhajska and W. Szybalski, personal communication). Termination at the *rrnD* terminator is also reduced 50% by lambda *N*-dependent modifications of RNA polymerase in an in vitro antitermination system, a result quantitatively similar to that obtained with strong nonribosomal Rho-independent terminators (8; A. Das, personal communication). These results indicate that *rrn* antitermination mechanisms may not reduce termination at *rrn* terminators as efficiently as the antitermination mechanism mediated by the lambda *N* antitermination protein. It is therefore possible that *rrn* and lambda antitermination mechanisms differ significantly in design and antitermination capability.

DNA sequences specifying *rrn* antitermination. The *rrn* leader regions contain sequences with proposed homology to the two lambda regions, called *nutR* and *nutL*, that specify lambda *N* protein-mediated antitermination. The most convincing of these homologies is a 14-base-pair region called BoxA. The homology of lambda BoxA sequences to an invariant region present in all *rrn* leader regions is shown in Fig. 1. Very significantly, a convincing homology to the consensus BoxA region is also present in an invariant sequence of *rrn* spacer regions just before the start of the 23S rRNA gene (Fig. 1). The BoxA sequences of spacer regions are the only spacer region sequences obviously homologous to *rrn* leader regions or lambda *nutR* and *nutL* regions. The presence of BoxA sequences in both leader and spacer regions suggests that similar events occur during the transcription of these two regions. Interestingly, the BoxA RNA transcripts from both the leader and spacer regions immediately precede regions that participate in very stable, long-range base-pairing interactions important to the processing of the rRNA precursor by RNase III and other RNases. BoxA sequences may in fact partially participate in the base-pairing interactions in these structures (3, 31). These observations raise the possibility that RNA secondary structure may be related to BoxA function. Notably, DNA sequence conservation in the seven *rrn* leader regions and five sequenced spacer regions is very stringent in the BoxA regions and in the sequences participating in the long-range base-pairing involved in the RNase cleavage of rRNA precursors, but the sequence conservation of other regions is less striking. It is noteworthy that a BoxA sequence is also present in the leader region of the *E. coli* tryptophanase (*tna*) operon (Fig. 1) and is important to the function of an antitermination mechanism that regulates this operon via tryptophan-dependent readthrough of a Rho-dependent terminator (27).

A mutation affecting BoxA in a region of lambda that specifies *N* protein-mediated antitermination (*nutR*) reduces

BoxA Sequences

lambda nutR	CCGCTCTTACACATTC
lambda nutL	ACGCTCTTAAAAATTA
φ21 nutR	TTGCTCTTTAACAGTT
φ21 nutL	AGGCTCTTTAACATCG
P22 nutR	ACGCTCTTTACCAATC
P22 nutL	ACGCTCTTTAACTTCG
tna leader	CCGCCCTTGATTTGCC
rrnA-F, H leaders	CTGCTCTTTAACAATT
rrnB,C,D,E,H spacers	TTGCTCTTTAAAAATC
CONSENSUS	N (t/c) GCtCTTtaaca (aT/tC) N position 1 … 14

		MUTATION	POSITION
rrnA leader	1.	T T	5,11
	2.	A (also G to A at -18)	-18,2
rrnB leader	3.	GG ACC	5,6,8,9,10
rrnC leader	4.	TA	2,3
lambda nutR	5.	T	2
tna leader	6.	G	9
	7-9.	G (or T, or A)	1
	10-12.	T (or A, or C)	2

FIG. 1. Summary of BoxA-like sequences and BoxA mutations with probable functional significance. φ21 and P22 are *E. coli* phages related to lambda. The sequence data in this figure have been compiled from published sequences (5, 6, 12, 18, 25, 27) and unpublished data (M. Cashel for the *rrnA* mutations; H. de Boer for the *rrnB* mutation; A. Borden and E. Morgan for the *rrnC* spacer sequence and *rrnC* mutation).

antitermination, whereas mutations affecting BoxA of the *tna* operon cause constitutive antitermination (Fig. 1). The reason for the opposite effects of BoxA mutations in these two systems is not yet clear. The effect on antitermination of several mutations in *rrn* leader Box A sequences (Fig. 1) has been analyzed by using operon fusion systems (M. Gottesman, M. Cashel, and H. de Boer, personal communications; unpublished data). These studies indicate that BoxA mutations decrease enzyme synthesis from downstream of both Rho-dependent and Rho-independent terminators and therefore adversely affect antitermination.

In another series of experiments, the lambda p_L promoter was fused to a promoterless but otherwise intact *rrnB* operon on a multicopy plasmid, and a series of deletion derivatives that affected portions of the *rrn* leader were made. The rates of synthesis of 5S rRNA and a tRNA encoded in the *rrn* spacer region were measured for each construction. Deletion of DNA upstream of the BoxA sequence had no effect on synthesis of these two small RNAs, whereas a deletion also extending into BoxA reduced synthesis of the RNAs (10). These results are also consistent with a BoxA requirement for antitermination.

Other experiments also suggest that BoxA is essential, and perhaps sufficient, for *rrn* antitermination. In a system where galactokinase is synthesized from a gene downstream of the lambda t_{R1} terminator, synthetic DNA containing BoxA was as effective in increasing galactokinase synthesis as was the intact *rrn* leader region (H. de Boer, personal communication). However, the interpretation of these experiments is complicated by the presence in the t_{R1} region of a prototype BoxA sequence and other sequences that participate in lambda antitermination. It is not clear from these experiments whether the lambda sequences themselves confer some degree of antitermination or whether lambda sequences can substitute for some deleted *rrn* sequences.

Although some experiments indicate that BoxA may be the only sequence required for *rrn* antitermination, the possibility that other sequences are also involved is raised by the properties of a 14-base-pair deletion beginning 26 base pairs downstream of the 14-base-pair BoxA sequence shown in Fig. 1. This deletion strongly reduces *rrnA* leader-mediated antitermination (M. Cashel, personal communication).

Proteins involved in *rrn* antitermination. Because the leader and spacer regions of *rrn* operons are partially homologous to sequences that participate in antitermination mediated by lambda *N* protein (*nutR* and *nutL*), host proteins involved in lambda antitermination might also function in *rrn* antitermination. Because of our poor understanding of the role of host proteins in lambda antitermination, no firm predictions of which host proteins are involved in *rrn* antitermination are possible based on sequence comparisons alone. However, mutations in host proteins that abolish lambda antitermination have been obtained, and their effects on *rrn* antitermination have been determined. The mutations examined were *nusA1*, *nusA*(Cs), *nusB5*, *nusC60* (RNA polymerase), and *nusE71* (a ribosomal protein). Of these, *nusA1*, *nusC60*, and *nusE71* were reported in one study to have no effect on the transcription of *rrn* operons (23). The use of operon fusions and hybridization of pulse-labeled RNA to probes specific for several regions of *rrn* operons indicated that the *nusB5* and *nusA*(Cs) mutations caused

premature termination of about half of the RNA polymerase molecules transcribing *rrn* operons and that some of the premature termination was probably within rRNA structural genes (24). In another study which used operon fusions and measurements of termination at the lambda t_{R1} terminator, the *nusA1* and *nusB5* mutations decreased antitermination caused by the *rrnA* promoter-leader region (M. Cashel, personal communication). In another approach, a high rate of transcription of the lambda *nutL* region was caused, with the hope that host proteins involved with lambda antitermination were depleted, thereby affecting other host functions dependent on these proteins (24). High-level transcription of the lambda *nutL* region caused about half of the RNA polymerase molecules to prematurely terminate within *rrn* leader regions as determined by hybridization of RNA to DNA probes specific for various regions of *rrn* operons (24). That efficient premature termination occurred in the leader region but not elsewhere is suggestive of a strong terminator in *rrn* leader regions.

An important reservation that must be kept in mind when considering studies with *nus* mutants is that available mutations eliminate lambda antitermination but may not abolish all functions of the proteins. Therefore, the mutations may not reduce the function of these proteins in rRNA synthesis. Mutations affecting *nus* proteins may also affect *rrn* operons indirectly rather than directly. Another difficulty in interpreting these experiments results from the observation that the *nusA1* and *nusB5* mutations affect premature termination in operons coding for proteins (29), raising the possibility that *nus* mutations may indirectly or directly affect termination in operons that do not have antitermination mechanisms.

In an unrelated approach, transcription complexes formed in crude extracts by using plasmid templates containing the p_L-*nutL* or p_L-*nutL-rrn* leader regions were isolated, and the proteins specifically bound to the transcription complexes were identified (J. Greenblatt, personal communication). These experiments suggested that the *rrn* leader region facilitates the association of NusB protein with the transcription complex, either directly or in association with other host proteins that recognize *rrn* leader regions.

In vitro experiments have also demonstrated that NusA protein by itself can cause efficient antitermination. Reduction of termination at t_{R1} at high NusA concentrations in vitro is quantitatively similar to the reduction seen when RNA polymerase first transcribes *rrn* leaders in vivo (C. Sigmund and E. Morgan, unpublished data). However, the effect of NusA on antitermination was not dependent on the presence of *rrn* leaders, suggesting that this effect of NusA in vitro is not completely representative of events in vivo.

Notably, a mutation in a novel *E. coli* gene has also been obtained, and it has been claimed that this mutation causes premature transcription termination in *rrn* operons but not other operons (16).

Other possible roles for antitermination. Since the best characterized antitermination mechanisms may exist primarily to regulate gene expression by conditionally affecting termination at a terminator preceding the regulated genes (6, 7, 27), it is possible that *rrn* antitermination serves a regulatory role by regulating termination at as yet uncharacterized terminators. The question of whether *rrn* leader and spacer regions modulate the transcription rate by regulating termination or by other mechanisms has not yet been carefully addressed.

Assay method ambiguities. Because *rrn* antitermination mechanisms in many cases cause only about twofold changes in measured gene expression or about a 50% reduction of termination, the sometimes ambiguous assay methods and experimental designs used for antitermination measurements may not always be sufficiently reliable or accurate for the task. For example, when operon fusions are used and enzyme measurements from different types of fusion operons are compared, relative enzyme measurements are unlikely to reflect exact relative mRNA synthesis rates when the mRNAs have large differences in structure. Existing studies on *rrn* antitermination have usually compared enzyme synthesis from mRNAs with different structures, as antitermination is measured by measuring the changes in enzyme synthesis that result from the introduction of large transcribed regions of DNA. Well-designed studies that use measurements of mRNA synthesis are not subject to this source of error, but the appropriate mRNA measurements have seldom been done in studies of *rrn* antitermination.

Another possible source of error is the effect of changes in transcript structure on terminator efficiency, an effect independent of antitermination that can be observed in vitro (C. Sigmund and E. Morgan, unpublished data). Most assay methods used also fail to distinguish convincingly whether measured changes in enzyme or RNA levels result from antitermination, inadvertent inactivation or introduction of terminators, or changes in the copy number of plasmids carrying operon fusions.

Many ambiguities inherent in the assay methods used to study *rrn* antitermination apply equally well to the methods commonly used to measure terminator efficiency. Therefore, an indirect way to determine the seriousness of these ambiguities is to determine the consequences of using different types of assay systems to measure the efficiency of a single terminator. An informal review (by the author) of published and unpublished data reveals that measurements of the efficiency of a single terminator, made by using different types of operon fusions (that do not incorporate known antitermination mechanisms), frequently yield disparate values for terminator efficiency. The measured values for terminator efficiency in fact frequently differ by an amount that approximates the effect that *rrn* antitermination has been reported to have on the same terminator or on similar terminators. It is therefore possible that some important conclusions about *rrn* antitermination will require revision when experiments are designed to minimize all sources of ambiguity. However, despite the possible problems with individual experiments, the proposal that *rrn* operons possess an antitermination mechanism has been substantiated by so many experimental approaches that it is almost certainly correct.

How *rrn* antitermination may work. It seems likely that *rrn* antitermination results from modification of RNA polymerase as it transcribes sequences in *rrn* leader regions. The modification of RNA polymerase probably endures as RNA polymerase transcribes *rrn* structural genes. Although studies of *rrn* spacer regions have not yet been completed, it seems likely from DNA sequence elements present in *rrn* spacer regions that *rrn* spacer regions reinstate modification of RNA polymerase molecules that have inadvertently become unmodified while transcribing 16S rRNA genes, or that these spacer regions modify RNA polymerase molecules that have by chance completely transcribed through 16S rRNA genes without being modified in *rrn* leader regions. It is likely that the modification of RNA polymerase efficiently suppresses premature termination at (probably) weak terminators in *rrn* structural genes, ensuring the equimolar synthesis of rRNA species, thereby facilitating the equimolar synthesis of ribosomal subunits and consequently increasing

cell fitness. The modification of RNA polymerase probably persists until RNA polymerase terminates at *rrn* terminators, which have evolved to be strong terminators and are inefficiently suppressed by *rrn* antitermination. Even though termination at *rrn* terminators is partially suppressed by *rrn* antitermination, excessive transcription of downstream genes is prevented. Based on this view, *rrn* antitermination, like the mechanism that couples transcription to translation in operons that code for proteins, exists to prevent unwanted premature termination within structural genes of a transcription unit. The existence of an antitermination mechanism therefore leaves the structural genes free to evolve in such a way as to maximize the function of the gene product, without the added constraint of minimizing premature termination.

Detailed but highly speculative models of how sequences specifying *rrn* antitermination result in the modification of RNA polymerase are easily constructed, but are premature at the present time. *rrn* antitermination is probably mediated by (at least) the BoxA sequences in *rrn* leader and spacer regions and by proteins including, but not limited to, NusA and NusB. More detailed speculations about how lambda *N* protein-mediated antitermination works can also be extended to *rrn* antitermination (see, for example, reference 14). Based on the known similarities and differences in DNA sequence and protein requirements of *rrn* and lambda antitermination, the measured differences in the antitermination efficiency of *rrn* and lambda antitermination, and the probable differences in functional roles of the two antitermination systems, it is the current prediliction of this reviewer that lambda *N* protein-mediated antitermination results from lambda *N* protein enhancement of an antitermination-competent transcription complex similar to that which forms in *rrn* leader and spacer regions.

ACKNOWLEDGMENTS

I thank my colleagues who offered their advice, criticisms, and unpublished observations to improve this review. I especially thank M. Cashel, C. Squires, and H. de Boer.

LITERATURE CITED

1. **Adhya, S., and M. Gottesman.** 1978. Control of transcription termination. Annu. Rev. Biochem. **47**:967–996.
2. **Aksoy, S., C. L. Squires, and C. Squires.** 1984. Evidence for antitermination in *Escherichia coli* rRNA transcription. J. Bacteriol. **159**:260–264.
3. **Bram, R. J., R. A. Young, and J. A. Steitz.** 1980. The ribonuclease III site flanking 23S sequences in the 30S ribosomal precursor RNA of *E. coli*. Cell **19**:393–401.
4. **Brewster, J. M., and E. A. Morgan.** 1981. Tn*9* and IS*1* inserts in a ribosomal ribonucleic acid operon of *Escherichia coli* are incompletely polar. J. Bacteriol. **148**:897–903.
5. **Franklin, N. C.** 1984. Conservation of genome form but not sequence in the transcription antitermination determinants of bacteriophages λ, ϕ21, and P22. J. Mol. Biol. **181**:75–84.
6. **Friedman, D. I., E. R. Olson, C. Georgopoulos, K. Tilly, I. Herskowitz, and F. Banuett.** 1984. Interactions of bacteriophage and host macromolecules in the growth of bacteriophage lambda. Microbiol. Rev. **4**:299–325.
7. **Gaffney, D., R. Skurray, and N. Willetts.** 1983. Regulation of the F conjugation genes studied by hybridization and *tra-lacZ* fusion. J. Mol. Biol. **168**:103–122.
8. **Ghosh, B., and A. Das.** 1984. *nusB*: a protein factor necessary for transcription antitermination *in vitro* by phage λ *N* gene product. Proc. Natl. Acad. Sci. USA **81**:6305–6309.
9. **Glaser, G., P. Sarmientos, and M. Cashel.** 1983. Functional interrelationships between two tandem *E. coli* ribosomal RNA promoters. Nature (London) **302**:74–76.
10. **Gourse, R. L., H. A. de Boer, and M. Nomura.** 1986. DNA determinants of rRNA synthesis in *E. coli*: growth rate regulation, feedback inhibition, upstream activation, and antitermination. Cell **44**:197–205.
11. **Holben, W. E., and E. A. Morgan.** 1984. Antitermination of transcription from an *Escherichia coli* ribosomal RNA promoter. Proc. Natl. Acad. Sci. USA **81**:6789–6793.
12. **Holben, W. E., S. M. Prasad, and E. A. Morgan.** 1985. Antitermination by both the promoter and the leader regions of an *Escherichia coli* ribosomal RNA operon. Proc. Natl. Acad. Sci. USA **82**:5073–5077.
13. **Howe, K. M., A. J. Newman, I. Garner, A. Wallis, and R. S. Hayward.** 1982. Effect of rifampicin in expression of *lacZ* fused to promoters or terminators of the *E. coli rpoBC* operon. Nucleic Acids Res. 10:7425–7438.
14. **Li, S. C., C. L. Squires, and C. Squires.** 1984. Antitermination of *E. coli* rRNA transcription is caused by a control region segment containing lambda *nut*-like sequences. Cell **38**:851–860.
15. **Liebke, H., and G. Hatfull.** 1985. The sequence of the distal end of the *E. coli* ribosomal RNA *rrnE* operon indicates conserved features are shared by *rrn* operons. Nucleic Acids Res. **13**:5515–5525.
16. **Liebke, H., and J. Speyer.** 1983. A new gene in *E. coli* RNA synthesis. Mol. Gen. Genet. **189**:314–320.
17. **Morgan, E. A.** 1980. Insertions of Tn*10* into an *E. coli* ribosomal RNA operon are incompletely polar. Cell **21**:257–265.
18. **Morgan, E. A.** 1982. Ribosomal RNA genes in *Escherichia coli*, p. 1–29. *In* H. Busch and L. Rothblum, (ed.), The cell nucleus. rDNA, part A, vol. 10. Academic Press, Inc., New York.
19. **Postle, K., and R. F. Good.** 1985. A bidirectional rho-independent transcription terminator between the *E. coli tonB* gene and an opposing gene. Cell **41**:577–585.
20. **Rosenberg, M., and D. Court.** 1979. Regulatory sequences involved in the promotion and termination of RNA transcription. Annu. Rev. Genet. **13**:319–354.
21. **Sarmientos, P., J. E. Sylvester, C. Contente, and M. Cashel.** 1983. Differential stringent control of the tandem *E. coli* ribosomal RNA promoters from the *rrnA* operon expressed *in vivo* in multicopy plasmids. Cell **32**:1337–1346.
22. **Sekiya, T., M. Mori, N. Takahashi, and S. Nishimura.** 1980. Sequence of the distal $tRNA_1^{asp}$ gene and the transcription termination signal in the *Escherichia coli* ribosomal RNA operon *rrnF* (or *G*). Nucleic Acids Res. **8**:3809–3827.
23. **Sharrock, R. A., R. L. Gourse, and M. Nomura.** 1985. Defective antitermination of rRNA transcription and derepression of rRNA and tRNA synthesis in the *nusB5* mutant of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **82**:5275–5279.
24. **Sharrock, R. A., R. L. Gourse, and M. Nomura.** 1985. Inhibitory effect of high-level transcription of the bacteriophage λ *nutL* region on transcription of rRNA in *Escherichia coli*. J. Bacteriol. **163**:704–708.
25. **Shen, W.-F., C. Squires, and C. L. Squires.** 1982. Nucleotide sequence of the *rrnG* ribosomal RNA promoter region of *E. coli*. Nucleic Acids Res. **10**:3303–3313.
26. **Siehnel, R. J., and E. A. Morgan.** 1983. Efficient read-through of Tn*9* and IS*1* by RNA polymerase molecules that initiate at rRNA promoters. J. Bacteriol. **153**:672–684.
27. **Stewart, V., and C. Yanofsky.** 1985. Evidence for transcription antitermination control of tryptophanase operon expression in *Escherichia coli* K-12. J. Bacteriol. **164**:731–740.
28. **Szeberenyi, J., and D. Apirion.** 1983. Initiation, processing, and termination of ribosomal RNA from a hybrid 5S ribosomal RNA gene in a plasmid. J. Mol. Biol. **168**:525–561.
29. **Ward, D. F., and M. E. Gottesman.** 1981. The *nus* mutations affect transcription termination in *Escherichia coli*. Nature (London) **292**:212–215.
30. **Young, R. A.** 1979. Transcription termination in the *E. coli* ribosomal RNA operon *rrnC*. J. Biol. Chem. **254**:12725–12731.
31. **Young, R. A., R. Macklis, and J. A. Steitz.** 1979. Sequence of the 16S–23S spacer region in two ribosomal RNA operons of *Escherichia coli*. J. Biol. Chem. **254**:3264–3271.