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Transcription Termination

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ABSTRACT: Chromosomes are organized into units of expression that are bounded by sites where transcription of DNA sequences into RNA is initiated and terminated. To allow for efficient stepwise assembly of complete transcripts, the transcribing enzyme (RNA polymerase) makes a stable complex with the DNA template until it reaches the terminator. Three general mechanisms of transcription termination have been recognized: one is by a spontaneous dissociation of the RNA at a sequence segment where RNA polymerase does not maintain its usual stable interaction with the nascent chain; another involves the action of a protein (rho factor in bacteria) on the nascent RNA to mediate its dissociation; and a third involves an action triggered by a protein that binds to the DNA at a sequence that is just downstream of the termination stop point. Transcription termination is important in the regulation of gene expression both by modulating the relative levels of various genes within a single unit of expression and by controlling continuation of transcription in response to a metabolic or regulatory signal.

KEY WORDS: RNA polymerase, intrinsic terminators, termination factors, attenuation, antitermination, elongation factors.

I. INTRODUCTION

The primary step of gene expression is the transcription of a segment of a DNA molecule to form discreet RNA molecules. Specific signals on the DNA direct the transcribing enzyme, an RNA polymerase, to initiate transcription at one end of the segment while other signals direct the enzyme to terminate transcription at the other end. These two signals encompass a unit of expression that can include one or more genes. Between the start and stop points, RNA polymerase catalyzes the sequential addition of nucleotides at an average rate of 43/s using the bases in one strand of DNA as a template to direct the selection of the specific nucleotide added. Transcription elongation is a stable process that allows RNA molecules as large as 100,000 nucleotides to be synthesized without dissociation of the nascent RNA. It is the stability of this elongation process that necessitates the involvement of special signals for termination at the end of a gene segment to prevent expression of adjacent distinct genetic units. The aim of this article is to present our current understanding of the mechanisms for transcription termination, including how the terminators are recognized and the ways in which the termination process can be regulated.

II. ELONGATION: THE PROCESS TERMINATED

Efficient transcription of a DNA segment depends on the stability of the ternary elongation complex consisting of the nascent RNA, RNA polymerase, and template DNA. When a transcript is released from this complex, it is unable to reattach in a way that allows continued transcriptional elongation. RNA release is, therefore,

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the commitment step of termination. Because premature release of incomplete transcripts would waste resources and could, by directing the synthesis of incomplete proteins, give rise to interfering products, the elongation complex must be stable throughout a gene sequence. Indeed, the experimental evidence indicates that transcriptional elongation is highly processive.[1,2] Most RNA polymerases allow the synthesis of RNA molecules with 1000 to 2000 nucleotides and certain transcripts synthesized by RNA polymerase II in eukaryotic cells have lengths in the 100,000-nucleotide range.[3] Because all chromosomes contain many genes, however, it is equally important that transcription be terminated efficiently at the end of a gene. Thus, terminators cause drastic alterations of the interactions that are responsible for stable elongation. RNA polymerases can exist in a state for elongation and can switch to an alternative state to allow termination. As pointed out by von Hippel and Yager,[4] the two states compete with each other: after addition of each nucleotide, there is a certain kinetic rate for addition of another nucleotide, $k_{forward}$, and a kinetic rate for termination, $k_{release}$. During elongation, $k_{forward} \gg k_{release}$, whereas at a termination site, $k_{release} > k_{forward}$. Thus, to understand termination, it is necessary to review the properties of the elongation complex.

A. Structure of Elongation Complex

A minimal elongation complex with *Escherichia coli* RNA polymerase consists of the core enzyme (subunits β, β', and 2α) bound to DNA and the nascent RNA.[5,6] There is no evidence for any covalent bonds formed among those three component molecules. The high stability is the consequence of a cooperative sum of a large number of weak bonds. Although binary complexes between DNA and RNA polymerase are held together primarily by ionic bonds, as indicated by their sensitivity to the concentration of counterions, the ternary elongation complex is stabilized further by a significant number of nonionic bonds, as they remain stable even in 0.5 *M* kCl.[2]

During elongation, the contact surface of the enzyme extends approximately 22 bp along the DNA, about 9-bp ahead and 13-bp behind the template residue in the nucleotide addition site, and to a point approximately 12 nucleotides from the 3′ end residue of the nascent transcript.[7] There is evidence, however, that these contacts are not the same at each step of elongation; molecular probing shows that the extent of contact varies for complexes arrested at different points, suggesting that there may be periodic or episodic expansion and contraction of contact domains along the DNA and RNA during elongation.[8]

The DNA double helix within the contact domain of the transcriptional elongation complex is unwound by about 1½ turns.[9] Presumably, the strands unwind to allow one of them to serve as a template for the binding of nucleoside triphosphates in the nucleotide addition site of RNA polymerase. Based on the analogy with DNA polymerase action, it has long been assumed that the 3′ end of the nascent RNA chain is base-paired with the template over the terminal 8 to 12 nucleotides of the transcript. Some recent experiments, however, have challenged the notion that the pairing extends beyond 2 or 3 nucleotides.[10] These experiments show that with certain isolated "stalled" complexes (stalled in the process of elongation by the absence of a NTP to pair with a specific template residue), the nascent RNA is susceptible to cleavage by various ribonucleases to within 2 or 3 nt of the 3′ end. Because these RNases do not normally cleave RNA paired with DNA under normal solution conditions, this result implies that only the last 2 or 3 residues are paired directly with the DNA and not up to 12 nt, as has been proposed. On the other hand, results from another set of experiments using a very different probe — in this case the reactivity of residues in the template strand with $KMnO_4$ — are more readily interpreted in terms of a hybrid helix extending for as many as 12 bp.[11] Neither experiment, however, establishes with certainty either of the two extreme views,2 vs. 12 bp of hybrid helix. There may be enough dynamic "breathing" of the hybrid helix in the stalled complexes to allow access by the nucleases, and the bases in the template strand could be protected from $KMnO_4$ reactivity by contacts of the DNA with protein within the isolated elongation complex. To resolve this issue definitively, it will be necessary to devise some other methods for probing the nature of the

association of RNA with DNA in the elongation complex. Whatever the true extent of pairing between the 3′ end of the nascent RNA and the DNA template strand, the enzyme has a functional group that displaces the RNA from the DNA so that the pairing never extends beyond a limit.[12] The addition of each nucleotide to the 3′ end of the transcript leads, on the average, to the displacement of 1 base paired to the DNA. The simplest mechanism to imagine is one in which the displacement proceeds uniformly 1 nucleotide at a time. The actual mechanism could involve displacements in blocks, however, with no nucleotides being displaced at some steps and several at others.[13] In this case, the extent of pairing between the nascent RNA and the DNA template would vary within the range of 3 to 12 nucleotides. Again, whatever the mechanism, the maximum extent of pairing is a fixed feature of the RNA polymerase. This is inferred from the observation that the extent of pairing is immune to torsional forces in the DNA that would favor increasing the extent of pairing with the nascent RNA.[12]

Because DNA has a helical secondary structure, there is a rotation about its axis during transcriptional elongation that is partitioned between the DNA itself and the RNA polymerase.[14] The way it is partitioned depends on the extent to which either of the components is constrained. Rotation of RNA polymerase will be constrained by the frictional drag of the nascent RNA, particularly when ribosomes are attached, whereas rotation of the DNA will be constrained by its attachment to fixed components, by the presence of other components attached to or acting on it, or by the extent of torsional strain in the DNA. Under conditions in which the RNA polymerase rotation is constrained (by the presence of ribosomes attached to the nascent RNA, for example) the DNA will rotate through the enzyme and transcription will tend to produce positive supercoils in the DNA ahead of the advancing RNA polymerase and negative supercoils behind it. In this case, DNA topoisomerases may be needed to remove excessive torsional stress in the DNA that could arise from RNA polymerase movement in opposite directions along the DNA or if the DNA is anchored at various points.[15] Because the direction of transcription does often change from one gene to another on a chromosome, the DNA topoisomerases play an important role in the orderly expression of genes.

B. Energetics of Elongation

Pure *E. coli* RNA polymerase can elongate chains as rapidly *in vitro* as in cells.[16] Thus, there is no requirement for an extra engine, such as a translocase driven by NTP hydrolysis, to facilitate its motion along the template. Chemical energy is available at each step from the formation of the internucleotide phosphoester bond at the expense of the phosphoanhydride bond in the NTP. Although the amount of energy that is released by this reaction is not known precisely, a good estimate can be made from the difference in the $\Delta G^{o\prime}$ of hydrolyses of the two kinds of bonds: ($NTP + H_2O \rightarrow NMP + PP_i$) vs. ($(N)_n$-$pN + H_2O \rightarrow (N)_n + NMP$). This is about 3-kcal/mol more negative for the first reaction, the hydrolysis of the phosphoanhydride bond.[17] Thus, that much energy is presumably available to move the enzyme from 1 template nucleotide to the next with the addition of a residue and for displacement of an RNA nucleotide base-paired with the DNA at each step. Although the DNA strands are separated within the RNA polymerases, the extent of unwinding remains the same, on average, as the enzyme moves along the template: One DNA-DNA base pair is formed for each base pair that is broken. Hence, the stepwise change should be isoenergetic on the average and the breaking of the DNA-DNA base pairs is not dependent on the energy released. For this reason, too, there is no need for a helicase driven by NTP hydrolysis to separate the double-stranded DNA as the enzyme moves along the template.

C. Variations in the Rate of Elongation: Pausing

Based on the time it takes for *E. coli* RNA polymerase to synthesize complete gene or operon-length products, the average rate for transcriptional elongation ($k_{forward\text{-}ave.}$) is about 43 $nt \cdot s^{-1}$. Similar values have been obtained for the synthesis of a polycistronic mRNA (the *trp*

operon mRNA)[18] and rRNA *in vivo*[19] or T7 early gene RNA *in vitro*.[16] This means that the average step time for elongation — the amount of time needed to add a residue — is 23 ms. Studies of the transcription process *in vitro*, however, indicate that the elongation process is uneven, and the value for $k_{forward}$ can vary enormously[20–22] — over 1000-fold from one step to another.[23,24]

Places where $k_{forward}$ is much lower than average constitute a pause site. Although considerable effort has been made to determine what features of a DNA sequence influence the rate of $k_{forward}$ and, particularly, what constitutes a pause site, we are still far from understanding the process. Sequences in the vicinity of the pause point clearly affect $k_{forward}$: those sequences can extend at least 14 bp ahead of and 25 bp behind the nucleotide residue where the pause occurs.[23,25,26] In some instances, the pause site is at a point where a stable stem structure could form near the 3′ end of the nascent transcript RNA. In those cases there is good evidence that the pausing is related to the formation of the stem structure, as mutational changes that decrease the predicted stability of the stem also decrease the extent of pausing.[23,27] How the formation of a double-stranded stem structure could affect elongation is not understood. One possibility is that the formation of a double-stranded structure near or within the point where RNA exits RNA polymerase may impede passage of the RNA through the exit site, thus blocking translocation. Another possibility is that formation of the stem somehow pulls the 3′ end away from the nucleotide addition step, thus blocking addition of the next nucleotide.

In some cases, the extent of the pausing is dependent on the relative concentration of one or more of the NTP substrates.[26] At such sites the sequence context of the DNA and the nascent RNA in contact with the enzyme could be influencing the affinities of substrates for the nucleotide addition site.

One of the clearest examples of where downstream sequences influence pausing has been found in some sites where RNA polymerase II from calf thymus pause. In this case, the downstream sequence includes two short runs of phased dA:dT tracts of the type that form bends in DNA.[28] Thus, this kind of a pausing could be the result of the impeding action of a discontinuity in the secondary structure of the DNA templates. This may be one out of several ways in which downstream DNA sequences can influence elongation, however, as the downstream sequences that affect pausing by *E. coli* RNA polymerase do not fit this pattern.[25] So far, no obvious pattern has emerged to explain how downstream sequences can affect pausing by *E. coli* RNA polymerase.

D. Elongation Factors

1. NusA

The rate of transcriptional elongation by core RNA polymerase can be modulated by other protein factors. In *E. coli*, the best characterized elongation factor is the product of the *nusA* gene, a protein with M_r of about 55,000.[29] It acts to prolong pausing at certain natural pause sites[30–33] and has a general inhibitory effect on chain elongation that can be overcome by high levels of nucleoside triphosphates.[34] Pure NusA protein binds reversibly to core RNA polymerase, either as a free enzyme or as part of a ternary transcription complex, but does not bind to holoenzyme.[35,36] Thus, it replaces sigma factor during elongation and could occupy the same binding site as sigma on the core enzyme.

NusA has two distinct effects on elongation: it increases the K_s for the nucleoside triphosphate substrates for RNA polymerase and it accentuates pausing at certain sites.[34] The first of these effects is general and leads to a retardation of chain growth when the concentration of NTPs is low. The mechanism of the alteration is not known; one likely possibility is that the binding of NusA to core RNA polymerase allosterically modifies the structure of the nucleotide addition site. The second effect is specific for certain pause sites. The best characterized examples are at pause sites where the RNA has the potential to form a stable stem loop with its 3′ end 8 to 10 nt from the 3′ end of the nascent chain (i.e., at hairpin-loop pauses).[23,27,33,37] In these cases, NusA could be exerting an allosteric influence on core RNA polymerase that stabilizes its interaction with an RNA stem structure in the exit site. Alternatively, NusA protein could be interacting directly with the RNA to stabilize the stem structure and its

overall interaction with the transcription complex. The attempts that have been made to detect a primary interaction of NusA with the nascent RNA in a complex that is paused at a specific site have given negative results.[38] However, enhancement of pausing at a site in λtR_1 (a rho-dependent terminator) with NusA was blocked by DNA oligonucleotides that bind to the single-stranded region on the 5′ side of the putative pause-inducing stem.[39] Thus, recognition of that part of a stem may be important in NusA action.

NusA has an essential function in *E. coli*.[40] As is described in subsequent sections, it is needed for the function of certain terminators and greatly enhances the efficiency of others. Before its identification as the product of the *nusA* gene,[41] the protein was isolated as a factor, called "L", that enhanced the yield of synthesis of large proteins in a partially fractionated, coupled transcription-translation system.[42]

One likely function of NusA is to retard continued elongation of RNA polymerase at critical control points in an operon to keep translation of the mRNA closely coupled to transcription. Many genes contain latent rho-dependent terminators that are quite effective when the transcripts are not being translated but are functionally masked by normal translation.[43] By causing RNA polymerase to pause at a point between the start point of translation and the first intragenic, rho-dependent terminator, NusA would allow the ribosome to bind and initiate translation before the RNA polymerase passes into the termination region with an unprotected nascent RNA.

The use of NusA to couple transcription with translation also occurs in the functioning of attenuators for amino acid biosynthetic operons. NusA enhances pausing at a site between the translation initiation codon and the transcriptional terminator in the *trp* and *his* operon leaders.[23,27] Because the functioning of these attenuators depends on the coupling of transcription with translation of the leader mRNA section, NusA is likely serving a critical role as that coupling factor. This role is also consistent with the finding that translation of the nascent leader transcript *in vitro* relieves the transcriptional pause.[44]

NusA also plays an essential role, along with some other host proteins, in mediating antitermination by the product of bacteriophage λ gene *N*.[45] Its name derives from this function, as a mutation in *nusA* was isolated that makes the host unable to use λ gene *N* to activate the expression of the λ delayed early genes. NusA, other host proteins, and λ N proteins combine together to form a *cis*-acting antitermination complex. Further details about this antitermination mechanism are considered in a later section.

2. TFIIS

Factors that affect elongation by eukaryotic RNA polymerases have also been identified. The best characterized of these is TFIIS, a protein that prevents RNA polymerase II from pausing excessively at certain very strong pause sites.[46–49] This protein, like NusA, binds to its cognate RNA polymerase. In primary sequence and function, however, it does not appear to be related to NusA. TFIIS has been isolated from a number of different organisms including several mammalian species,[47,48] *Drosophila*,[50] and a yeast.[51] The SII polypeptide has M_r of 31,000 to 34,000.

Besides acting to prevent pausing when it is present from the start of a transcription reaction, TFIIS will also cause RNA polymerase molecules that have become stalled to resume elongation.[52,52a,52b] In the process of reactivating the polymerase in a stalled complex, TFIIS effects the removal of nucleotides at the 3′ end of the transcript by a hydrolytic reaction that is inhibited by α-amanitin, a general inhibitor of RNA polymerase II function. This process appears to move the polymerase backward out of a stuck or jammed configuration and thus allows elongation to resume.

The cDNA molecules that encode TFIIS have been isolated from a number of organisms.[53–56] The predicted amino acid sequence for mouse TFIIS is similar to that of the yeast *DSTI* gene.[57] *DSTI* encodes DNA-strand transfer protein-α, which has activities *in vitro* that are similar to those catalyzed by *E. coli* RecA protein. This similarity suggests that perhaps TFIIS might act to facilitate a reverse motion by RNA polymerase by causing the part of the nascent RNA that was just displaced from the DNA template to be transferred back to pairing with the template strand.

If the extent of unwinding of the DNA is constant, the very 3′ end would then become accessible to a nucleolytic activity within the polymerase, an activity that is similar to the 3′ to 5′ proofreading nucleases of DNA polymerases. Pausing at certain sites is apparently a stochastic process that occurs with a certain fraction of the RNA polymerases that reach the site. In some cases, the enzyme molecules that stall become locked in a configuration that does not allow continued nucleotide additions. Thus, to get out of this jam, TFIIS causes the enzyme to move back on the template. The latent ribonuclease then cleaves the displaced 3′ end, allowing the newly generated 3′ end to come into register with the template at the nucleotide addition site. Although there may be a certain probability that the enzyme will get stuck again at the same site, TFIIS could allow the polymerase to back up and try again. Eventually, most molecules will be able to proceed past that site. Figure 1 shows a diagram of this model for TFIIS action.

Fractionation of HeLa extracts has separated two other components that have effects on elongation by RNA polymerase II. One, TFIIF is also an initiation factor.[58–60] It consists of two polypeptide components with M_r of 30 and 78 kDa that bind tightly to RNA polymerase II. Because of that property, this factor is also known as RAP30/74 (RNA polymerase-associated proteins).[61] As an elongation factor, TFIIF stimulates the overall rate of elongation, and unlike TFIIS, it does not specifically affect the extent of pausing.

The other component from fractionated extracts that affects elongation is TFIIX. It has not been purified to homogeneity so its composition is unknown. Like TFIIF, it stimulates elongation but is functionally distinct, as it can further stimulate elongation of complexes that are saturated with TFIIF.[58,59]

III. INTRINSIC TERMINATORS

In spite of the high stability of the transcription complex during most steps of elongation, there are some points where the stability drops sufficiently for the transcript to dissociate spontaneously. These are the transcriptional stop points of an intrinsic terminator. Spontaneous termination at defined, functional points is a characteristic of many, but perhaps not all, RNA polymerases. Certainly, *E. coli* RNA polymerase,[17] RNA polymerase III from eukaryotic cells,[62] and the bacteriophage T7 RNA polymerase[63] respond to such sites. There is no evidence, however, that RNA polymerase I of eukaryotic cells terminates transcription spontaneously. Although RNA polymerase II has been shown to terminate with moderate efficiency at certain sequences,[64,65] a role for these intrinsic terminators as part of a natural (biologically functional) terminator has not been demonstrated.

A. Sequences of Intrinsic Terminators

The best characterized intrinsic terminators are the ones recognized by *E. coli* RNA polymerase. A large number have been identified and an analysis of their sequences have revealed some distinct motifs. These are characterized by about 20 bp of a G+C-rich sequence with an interrupted dyad symmetry preceding (in the direction of transcription) a sequence of about 8 bp with a run of dA residues on the template strand.[66–68] A typical example is the terminator for the *E. coli trp* operon attenuator (*T trpA*) shown in Figure 2a as a double-stranded DNA sequence. There is considerable evidence that these sequence motifs are important parts of the terminator. However, other features of the sequence within 30 bp of the transcription stop point that are not evident as an obvious consensus sequence are also important. The sequence following the run of T:A base pairs is in this category. Changes in the sequence 3- to 5-bp downstream from the stop point for the T7 early gene termination (T7Te) can cause the efficiency of the terminator to drop from 65% to 10%.[69]

A terminator is operationally defined as a point where the rate of release of an RNA transcript is greater than the rate of addition of the next nucleotide, $k_{forward}$.[4] The sequences at an intrinsic site must have features that can allow a great increase in $k_{release}$ and/or a great decrease in $k_{forward}$, when compared with "average" sequences. At least three aspects of the terminator sequences have features that are likely to con-

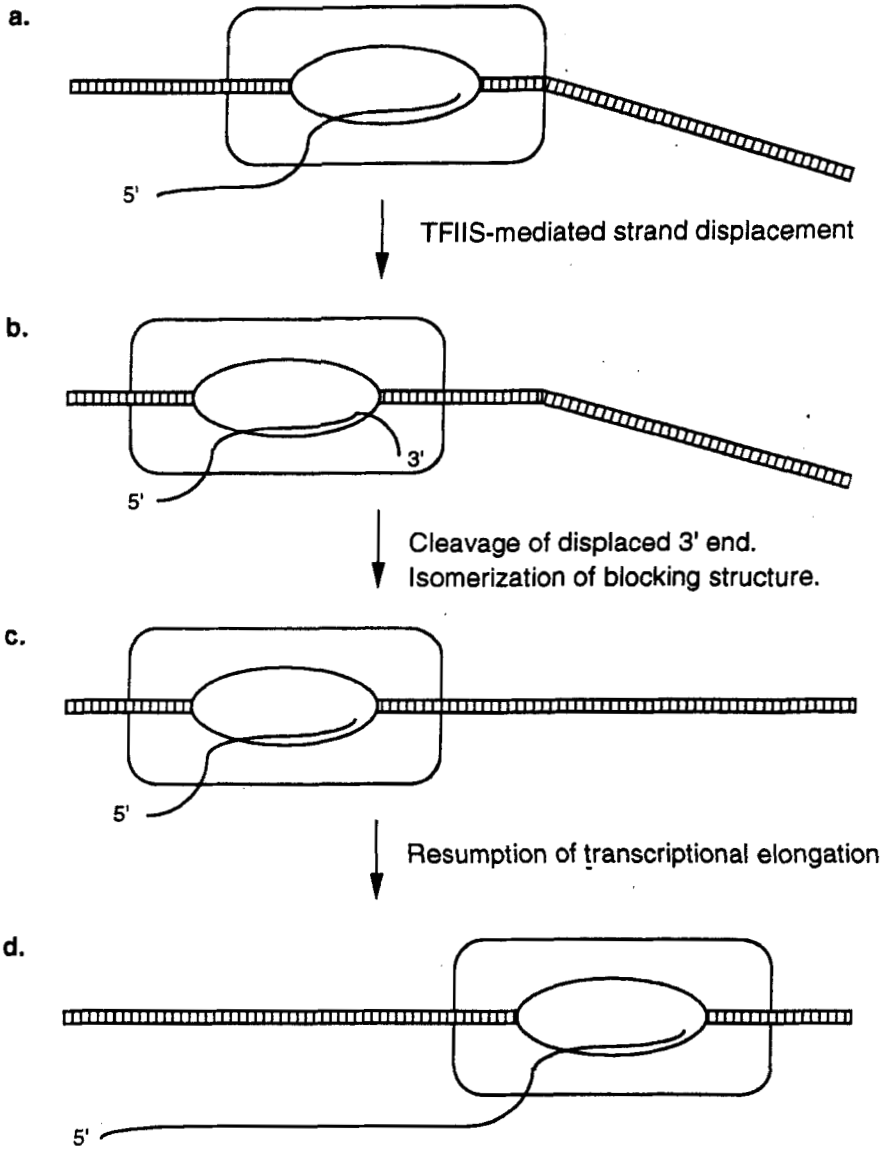

FIGURE 1. Reactivation of stalled RNA polymerase II by TFIIS. (a) Represents an elongation complex that has been stalled by a bend in the DNA structure. Reaction a to b: TFIIS mediates a backward translocation of RNA polymerase II that involves displacement of the nontemplate DNA strand with the nascent RNA and the concurrent displacement of the 3′ end of the nascent strand. Reaction b to c: the 3′ end of the transcript is available for cleavage by action of a nearby RNase domain or subunit, thus creating a new 3′ end point that could be used for resumption of polymerization. If the blocking structure had been removed (for instance by isomerization of the bend as indicated in b to c), RNA polymerase would be able to pass by the site by normal polymerization (reaction c to d). (This scheme is based on a model proposed by Reines, D., *J. Biol. Chem.*, 267, 3795, 1992. With permission.)

tribute significantly to those changes. The first is the G + C-rich sequence with the interrupted dyad symmetry. That sequence would have been transcribed by an RNA polymerase that has reached the termination end point and gives rise to a segment of RNA that would be capable of forming a stable stem-loop secondary structure. This is indicated in Figures 2b and 2c. Whether

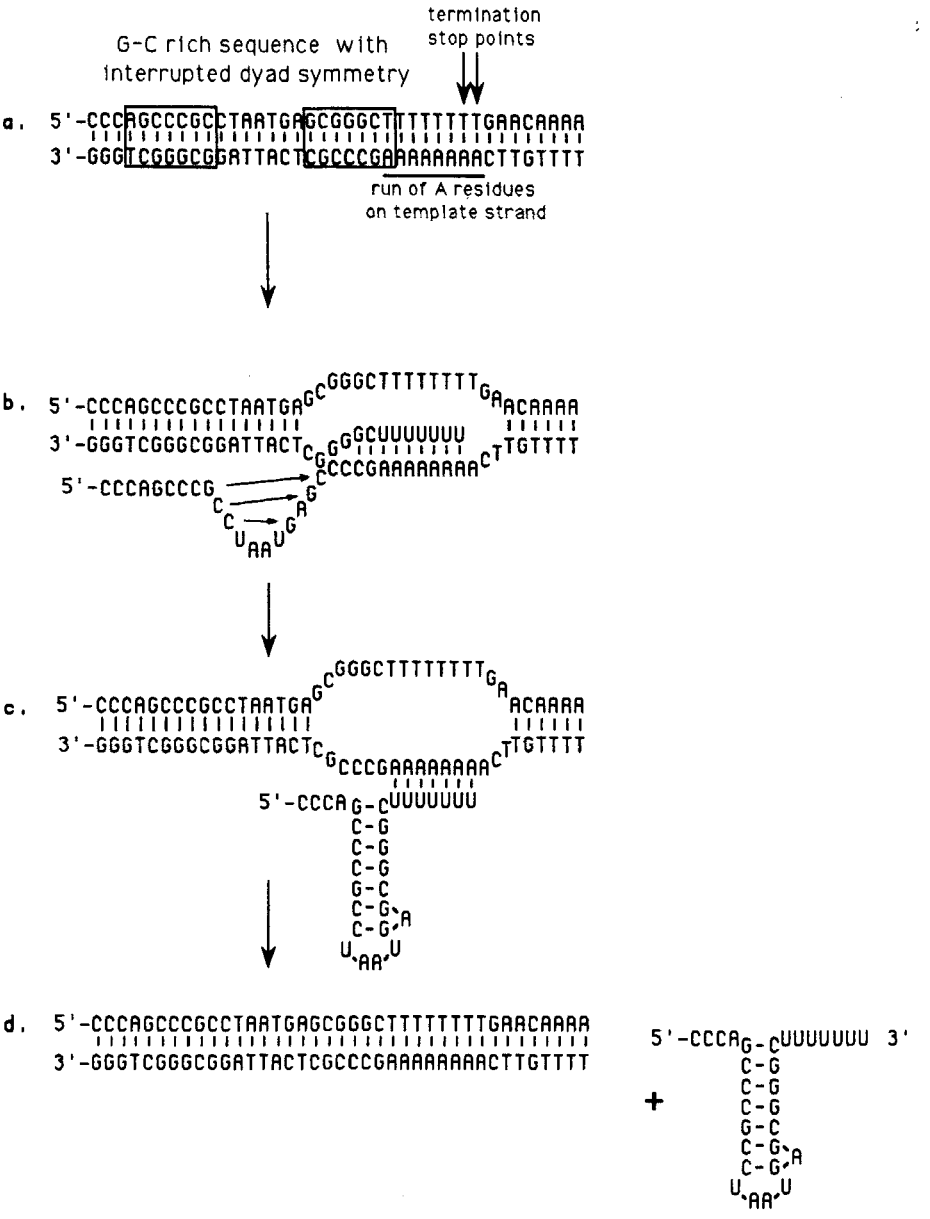

FIGURE 2. Sequence of the core region of *T trpA*, with a schematic representation of the RNA and DNA interactions that might occur at various steps of the termination process. (a) Shows the placement of the characteristic features of intrinsic terminators. (b) Shows the DNA bases that would be separated in the elongation complex and the pairing between the template strand and the 3′ end of the RNA that has been elongated to the first of the two termination stop points. Presumably, there is enough conformational freedom in the nascent RNA for the initiation of stem-loop formation through pairing of residues, as indicated by the arrows. (c) Shows the complex after the stem-loop structure has formed. (d) Shows the DNA and RNA after release of the RNA.

the structure actually forms in the nascent RNA in a transcription complex at a termination end point has not been demonstrated but there is abundant indirect evidence that it does. First and foremost, mutants that change the sequence in a way that affect the pairing of the stem affect termination.[70,71] For instance, a change that interrupts the pairing in the stem decreases the ef-

ficiency of termination, but most of the efficiency can be recovered if a second change restores the pairing.[71] Second, the mutational change has its effect only if it is in the template strand, thus ruling out possible models that involve a structure in the nontemplate DNA strand rather than the transcript itself as the feature that is detected.[72,73] Third, there is a strong correlation between the predicted stability of part of the RNA structure and termination efficiency of variants with changes in the stem. Because the sequence of the loop part of an RNA stem-loop also influences the stability of the RNA secondary structure,[74] it is not surprising that changes in the loop sequence also affect termination efficiency.[71] In this case, however, the rules for contributing to loop stability have not been fully elucidated, making it harder to predict how various changes are correlated with termination efficiency. Fourth, the presence of DNA oligonucleotides that are complementary to the 5′ strand of the stem specifically reduces efficiency of termination at an intrinsic terminator,[32] presumably because the binding of the oligonucleotide preempts formation of the stem.

Mechanistically, how might the formation of a stem structure in the nascent RNA contribute to the termination process? As mentioned earlier, some pause sites also occur at points where the nascent transcript could form a stem-loop structure. Thus, it is likely that stems associated with terminators also cause pausing, although this has not been directly demonstrated for a stem in an intrinsic terminator. They would thus contribute to termination by decreasing the value of $k_{forward}$ for RNA polymerase at a termination stop point. In addition, the stability of the elongation complex is not uniform along a template. Arndt and Chamberlin[75] have shown that ternary complexes with RNA polymerase arrested or paused at certain points that are not termination stop points dissociate at rates that are significantly higher than at other points. In all cases, these low stability pause sites are at points where a short stem could form in the nascent RNA with the stem ending within 6 to 10 residues at the 3′ end of the transcript in the paused complex. This result suggests that formation of the stem leads to instability of the ternary complex. Because the position of these partial ''destabilizing'' stems are exactly at the position of the stems in terminated transcripts, the termination RNA stems likely contribute to an increase in $k_{release}$ as well as to a decrease in $k_{forward}$ at the termination stop point.

The second aspect of the sequence for an intrinsic terminator is the run of dA residues in the template strand (Figure 2a). Because termination stop points usually occur after several of the dA residues have been transcribed, the RNA molecule will have a run of U residue at their 3′ ends (Figure 2b). Again the importance of the run of dA residues has been tested by mutational analysis.[70] In the conventional view of the transcriptional elongation complexes, most or all of these 8 terminal U residues would be base-paired with the dA residues in the template strand. However, as pointed out in the section on elongation, many of the U residues may already be displaced from the template and within the RNA exit site of RNA polymerase instead.[10]

There is excellent physical chemical evidence that a hybrid helix consisting of rU residues paired to dA residues is significantly less stable than most other hybrid helices.[76] The possible reason is that a run of dA residues does not fit well into A-form double-helices, the exclusive form that is taken up by helices that contain at least one RNA strand. Thus, poor pairing of the 3′ end of a RNA to the template could readily contribute to an increase in $k_{release}$. This basic property of RNA-DNA duplex helices is perhaps one of the most compelling reasons to believe that the normal elongation complex does involve a hybrid helix of 8 or more bp.

The third aspect of the intrinsic terminators is the sequence following the run of dA residue in the template strand.[69,77] Although this sequence is not transcribed to become part of the terminated transcript, it is within or just ahead of the contact point between the enzymes and the DNA in the transcription complex at the termination site. Because downstream sequences are known to affect pausing at nontermination sites,[25] a major role could be to contribute to a decrease in $k_{forward}$. Those sequences could be influencing either the unwinding of the DNA or just the progression of the enzyme along the DNA. Alternatively, because downstream sequences are within the contact site, there could be some variations in the stability of the binding of RNA

polymerase related to sequence variations in the contact points. Thus, the downstream sequence could conceivably increase $k_{release}$ at a termination stop point.

B. Models for Spontaneous Termination

A specific model for the mechanism of termination at an intrinsic site has been proposed by von Hippel and Yager.[4] The critical postulates of the model are that during elongation, the nascent RNA is held to the DNA template by a hybrid helix that is 12 bp in length and that the termination kinetics are governed by the equilibrium thermodynamics of the ternary complex. The variations in the stability of that complex are described primarily in terms of the free energy of formation of the separated DNA strands (the DNA "bubble") and the free energy of formation of the RNA-DNA hybrid. This latter term is considered to consist of the energy of the 12-bp hybrid corrected for any change in the extent of that hybrid caused by formation of RNA-RNA base pairs in an RNA-stem near the 3′ end. Therefore, one of the basic driving forces of this mechanism is the formation of the stem structure at the expense of the hybrid helix. This was a concept first proposed by Farnham and Platt[78] but analyzed in more extensive detail by von Hippel and Yager.[4] Although there is a fair correlation between calculated termination efficiencies using these components and actual termination efficiencies, there are enough discrepancies to realize that some of the simplifying assumptions of the model are not valid.

An implied feature of the von Hippel-Yager model is the assumption that the elongation barrier, the activation energy for the addition of another residue, does not vary extensively. The fact that the step time for elongation can vary considerably at different steps during elongation, however, indicates that the elongation barrier is not uniform. Another assumption is that the free energy of the ternary complex does not contain a term for the binding of the RNA to the exit site on the template. This is a term that also could vary considerably with changes in the sequences and structure of the RNA at each step. It is this term that may account for the large variation in the ways different terminators respond to the ionic conditions of the reaction, with some terminators being more sensitive than others to the monovalent salt concentration and magnesium concentration.[79]

The key structural element of the von Hippel-Yager model is the proposed isomerization of the nascent RNA within the elongation complex that reduces the extent of contact of the RNA 3′ end with the template DNA strand. Clearly, the reduction of that contact from 12 bp to 8 or fewer would decrease the stability of that interaction. Hence, it is critical to know whether the extent of the hybrid is normally 12 bp during most steps of elongation and whether the RNA-RNA stem structure can involve bases that are within 12 nt of the 3′ end of the transcript.

The mechanism for termination clearly depends on the structure of the transcription complex during elongation. In the major alternative model for elongation,[10] only 2 or 3 bp hold the 3′ end of the nascent RNA to the template. In this case, the formation of a stem structure in the RNA within 8 nt of the 3′ end would not likely affect the pairing with the template. Thus, termination would occur as a result of a large decrease in the binding of the nascent RNA to the RNA product site. The RNA exit site could have a low affinity for an RNA with a stem followed by 5 or 6 U residues. The energetics for the formation of the structure and the binding of the protein to the U residues could follow the same pattern as those that are predicted to affect the attachment of the RNA to the DNA. The correlations that are shown by von Hippel and Yager[4] fit either model. The one strong argument in favor of the model in which the stability of the interaction of the nascent RNA with the DNA is the most crucial feature is the clear evidence for the low stability of the rU:dA hybrid helix.[76] In the alternative, a poor interaction between rU residue and the exit site of RNA polymerase is merely conjecture at this point. On the other hand, proteins do show distinct preference in binding to nucleic acids with different secondary structures. Hence, the formation of a double-stranded stem near the 3′ end of the nascent transcript could reduce its affinity for the RNA exit site.

Because there is some sort of exit site on the polymerase, the ability to form a secondary structure in the RNA that interacts with that site is relevant for both models.

The overall process of transcription termination also includes the release of the RNA polymerase. Attempts to determine whether that step is simultaneous with release of RNA or occurs subsequently has given ambiguous results.[80] Using a kinetic approach, however, Arndt and Chamberlin[81] were able to show that the addition of excess sigma factor significantly increases the rate of enzyme recycling in a system that involves synthesis of a 160-nt transcript from a DNA template with a strong promoter and efficient intrinsic terminator. This result fits well with a model in which sigma is directly involved in catalyzing release of core RNA polymerase from DNA at a step after release of the RNA. Thus, in its general capacity of decreasing the affinity of core for nonpromoter DNA sequences,[82] sigma factor can also be considered a termination factor.

IV. TERMINATION FACTORS

The process of purification separates RNA polymerases from a number of cellular components that are either required for or control various steps in the transcription process. Although many highly purified, "basic" RNA polymerases can recognize cognate intrinsic terminators, they often require the intercession of factors to terminate RNA synthesis at other functionally important sites. In addition, various factors are known to modulate function at intrinsic terminators.

There are three well-characterized protein factors that are essential for function during transcription of their cognate terminators. These are rho factor,[83] which acts with *E. coli* RNA polymerase on a number of important termination sites in the *E. coli* genome; transcription termination factor I (TTFI),[84] which acts with RNA polymerase I for function at sites at the ends of the DNA sequences encoding the large ribosomal RNA precursors; and vaccinia termination factor (VTF),[85] which is necessary for causing purified vaccinia virus RNA polymerase to terminate at distinct signals at the end of genes that function early during vaccinia viral development. Although these factors have the same basic function, each has a distinctly different mechanism. Table 1 is a summary of these factors along with some factors that affect elongation and can regulate termination.

A. Rho-Dependent Termination

The basic role of a termination factor is to provide a mechanism for dissociating a nascent transcript at a site lacking all the requisite sequence elements of an intrinsic terminator.[86] A special mechanism is required because of the high stability of the transcription complex during elongation through most sequences. Rho factor accomplishes this feat by binding to the nascent RNA and by using the hydrolysis of nucleoside triphosphates — primarily ATP — to fuel an action on the RNA that dissociates it from the transcription complex.[80,87,88] The specificity of rho action is dictated in large part by the sequence and structural requirements for its binding to RNA. However, it is also determined by other features of the DNA sequence at or near the transcription stop points, including those that govern the step time for nucleotide addition to the nascent chain (the extent of pausing),[24,33] as well as those that influence the structure of the transcript from the rho-factor binding site to the point where the nascent RNA emerges from RNA polymerase.[89] Altogether, the sequences that form a rho-dependent terminator extend from at least 60-bp upstream to about 20-bp downstream of the termination stop points. Analysis of the sequences of several rho-dependent terminators has not revealed a common motif or consensus. The one sequence characteristic that correlates well with rho-dependent terminators is a compositional bias of a relatively low G and high C content in the part of the transcript that interacts with rho.[90] This bias reflects the requirements for binding of rho to RNA, as rho is specific for single-stranded RNA and has an especially strong affinity for unpaired C residues.[91]

Rho is a hexamer of a single polypeptide with 419 amino acid residues.[92,93] Individual subunits appear to have a compact globular shape with a diameter of about 42 Å.[94] The subunits are or-

TABLE 1
Various General Termination/Antitermination Factors

Name	Function	Size (M_r)	Site of action
Prokaryotic			
rho	Required factor for many terminators	$(46,000)_6$	Binds to nascent RNA; uses NTPase to effect dissociation
NusA	Pause-enhancing factor increases efficiency of intrinsic terminators	55,000	Binds to RNA polymerase scans RNA structure
NusG	Possible cofactor for rho-dependent termination	20,000	Binds RNA polymerase core
NusB S10	rrn gene antiterminators	15,700 12,000	Binds to RNA sequence signal (boxA)
Eukaryotic			
TTFI	RNA polymerase I termination factor	105,000	Binds to downstream DNA site
MT	Mitochondria RNA polymerase termination factor	~35,000	Binds to downstream DNA site
TFIIS (SII)	Catalyzes reactivation of stalled RNA polymerase II	34,000	Binds to RNA polymerase II
VTF	Vaccinia virus RNA polymerase termination factor	95,000 + 31,000	Interacts with specific sequence in nascent RNA
La	RNA polymerase III termination factor	47,000	Binds to 3' end of transcripts

ganized in a ring structure with a linkage relationship that consists of three fairly stable dimers that are more loosely associated to form the hexamer.[95] Both sequence and functional analyses indicate that the subunit has a distinct domain for binding RNA and a domain for binding nucleoside triphosphate cofactors.[96,97] The fact that rho can protect 70 continuous residues of poly(C) from digestion with pancreatic RNase suggests that the RNA binding site in the hexamer consists of a continuous groove that extends across all six subunits.[87,94] Binding studies with oligo(C) molecules with chain lengths less than 22, however, have indicated that only three subunits in the hexamer can bind RNA tightly at one time.[98] Because the subunits have identical sequences and only one distinct RNA binding domain has been found per subunit, this alternation of function may be an example of half-of-the-sites reactivity whereby binding to the site on one subunit of a dyad pair alters the structure of the site in the adjacent subunit via allosteric changes transmitted across the dimerization interface. This alteration of structure could still allow polymeric RNA to fit in the domain and attain protection but not have the extensive bonding that holds the segment of RNA in the other subunit. Although the mechanistic consequence of this type of binding is not yet clear, this alteration of binding function may provide the means of dynamically linking changes in RNA binding with ATP hydrolysis.

The binding of ATP in the hexamer also shows a half-of-the-site reactivity.[99] Three ATP molecules bind with high affinity, whereas three more can also bind but with a much lower affinity.[100] When rho is bound to RNA, the high-affinity ATP bonding sites become active sites for ATP hydrolysis. The overall process of hydrolysis of ATP to ADP and Pi leads to an im-

portant, but ill-defined, structural change in the RNA.[87] Functionally, the interactions between rho and RNA that are coupled to ATP hydrolysis lead to dissociation of structures attached to the 3′ end of the RNA.[88,101] This reaction causes termination when the "structure" attached to the 3′ end of the RNA is the transcription complex of RNA polymerase and template DNA.

The mechanism used to dissociate the transcription complex is not known. However, the process is regulated by sequences in the DNA and in the nascent transcript.[102] First, rho has strong preferences in its binding to RNA. The critical features of this preference is a sufficiently large segment of RNA lacking base pairing and the presence of some C residues. Another aspect of the specificity is a kinetic match between the action of rho on the RNA and the motion of RNA polymerase on the template[103]; rho causes RNA polymerase to terminate preferentially at points that are natural pause sites.[24,33,86] This result indicates that rho is acting primarily as an RNA release factor with the stop point specificity being determined by where rho is bound to the nascent RNA and where the RNA polymerase is on the DNA after the bound rho has started to act.[80] The positioning of the RNA polymerase after the rho binding site has become available in the nascent RNA is dependent on the rules that govern transcriptional elongation. Thus, important components of a rho-dependent terminator are the sequences that govern the rate of elongation. This is a characteristic that is shared between rho-dependent and intrinsic terminators. There is no evidence that rho affects the elongation pausing specificity of the polymerase.[33] There is also a dynamic component to the interaction of rho with the RNA that is governed by sequences between the binding site and the 3′ end of the transcript. The critical aspect of this sequence is the extent and stability of base-paired secondary structures in the region that could hinder the access of rho to the transcription complex.[89] Evidence for a dynamic interaction also comes from studies with mutant forms of both rho and RNA polymerase.[103] One mutant form of rho that has poor termination activity was found to be normal in its ability to bind to mRNA and to hydrolyze ATP with an unstructured RNA cofactor, but was deficient in its ability to hydrolyze ATP with a structured RNA. The mutant rho did cause termination when the rate of elongation by RNA polymerase was decreased either by lowering the concentration of NTPs or by using a mutated RNA polymerase with increased K_m for NTP substrates.[103]

Currently, it is not known whether any specificity is imparted by the sequences in the DNA near the stop point, aside from the effect these sequences have on the kinetic match. If rho had a specific interaction with the sequences at the 3′ end of the transcript, that specificity would be evident in a consensus or bias in the 3′ end sequence of rho-terminated transcripts, but none has been observed. Another influence that sequences near the stop point could have is on the stability of the ternary complex. Arndt and Chamberlin[75] have shown that the ternary complex is intrinsically less stable at some points than at others. The ease of dissociation could readily make certain pause points in the termination region be preferred stop points. However, the extent that this influence contributes to the specificity of a rho-dependent terminator is not known.

The lack of a stringent sequence requirement for a rho-dependent terminator raises the possibility that such terminators might be fairly frequent in DNA sequences. In fact, rho-dependent terminators are commonly found within genes of *E. coli* as well as at the ends of genes and operons, their expected locations.[104] The ones within genes, however, usually function only when the nascent RNA is not being translated by a ribosome. Because rho has to bind to RNA for it to terminate transcription, it will not be able to gain access if the RNA is blocked by a ribosome that is in the process of translating the mRNA as it emerges from the RNA polymerase. This feature of the rho mechanism thus accounts for the high specificity of its action: terminators will be limited to those sections of the DNA that do not encode functional parts of mRNA, such as at the ends of operons. This raises a question of what protects rho from acting on transcripts that are not translated. This is particularly relevant to the transcription of ribosomal RNA. Ribosomal RNA genes may be naturally devoid of rho-dependent terminators because the RNA product is highly structured and has very few segments that might serve as good binding sites for rho. Indeed, rho

has poor affinity for isolated rRNA molecules.[91] In addition, ribosomal proteins bind to the nascent rRNA and this could afford further protection. However, rRNA operons appear to have been devised with a mechanism that gives them even further immunity to rho action.[105,106] Sequences near the start of the genes establish an antitermination mechanism. How this mechanism operates is not yet known but one possibility is that it increases the rate of transcriptional elongation by preventing pausing.

B. TTFI

Termination of transcription of DNA encoding ribosomal RNAs by RNA polymerase I in eukaryotic cells depends on a sequence-specific DNA-binding protein called TTFI (polymerase I termination factor).[84] Mouse cell TTFI binds tightly to DNA molecules containing the sequence motif of AGGTCGACCAG(A/T)(A/T)NTCG.[107] This motif, called a Sal box because it contains a *Sal*I restriction site, is found 18-bp ahead (downstream) of the DNA point encoding the 3′ end of pre-rRNA and is a necessary sequence element for termination of transcription by RNA polymerase I. When a DNA fragment is transcribed by either mouse or yeast RNA polymerase I starting at a site upstream from the Sal box, virtually all the polymerase molecules read through the sequence in the absence of TTFI, whereas most terminate at a point 11-bp preceding (upstream of) the start of the sequence in the presence of TTFI.[108] When the same DNA fragment is transcribed by any one of a number of other RNA polymerases from various sources (including RNA polymerases II and III from eukaryotic cells as well as *E. coli* and T3 RNA polymerases), all read through the Sal box region without termination either in the presence or absence of TTFI, although both the *E. coli* and RNA polymerase III do terminate to some extent at an upstream sequence that has a weak resemblance to an intrinsic terminator for those enzymes.[108]

Mouse TTFI consists of a polypeptide with an apparent molecular mass of 105 kDa.[84] Treatment with low levels of proteinase K yields a fragment that can still bind to DNA with a Sal box, as demonstrated by gel retardation assays, but can no longer cause termination of transcription.[84] This experiment suggests that the TTFI polypeptide has separate DNA-binding and termination domains. This interpretation is also consistent with the finding that the function of the Sal box sequence is orientation dependent; it does not cause termination of transcription when it is inverted.[107] TTFI bound to DNA is not merely serving as a roadblock. Instead, when it binds to the asymmetric Sal box sequence with the right orientation, it presumably brings the separate termination-promoting domain into a position where it can make contact with an elongating RNA polymerase I. The bound protein may serve as a partial roadblock to slow down the rate of elongation, whereas the other domain mediates the release of the nascent RNA from the DNA template. Clearly, the mechanism used by TTFI is very different from that used by rho and represents another important paradigm of a basic mechanism of transcription termination.

The termination of transcription at the ends of the DNA sequence encoding rRNA in mitochondria of animal cells occurs by a mechanism that has many features that are similar to those used to terminate synthesis of the rRNA transcripts in the nucleus. The signal is a 13-nt sequence that occurs just downstream from the transcription stop points.[109] Extracts of mitochondria contain a polypeptide (or polypeptides) with M_r from 33,000 to 36,000 that causes termination to occur at that point during transcription of the rDNA *in vitro*.[110] This same fraction of size-resolved polypeptides also contains a component that binds tightly to a DNA fragment containing the 13-nt sequence.[110,111] These results imply that termination is caused by the protein that binds to the 13-nt DNA segment. A naturally occurring mutation in the 13-nt sequence is found in human beings with a mitochondrial function disease known as MELAS.[110] This mutation greatly reduces the efficiency of rRNA transcription termination and the affinity of binding of the purified 33 to 36 kDa protein. This observation is further strong evidence that the DNA-binding protein is the termination factor. This factor is known as MT. The fact that the defect leads to a rather severe pathological condition also provides compelling biological evidence for the im-

portance of transcription termination in the orderly expression of genes.

The terminator for mitochondrial rRNA transcription differs from its nuclear counterpart in one important aspect; it functions in either orientation,[109] whereas the nuclear rRNA transcription termination sequence is undirectional. Because most mitochondrial gene expression mechanisms retain characteristics that reflect the eubacterial origins of mitochondria, the existence of this DNA-binding, roadblock-like mechanism for termination is very interesting. No similar mechanism has been recognized for a eubacterial (prokaryotic) terminator. In particular, the rRNA transcription terminators in *E. coli* appear to be potent intrinsic terminators. This suggests that a special system evolved for mitochondrial rRNA genes or perhaps the mitochondria expropriated the system that had evolved for terminating rRNA transcription in the nucleus.

Proteins that bind tightly to specific DNA sequences can act as blocks to elongation with *E. coli* RNA polymerase[112] and RNA polymerase II,[113] but these proteins do not cause termination by themselves because they do not mediate release of the RNA. Because rho factor can mediate the release of RNA from RNA polymerase arrested during elongation at a block,[112] however, a specific DNA-binding protein could reasonably be a component in a subclass of rho-dependent terminators.

C. VTF

A termination factor that functions with an RNA polymerase that synthesizes precursors to mRNAs in eukaryotic cells has been isolated from vaccinia virus.[85] This factor, called VTF, acts to terminate transcription by vaccinia RNA polymerase at a position that is 50 bp downstream from the sequence TTTTTNT (on the nontemplate DNA strand).[114] Although this sequence is similar to the part of an intrinsic terminator where RNA release occurs, pure vaccinia RNA polymerase transcribes the segment of DNA with that sequence without releasing the nascent RNA chain.

VTF contains two subunit polypeptides of M_r 95,000 and M_r 31,000.[85] It has the remarkable property of also being the vaccinia mRNA-capping enzyme. The two reactions appear to be catalyzed independently by the same protein. This is suggested by the findings that VTF still terminates transcription when the capping reaction is inhibited by pyrophosphate or when VTF is added to a reaction mixture containing an elongation complex with an uncapped 390-nt nascent transcript.[115]

The upstream position of the sequence signal for termination suggests that it might be recognized at the level of RNA. This hypothesis is supported by the observation that VTF loses its ability to terminate transcription when the reaction mixture contains BrUTP in place of UTP.[116] Because BrUTP is readily incorporated into RNA by vaccina RNA polymerase, the substitution of BrU for U is likely to change the structure or properties of the -UUUUUNU- transcript of the upstream signal. Recently, Hagler and Shuman[117] have shown that the final 18 residues of the nascent RNA are well protected within the vaccinia RNA polymerase. Thus, a sequence that is 50 nt from the transcriptional stop point should be fully accessible to bind to VTF.

From the evidence at hand, VTF shows some similarities with rho factor in recognizing the nascent RNA. It has not yet been demonstrated, however, whether VTF is an RNA-dependent NTPase or whether it can directly dissociate transcription complexes by an ATP-hydrolysis-dependent reaction in the absence of further transcriptional elongation. The position and function of the upstream signal is also reminiscent of an important 3′ end formation characteristic in the synthesis of mRNAs in the nucleus of eukaryotic cells. The addition of poly(A) is triggered by the RNA sequence AAUAAA approximately 30-nt upstream of the site where cleavage of the nascent transcript occurs. Because the cleavage leads to instability of the remaining nascent nuclear pre-mRNA transcript and eventual termination of RNA polymerase II transcription, it is important to demonstrate whether VTF is actually causing dissociation of the vaccinia RNA polymerase at the termination point or whether the polymerase is continuing to elongate a transcript that is rapidly degraded by a latent nuclease. The current evidence does not eliminate that possible mechanism, unlikely as it may seem.

V. AUXILIARY TERMINATION FACTORS

Although some pure RNA polymerases can spontaneously terminate transcription at certain sites, the efficiency of that process is often enhanced significantly by the presence of additional separable factors. The best characterized of these auxiliary termination factors is the *E. coli* NusA protein.[118] However, the La protein from animal cells affects termination by RNA polymerase III,[119] and a factor identified as τ (tau) enhances and modifies recognition of some fairly strong intrinsic terminators for *E. coli* RNA polymerase.[120] A role for the NusG protein in *E. coli* for the function of some rho-dependent terminators has also been implied.[121]

A. NusA

The sequence of the tR_2 terminator of coliphage λ matches well with the identified motifs of intrinsic terminators, yet pure *E. coli* RNA polymerase terminates there with only about 45% efficiency. This efficiency can be increased to 90% by the presence of saturating amounts of NusA protein.[118] NusA has a similar effect on the *in vitro* function of a number of intrinsic terminators, including some that are much weaker than tR_2 and some that are much stronger. One example of this latter type is a terminator at the end of an *E. coli* rRNA operon rrnB T1. In the absence of NusA, the termination efficiency at this terminator is 80%, when it is transcribed *in vitro* with pure RNA polymerase. In the presence of NusA, however, the termination efficiency is 95%. Because NusA is an elongation factor that increases the rate of pausing at certain sites, its likely function at these terminators is to decrease the values of $k_{forward}$ for addition of the next nucleotides when RNA polymerase is at that termination stop point. The enhanced pausing at that point would give more time for the conformational change that leads to release of the nascent transcript. As pointed out earlier, NusA protein interacts reversibly with core RNA polymerase and somehow stabilizes the interaction that causes the putative RNA stem-loop structure to slow the elongation process. There is as yet no strong evidence that NusA interacts directly with the RNA.

NusA has a strong effect on the recognition of a terminator in the ribosomal protein S10 operon leader of *E. coli*.[122] The sequence of this terminator does not resemble a typical, strong intrinsic terminator. Consequently, the efficiency of termination during transcription of that DNA with highly purified *E. coli* RNA polymerase is low in the absence of NusA but is about 50% with NusA present. Thus, the terminator is operationally a NusA-dependent terminator. The transcripts end at points in a region that contains two short stretches of dA residues separated by a dG-dC dinucleotide on the template strand. This part of the terminator is actually not too dissimilar from that of several strong intrinsic sites. The element that is lacking is the high content of G and C residues preceding the stop points. However, the upstream sequence does encode an RNA that could form a moderately stable stem-loop structure. Although this structure may not be sufficiently stable to be part of a pause signal that would make $k_{forward} < k_{release}$, it could have the requisite features for NusA to enhance pausing. This effect of NusA on $k_{forward}$ would then be sufficient to tip the balance in favor of release at that site.

Another interesting property of the S10-operon leader terminator is that ribosomal protein L4 enhances the effect of NusA substantially, yielding an overall termination efficiency of about 70%.[122] This effect is consistent with a role of L4 as an autogenous negative regulator of the expression of the S10 operon; L4 is the product of the third gene of that operon. Because L4 is an RNA-binding protein, its likely mechanism is to bind to some structure in the S10 leader RNA and thereby modify the way the transcript folds, perhaps enhancing the formation of the terminator stem structure. L4 bound to the nascent RNA could also modify the elongation properties of RNA polymerase.

In spite of its general ability to enhance pausing, NusA does not increase the efficiency of termination at rho-dependent sites. In fact, it has an antagonistic effect at some rho-dependent terminators[123–125] and no obvious effects at other terminators.[43] The inhibitory effect at tR1 occurs even though NusA strongly enhances pausing at one of the major termination stop points in that site.[33,124] Because rho acts dynamically to dis-

sociate transcripts at points where it can catch RNA polymerase molecules by its action along the nascent transcript, a modulation that would increase the extent of pausing at a normal termination stop point would be expected to increase the efficiency of rho action there. This is clearly not the case and suggests that some other effect of NusA on the reaction is overriding its expected stimulatory effect via pausing. NusA could interfere with the binding of rho to the RNA or block rho's access to RNA polymerase along the RNA. Rho has been shown to bind to NusA and to complexes of core RNA polymerases with NusA.[126] Although such an interaction might be a means of bringing rho into close proximity with the mRNA molecules it will act on, the binding could also be a means for diverting (or subverting) an action of rho on the transcription complex; an affinity for the NusA-core domain might prevent an incoming rho molecule from reaching its target for termination, the point of attachment of the nascent RNA to the RNA exit site or to the DNA template.

Another activity affecting termination at *E. coli* intrinsic terminators has been purified partially and may be a new factor tentatively called tau.[120] This activity, like NusA, increases the efficiency with which pure *E. coli* RNA polymerase terminates at the major early gene terminators for coliphages T7 and T3. It differs from NusA because it produces transcripts that are slightly shorter than those terminated by action of pure RNA polymerase by itself. These shorter RNAs have 3′ end sequences that are closely similar to the RNA molecules isolated from T7 and T3 infected cells. Because the "tau" activity has not yet been purified to homogeneity, it could be a mixture of components including possibly NusA and a nuclease or it could truly be a new factor.

B. NusG

Another auxiliary termination factor in *E. coli* is the product of the *nusG* gene. NusG was isolated as a 21-kDa protein that makes antitermination by λN protein more processive during transcription of λ genes *in vitro*.[127] NusG by itself has some minor effects on the moderately potent intrinsic terminator in the attenuator region preceding the gene for the β subunit of *E. coli* RNA polymerase (*TrpoBa*); it reduces efficiency from 62% to about 54%,[128] whereas at λtR1, a rho-dependent site, it exerts a slight enhancement of termination.[127] As an intrinsic terminator, *TrpoBa* is unusual in that its function is affected negatively rather than positively by NusA.[128] As with NusG, the effect is only slight and the function of both factors together is additive causing the termination efficiency to drop from 62% in the absence of factors to 42% in the presence of both.

There is evidence that NusG may play a significant role in the *in vivo* function of some rho-dependent terminators. This is from assays for the function of five separate terminators under conditions in which the cellular content of NusG has been depleted severely.[121] The extent of termination at three different rho-dependent terminators including λtR1 was severely reduced. However, the function of the terminator in IS2 and of a rho-independent terminator were not strongly affected. The strong effect of NusG loss on the function of λtR1 *in vivo* is in contrast to the lack of a NusG dependence for termination at that site *in vitro*. *In vivo*, NusG could be acting as an auxiliary factor to allow rho to function under conditions that are not ideal, conditions that are not duplicated for the reaction *in vitro*, which has been optimized for rho function without NusG. Another possibility is that the effect of NusG is indirect; it could play an essential role in dissociation of the ribosomes from mRNA. Because ribosomes readily occlude the sites for binding of rho to the nascent RNA, a defect in ribosome release could interfere with some rho-dependent terminators but not others. Clearly, the issue needs to be resolved.

C. La Protein

Eukaryotic cells contain a protein that binds tightly to precursors of tRNAs and 5S rRNA. This protein, called La because it reacts with anti-La lupus antibodies, has been purified from a number of sources and consists of a polypeptide with M_r of 47,000 to 50,000.[129] It has been shown to act as a termination factor for RNA polymerase III in cell extracts that have been depleted of

endogenous La protein by immunoreaction with La-antibodies.[119] Although highly purified RNA polymerase III is able to terminate transcription at a number of cognate terminators by itself,[62] La appears to be necessary for efficient and accurate termination.

The RNA molecules bound by La are products of RNA polymerase III. They are characterized by the presence of a short string of uridine residues at their 3′ ends. Purified La exhibits an ATPase activity in the presence of RNA and catalyzes the ATP-dependent dissociation of an RNA-DNA duplex structure in which the RNA has several U residues at its 3′ end that are base paired to DNA oligonucleotide.[130] Thus, La has many of the properties of *E. coli* rho factor. Not enough is known yet, however, to conclude whether La acts, like rho, by binding to a free single-stranded region of the RNA and effecting release through an action along the RNA driven by ATP hydrolysis. Alternatively, it could start the release reaction by binding to the very 3′ end of nascent transcripts that have several U residues. Because of the low affinity of rU-dA hybrid helices, these 3′ ends might have a tendency to unwind partially and thus momentarily expose the part of the transcript to which La has high affinity. The ATPase/helicase function of La could be what finishes the release process.

VI. CONTROLLING TERMINATION

The level of expression of many genes is controlled by the function of upstream terminators. In bacteria, this type of control is important for the expression of biosynthetic operons. Also, some viruses use mechanisms for bypassing terminators as a way of changing the pattern of gene expression during their developmental cycles. The best-studied examples of these are, respectively, the terminator at the end of the leader gene of the *E. coli trp* operon *TtrpA*[131] and the terminators that are downstream from three coliphage promoters, P_L, P_R, and $P_{R'}$.[132] The former is controlled by an attenuation mechanism, whereas the latter are controlled by antitermination mechanisms.

A. Attenuation Control

TtrpA, is a prototypical intrinsic terminator. The extent to which it functions, however, can vary as a result of interactions between the nascent leader RNA and ribosomes that affect the formation of the terminator stem structure in the RNA.[131] These interactions are governed in turn by the levels of tryptophan in the cell. In the absence of ribosomes translating the leader RNA, which is the usual *in vitro* situation, that stem structure forms readily and the terminator functions with high efficiency. The terminator also functions well *in vivo* when ribosomes are able to translate the leader RNA without hindrance. When cells are starved for tryptophan, however, ribosomes stall at the two tandem *trp* codons in the leader RNA and thereby allow another structure to form in the nascent leader RNA that preempts formation of the terminator stem structure. Hence, when the concentration of tryptophan is low in the cell, the terminator does not function well, allowing transcription of the genes that encode the tryptophan biosynthetic enzymes. This control mechanism neatly exploits the role of the structure of nascent RNA as part of the termination signal and the interplay between the ribosome and the nascent RNA. A similar mechanism is used for a large number of operons for the biosynthesis of amino acids in *E. coli* and related organisms.[131]

Attenuation of an upstream terminator is a likely feature of the control circuit for the expression of nucleotide biosynthetic pathway genes. In the *pyrBI* operon, the position of the ribosomes on the leader RNA is controlled by transcriptional pausing at a site that is upstream of the terminator.[133] Like the *trp* attenuator, the *pyrBI* attenuator has a strong intrinsic terminator. In this case, however, the translational reading frame of the leader extends into the terminator stem structure, thus, termination can be suppressed if the ribosome translates the RNA so close to the RNA polymerase that the nascent RNA is unable to form the stem structure. The critical feature of this control circuit is the closeness of the coupling of translation to transcription. This depends on the extent of pausing at an upstream site in a

region encoding several U and C residues in the transcript. Thus, the extent of pausing responds to the concentrations of UTP and CTP. When their concentrations are low the enhanced pausing ensures a close coupling of translation and a suppression of termination by the closely coupled ribosomes. In contrast, when the level of UTP is high, the pause is very brief and RNA polymerase is able to get well ahead of the ribosome, thereby allowing the termination stem to form unimpeded. Again, this system exploits the structural feature of the signal for an intrinsic terminator and the interplay between ribosomes and the nascent transcript.

As a rule, the terminators in the attenuators for biosynthetic operons in *E. coli* are rho independent. In *E. coli* there are two nonbiosynthetic operons that are known to have rho-dependent terminators in their attenuators. These are the *tna* and *liv* operons.[131] The best studied of these, the *tna* operon, encodes at least two products that allow growth on tryptophan as the sole source of carbon and energy.[134] High levels of tryptophan induce expression of this operon by suppressing the action of the rho-dependent terminator in the attenuator.[135] The involvement of ribosomes is strongly suggested by the fact that mutational changes of the single *trp* codon in the *tna* leader open reading frame to either a stop codon or a different amino acid abolishes induction by tryptophan.[136] The exact mechanism of the induction is not clear but could be a result of ribosome occlusion of the binding site for rho on the RNA. This hypothesis is supported by the finding that deletion of a 22-bp segment of DNA immediately following the translation termination codon of *tnaC* leads to constitutive expression of the downstream gene.[136] Because the RNA encoded by this 22-bp segment is C rich, it is likely to be a critical part of the binding site for rho (the *rut* site). High levels of tryptophan could allow rapid translation through the single *trp* codon, increasing the chance that the ribosome will be positioned at the termination codon where it could block binding to the RNA. This interpretation holds the implication that the ribosome is kinetically delayed at the single *trp* condon in the *tna* attenuator with noninducing levels of this amino acid, levels that are sufficient for normal translation of all other genes containing *trp* codons.

A special case where the function of rho-dependent terminators is used to control the expression of a gene is with the *rho* gene itself; *rho* is preceded by several rho-dependent terminators that respond to the overall level of rho protein in the cell.[137,138] These terminators thus form part of an autogenous regulatory circuit that exploits the functional activity of the gene product. This circuit maintains a constant level of rho with changes in the number of copies of *rho* gene in the cell and allows overexpression of defective rho factors encoded by *rho* mutants.

Rho-dependent terminators also function to help protect cells from certain conditions of environmental stress.[104] Many genes contain latent intragenic rho-dependent terminators that do not function under conditions of normal expression but become unmasked when translation is blocked by starvation for amino acids or is terminated prematurely.[43] This feature serves as a feedback mechanism that causes transcription of an operon to be terminated if the mRNA is not being translated and thus prevents needless waste of energy resources for the synthesis of unused RNA molecules. Mechanistically, the presence of ribosomes that closely translate a nascent RNA will normally block binding of rho to the RNA or prevent access of rho to RNA polymerase along the RNA. Starvation for amino acids allows RNA polymerase to move well ahead of the translating ribosome, thus exposing rho binding sites on the "naked" nascent mRNA.

B. Antitermination

Bacteriophage λ and related phages use mechanisms for inhibiting termination of transcription as a means of activating the expression of genes in the middle and late periods of their developmental cycles.[132] These antitermination mechanisms are specific for RNA polymerase molecules that have either started at special promoters or have passed a special sequence element. The simpler of the two systems in λ is

mediated by the product of the λ*Q* gene.[139] Q antitermination is specific for transcription by RNA polymerase molecules that have initiated at a λ promoter, $P_{R'}$, that is situated between genes *Q* and *S*. This promoter has a special sequence (*qut*) that allows RNA polymerase molecules that have started there to interact with Q protein.[140,140a] Critical residues of the sequence are both upstream and downstream from the transcription start point.[141] The transcribed downstream residues include a signal that causes RNA polymerase to pause just after initiation. This pause apparently allows Q to interact with RNA polymerase. Once bound, the Q-modified enzyme is released from the pause and is able to bypass most transcription terminators, both intrinsic and rho-dependent. This function of Q is enhanced *in vitro* by NusA protein.

The other λ antitermination system depends on the product of λ*N* gene and a number of host proteins, including NusA, NusG, ribosomal protein S10 (NusE), and NusB.[142] N antitermination is established on RNA polymerases that have passed a downstream sequence element called *nut*.[143] Unlike the Q system, N antitermination is not strictly dependent on the promoter and can be established after transcription of as many as 250 bp of DNA.

The mechanisms used by these proteins to cause RNA polymerase to bypass terminators is not well understood. In both cases, the recognition of the antitermination signal causes the RNA polymerase to be modified. With the N system the modification involves the formation of a complex of proteins, including N, NusA, NusG, S10, and NusB associated with the RNA polymerase.[142] Parts of this complex forms through a direct interaction of proteins with the *nut* sequence on the nascent transcript.[144,145] The *nut* sequence consists of two parts: a conserved 9-nucleotide sequence called box A, which is found not only as part of λ*nut* sites but also in bacterial *rrn* operon antiterminator signals; and a 15-nt nucleotide sequence called box B, which encodes an RNA that would form a short stem, structure with an A-rich loop.[45] In the current model, NusB and S10 bind to the box A sequence on the RNA, whereas N recognizes the box B stem-loop structure.[144,145] These proteins are held together through interactions with core RNA polymerase that are stabilized by NusA and NusG as a ribonucleoprotein complex that stays attached to the elongating RNA polymerase.[142]

With the Q system, the modification presumably involves the formation of a complex with Q and NusA. One known consequence of the change is that the RNA polymerase does not remain at pause sites as long as the unmodified enzymes.[146] In other words, the modification increases the overall rate of transcriptional elongation. Because NusA itself enhances pausing at many sites, Q and N act not only to suppress pausing but to suppress as well the normal effect of NusA.

A major component of the termination process is the pausing by RNA polymerase at the termination region. Because the termination stop points are at the sites when $k_{forward} < k_{release}$, changes that would increase the intrinsic rate of elongation ($k_{forward}$) at specific sites could cause $k_{forward}$ to become greater than $k_{release}$. This could account for the effect of the antitermination factors at both intrinsic and rho-dependent terminators. In addition, however, the presence of the antitermination proteins attached to RNA polymerases could prevent rho in its action along the RNA from reaching the critical sites on RNA polymerase.

Part of the recognition of the *nut* signal to form the N-antitermination complex occurs by an interaction of N protein with the *nut* sequences in the transcript. This sequence forms a specific stem-loop structure[147] that is recognized by N protein in the transcription complex.[144,145] Because the *nut* site on the transcripts initiated at P_R of λ is situated within the two segments of the nascent transcript that form the binding site for rho to cause termination at tR1,[148] the binding of N and antitermination factors to the site can directly prevent rho from acting at tR1. This mechanism by itself, however, would not explain how the N-antitermination complex blocks rho action at further downstream sites.

The ability of RNA polymerase to terminate transcription can be intrinsically modulated by interactions established at the promoter and with the sequence at the 5′ end of the transcript.[149,150] It is possible that sequences at the 5′ end of the transcript can fill sites on the RNA polymerase

that either influence how the enzyme interacts with segments near the 3′ end or influence allosterically the nucleotide addition properties of the enzyme. The antitermination mechanism could well be just an enhancement of intrinsic properties of RNA polymerase by making use of proteins to recognize specific sequences in the RNA and to stabilize these intrinsic interactions with RNA polymerase.

C. *rrn* Operon Antitermination

RNA polymerase molecules that are engaged in transcribing the DNA sequence of a ribosomal RNA operon in *E. coli* are modified in a way that makes them bypass certain terminators. These include terminators that have been inserted in the rRNA gene (*rrn*) DNA sequences.[151] These polymerase molecules, however, do not bypass all terminators and do terminate transcription at the strong intrinsic sites at the ends of the *rrn* operons.[105] The probable purpose of the modification is to ensure that transcription of the rRNAs is immune from rho action.

The modification is established by recognition of a sequence signal that is nearly identical to one part of the *nut* signal of phage λ.[105] All *rrn* genes contain box A sequences in two locations.[152] The first is just downstream from the start point of transcription, the other is in the intergenic region between the 16S and 23S rRNA genes. Recently, Nodwell and Greenblatt[153] have shown that a heterodimer of NusB and S10 protein binds to the box A sequence RNA of the *rrn G* operon. They propose that the mechanism of transcriptional antitermination in the *E. coli rrn* operons, like that for λN protein, involves formation of a ribonucleoprotein complex on the box A RNA segment that is carried along with the elongating polymerase. The one known difference is that the *rrn* gene antiterminators do not seem to involve an N-like protein, perhaps because they do not require the added stability that N can impart to the λ complex. A role for NusB in *rrn* operon antitermination is also suggested from the observation that mutations in the *nusB* gene reduce the frequency of completing transcripts initiated at *rrn* promoters.[154]

D. Inducible Gene Expression by Antitermination: The *bgl* Operon

Although the expression of many operons in *E. coli* are known to be controlled by regulating the activity of transcriptional terminators in response to a metabolic signal, nearly all the known examples involve complex circuits that involve governing the interaction of ribosomes with the nascent transcript. In the case of the *bgl* operon, however, the induction of its expression by aromatic β-glucosides involves the inhibition of the function of terminators by a specific regulatory protein, not a ribosome.[155] This protein is BglG, the product of the first gene in the operon. It is an RNA-binding protein[156] that acts to inhibit termination at two intrinsic terminators, one located upstream of the *bglG* gene, the other in the intercistronic region situated between *bglG* and the two other genes of the operon, *bglF* and *bglB*. It binds specifically to a 32-nucleotide bulged-hairpin structure present in the two termination structures and, in so doing, interferes in each case with the function of the stem structure in the mechanism of termination.

The function of BglG is controlled through phosphorylation by action of BglF, a phosphotransferase β-glucoside membrane transporter.[157] In the absence of inducing β-glucosides, BglF catalyzes phosphorylation of BglG, converting it to a monomeric form that does not bind to its target RNA,[158] thus allowing the terminators to function. In the presence of β-glucosides, BglG becomes dephosphorylated, allowing it to dimerize and bind to its targets on the transcripts and thereby inhibit termination. The increased readthrough of the terminators raises the level of expression of *bglF* and *bglB*, the genes encoding the products responsible for metabolism of β-glucosides.

The antitermination mechanism resembles those for the *rrn* operon and the λ genes controlled by N in that the regulatory protein binds to a specific site on the nascent RNA. The function of BglG is regulated by a metabolic signal.

however, whereas the others appear to involve blanket regulations.

E. Anti-Rho-Dependent Termination

Bacteriophage P4 encodes a 21-kDa protein, the product of the *psu* gene, that antagonizes rho-dependent transcription termination.[159] Although its mechanism is unknown, the generality of its function could be easily accomplished by binding directly to rho itself or by catalyzing a chemical modification of rho.

The role of *Psu* protein as an antiterminator in the developmental cycle of P4 is most pronounced when the expression of late genes of P4's helper phage P2 is blocked by a polar mutation. However, with wild-type P2 as helper, the phage yield of P4 *psu* mutations is reduced three- to fivefold relative to that of P4 psu^+. Thus, P4 makes use of a general antitermination function to overcome the interference of rho in expression of the late genes.

F. Eukaryotic Attenuators

The transcription of several genes in eukaryotic cells is controlled by a mechanism that influences the continuation of RNA chain growth at steps shortly after initiation.[160] These genes have sequences that are serving the same functional role as the attenuators in *E. coli*. However, in some cases at least, these eukaryotic "attenuators" differ from their bacterial counterparts in that they contain transcriptional arrest sites rather than true terminators; the RNA polymerase molecules remain engaged in a ternary complex that can resume elongation when provided with the appropriate signal.

This kind of mechanism is important in the induction of a heat shock gene in *Drosophila melanogaster*.[161] The promoter for the *hsp70* gene allows RNA polymerase II to initiate transcription in uninduced cells and to synthesize ~25 nucleotides at which point the enzyme ceases further elongation. Upon receiving the heat-shock induction signal, transcription elongation resumes in a way that allows both the enzyme to escape from the transcription arrest site and further RNA polymerases, which initiate subsequently, to transcribe that region without pausing. The response to the heat shock depends on a specific sequence signal, a "GAGA" element, that resides upstream from the TATA sequence element in the promoter.[162] The fact that the pause occurs fairly close to these promoters and is regulated by untranscribed upstream sequences suggests that the signal may be affecting some late stage in the initiation process. Whatever the mechanism, it is a clear example of a control at the level of transcriptional elongation. One advantage of the mechanism is that it allows the gene to be poised for a rapid increase in transcription.

Another example of a regulatory system that apparently involves release from a transcriptional pause is in the infectious cycle of the human immunodeficiency virus (HIV).[163] A transcriptional arrest or termination site exists just downstream from the promoter for HIV in its long terminal repeat (LTR). In this case, arrest occurs after the host cell RNA polymerase II has synthesized ~57 nucleotide transcripts. Expression of HIV genes is regulated by the virally encoded *trans*activator protein, Tat. The target for Tat function is the *trans*acting responsive region (TAR) in the sequence from 19 to 42 nucleotides downstream from the startpoint of transcription in the HIV LTR. Tat is a small protein, with about 100 amino acid residues, that binds to the RNA transcript of the TAR regions.[164,165] This transcript forms a special stem-loop structure with a bulge from the stem.[166] Because Tat could readily bind to this structure in the nascent transcript with RNA polymerase II arrested at the site 57-nucleotides downstream from the transcription start point, its function could be to cause the arrested polymerase to resume elongation. Alternatively, because polymerase may actually be terminating transcription, the binding of Tat to the nascent transcript could convert RNA polymerase II to a form that bypasses the terminator. In either case, Tat would enhance the expression of the downstream genes.

Tat protein bears both a structural and functional resemblance to the λN antitermination protein.[144] N is also a small protein that binds specifically to a transcript containing a particular stem-loop structure. In addition, the RNA-bind-

ing properties of both proteins depend on segments that are rich in arginine residues.[165] Finally, both proteins modify the transcriptional elongation properties of the RNA polymerase with a nascent transcript that has the appropriate stem-loop target structure. Thus, the N-antitermination system serves as a useful paradigm for elucidating the mechanism of Tat functions.

Mechanisms for controlling expression by blocking or terminating transcription at an attenuator situated just downstream from a promoter occur in the developmental cycles of other viruses, including SV40 and adenovirus 2.[167,168] A candidate protein that could serve the function of overcoming the transcriptional block in the later stages of adenovirus infection is a product of an adenovirus gene that is essential for replication.[169] This protein has been shown to bind to the isolated, attenuated transcript and could thus be an antitermination factor.

VII. PERSPECTIVES AND SUMMARY

In spite of the important advances that have been made recently, the overall understanding of the termination process lags behind that for the initiation process. There are two major reasons for this lag. The first is based on the perception that the most commonly used mechanism for controlling gene expression involves the initiation of transcription. The realization that the expression of many genes is controlled at steps subsequent to initiation has given some impetus to studying the basic mechanisms for elongation and termination. The second reason is the technical difficulties involved in setting up suitable model systems for termination; the basic biochemical properties of initiation have to be solved to make substrates for the termination process. It is this latter requirement that is largely responsible for the paucity of solid biochemical evidence concerning the mechanism of termination of transcription with RNA polymerase II. This should change soon, as most of the components for initiation have been identified, isolated, and partially characterized. With the ability to achieve efficient initiation at specific promoters on DNA *in vitro*, attention can be turned to isolating the components that are necessary to complete the transcription cycle.

From the studies that have been made with partially purified systems, a few of the characteristics of RNA polymerase II mechanisms are apparent. There is some limited evidence that RNA polymerase II does respond to intrinsic terminators.[64] This includes both endogenous terminators, as in the attenuator downstream from the adenovirus major late promoter,[155] and prokaryotic intrinsic terminators inserted into a eukaryotic transcription unit. However, the characteristics of intergenic terminators in metazoan eukaryotic cells have some earmarks of a mechanism involving a rho-like factor. Several genes have been shown to contain termination sites that are downstream from the regions that encode the 3′ ends of mature, processed transcripts.[170] In some cases, the terminator appears to consist of a number of partially efficient sites that are spread over several hundred base pairs of DNA. The functioning of some of these RNA polymerase II terminators has been shown to be coupled to the functioning of the 3′ end maturation signals;[171] defects in the signal that prevent cleavage of the nascent transcript also prevent the functioning of the downstream terminators. This observation has inspired an attractive model for transcription termination. Because the cleavage reaction creates a new 5′ end for the nascent transcript, that new end could be the entry point for a rho-like termination factor. Once on the RNA, the factor would use an ATP hydrolysis reaction to fuel its translocation along the RNA to reach RNA polymerase II and to cause dissociation of the transcription complex. The attractive feature of this model is that the presence of the cap at the 5′ end of the uncleaved nascent transcript would serve as a marker to keep the transcript immune from action of the factor. In addition, it would assure that termination occurred at some point soon after the functional units of the transcript have been cleaved in the maturation reaction. This model makes several predictions that should be testable once the appropriate purified transcript system has been developed. However, this model also predicts that the system will depend not only on initiation factors, but on capping enzymes and the polyadenylation maturation system as well. Assembling

all these components in a reconstituted system is a daunting task.

One critical feature of this proposed model for RNA polymerase II termination is the establishment of biochemical immunity to a signal at the promoter. This is reminiscent of the λQ and *E. coli rrn* antitermination mechanisms. There is also already strong experimental evidence that eukaryotic cells have mechanisms that set a pattern of maturation of the transcripts at their 3′ ends that is established at certain promoters for RNA polymerase II. Although most transcripts synthesized by that enzyme are destined to become mRNA molecules, some become small nuclear RNAs. These two classes of RNAs are processed differently at their 3′ ends, and the ability to recognize the specific processing signals depends on interactions that are established at their promoters. Transcripts initiated at a snRNA gene promoter are processed at their 3′ ends in response to a downstream signal. However, transcripts that are initiated at a regular promoter for a gene that yields a mRNA are not processed in response to that same signal.[172] Somehow initiation at the snRNA promoter alters the RNA polymerase so that the processing enzymes can gain access to the transcript on passing through the end-formation processing signal.

The synthesis of RNA by DNA-dependent RNA polymerase can be terminated in a number of mechanistically distinct ways. There are now several good model systems for studying the details of two of the fundamental mechanisms used by *E. coli* RNA polymerase and although there is a pretty good understanding of these mechanisms at a superficial level, many of the specific steps have yet to be worked out. Because the fundamental process of transcription is the same for all RNA polymerases, it seemed likely that the mechanism used for termination might be basically the same for all enzymes. This assumption has already been proven wrong with the finding that the mechanism for termination of transcription with RNA polymerase I is fundamentally different from any of the known mechanisms for the bacterial terminators. Similar surprises may also come from elucidation of the mechanisms used by RNA polymerase II of eukaryotic cells. At the least, a thorough understanding of the known mechanisms has provided a framework for defining the basic features of the termination process. This should help guide us in the further quest of resolving the details of the known mechanisms and in discovering the nature of the remaining major unknown mechanisms.

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