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RNA Polymerase Elongation Factors

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Abstract

The elongation phase of transcription by RNA polymerase is highly regulated and modulated. Both general and operon-specific elongation factors determine the local rate and extent of transcription to coordinate the appearance of transcript with its use as a messenger or functional ribonucleoprotein or regulatory element, as well as to provide operonspecific gene regulation.

Keywords

antiterminator; termination; pausing

INTRODUCTION

The movement of RNA polymerase (RNAP) as it transcribes DNA is not uniform and inevitable, but instead it is modulated by regulatory influences that accelerate it or slow it or determine if it stops altogether. For bacterial RNAP the best-characterized examples are antiterminators, operon-specific genetic regulatory elements that allow genes downstream of a terminator to be expressed. Other processes coordinate transcription with the use of the transcript—usually of course as messenger or “structural” RNA, although RNA can be a regulatory element itself. Thus, in regulation by an attenuator RNA or certain riboswitches, the rate of RNA synthesis is critical to the function. A global view is that utilization of the emerging transcript is a precisely evolved pathway of interactions with the translation apparatus, processing factors, and the RNA degradation machinery. Disruption of this pathway can interfere with RNA function and cause potentially deleterious free RNA to accumulate; in response, the cell has a mechanism to stop futile transcription. Furthermore, transcription can be blocked by accident, for example, when a noncoding lesion like a thymine dimer is encountered in the DNA template strand or during clashes with processes of replication and recombination. In these cases cellular processes remove the transcription complex.

We consider first the establishment and structure of the transcription elongation complex, and then elongation factors that act upon it. Structural and functional studies have revealed how nucleic acids move through RNAP and what interactions between nucleic acids and enzyme stabilize the complex. This provides a basis to understand the activity of both intrinsic modulatory signals in the DNA—i.e., pause and termination signals—and regulatory proteins. Transcription termination and release of the transcript can be described in some detail, and several specific classes of pause-inducing sequences that provide signals and substrates for regulatory interactions are known. The most complete description of a regulatory function in

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transcription elongation is antitermination in *Escherichia coli*, mediated by the bacteriophage λ gene *N* and *Q* proteins, the cellular RfaH protein, and the cellular ribosomal RNA gene antitermination system that is analogous to that of λN. Several proteins discovered through studies of antitermination—primarily NusA and NusG—determine the nature of pausing. These are essential in *E. coli* and highly conserved in bacteria, and although some biochemical activities and even atomic structures are known, it is difficult to describe their precise cellular function. Specialized proteins deal with accidental disruption of the elongation reaction: Gre and Mfd factors catalyze, respectively, the removal of an aberrant RNA 3′ end so that RNA synthesis can be restarted from an active primer, and the release of enzyme irretrievably blocked by a lesion in the DNA template strand. Other recent reviews have considered in greater detail some of the topics discussed below, as well as topics not discussed (e.g., attenuation) (11,94,109,150).

BASIC PROCESSES OF TRANSCRIPTION

Initiation of Transcription

Once an open promoter complex is formed, the initial stage of RNA synthesis occurs with melting of downstream DNA and scrunching of DNA of the growing bubble into the enzyme, during which process the contacts of the initiation factor sigma with promoter elements are maintained. Energy stored as scrunched DNA may be used to break sigma-promoter contacts, thus effecting the transition to elongation (49,108,135). When this transition fails, the RNA aborts, a feature common to in vitro transcription by most RNAPs. Importantly, Gre proteins (see below) suppress abortive initiation and promote elongation.

Elongation of the RNA Chain

The elongation cycle comprises three basic steps (142): (*a*) Binding of a template-complementary nucleoside triphosphate (NTP) into the active site; (*b*) chemical reaction of the RNA chain 3′-OH with the NTP α-PO_4, catalyzed by a pair of bound Mg^{2+} ions, resulting in one NMP addition to the RNA and liberation of pyrophosphate; and (*c*) translocation of the nucleic acid assemblage to place the next template base in the active center. The elongation complex of RNAP (Figure 1) is stabilized by several sets of interactions among protein and nucleic acids (58,93,141): (*a*) downstream duplex DNA is bound within the enzyme; (*b*) about nine nucleotides of RNA at the growing end are annealed to the template DNA strand, forming a 9-bp RNA/DNA hybrid enclosed by protein; and (*c*) an additional ~5 nucleotides of RNA upstream of the hybrid are bound in a protein channel until the RNA emerges 14 nucleotides from the growing end. Despite the high stability of the complex to dissociation, the various interactions allow lateral mobility of DNA and RNA through the complex during translocation. In fact, the RNA alone has some mobility, showing an ability to slip relative to the template; this is observed at positions where a template homopolymer sequence is longer than 9 bp, so that the slipped position is not destabilized by DNA/RNA mismatches in the hybrid (145).

A remarkable activity of transcribing RNAP is to move backward along the template, or backtrack, reversing the translocation steps that assembled the RNA chain but not depolymerizing the chain itself; the RNA 3′ end protrudes as single-stranded RNA from the secondary channel, an aperture that connects the active center to the outside and is believed also to be the entry site of the NTP substrate (57,95). Backtracking is likely a response to some failure of the RNA 3′ end to be elongated, as might occur, for example, through chemical damage or misincorporation. Backtracking is strongly stimulated by weak or disrupted pairing in the RNA/DNA hybrid where templating of the RNA occurs (95), consistent with its being a response to an aberrant hybrid.

Transcription Pausing

The average transcription rate of *E. coli* RNAP is in the range of 50–100 nucleotides per second, equaling a translocation step time of 10–20 ms. But the enzyme pauses frequently, remaining at the same template site for up to tens of seconds or more. Some of these pause events are significant and are detected both in vivo and in vitro, as described below. In addition to known authentic pause sites, however, single-molecule assays of transcription in vitro display continuous, low-efficiency pausing, apparently from common sequence features that modulate the elongation rate (2,44,88). Possibly this ubiquitous pausing reflects a finely detailed evolution of transcription rate to match the particular fate of the transcript. However, it cannot be excluded that much of this is an unnatural property of the purified transcription system.

A major reason for interest in transcription pausing is that antiterminators antipause (6,53, 106,153), meaning they reduce the half-life and/or efficiency of pausing at some sites. Furthermore, mutations in RNAP core subunits can either enhance or reduce pausing (29). It has long been thought that antipausing could explain antitermination (153), because the uridine-rich segment of intrinsic terminators induces pausing at the release site even in the absence of the hairpin, and certain Rho terminators are strong pausing sites in the absence of Rho (64,85). Furthermore, it is plausible that the pause is kinetically necessary to provide time for the termination event. It is impossible by standard methods to measure a pause associated with a termination event of an active intrinsic terminator, so that no computational resolution of this proposal by considering pausing kinetics is possible, but other evidence that antipausing can underlie antitermination is described below. For neither core subunit mutations nor most antiterminators is it known if the effect is only on specific classes of pause sites, where their effects are well documented, or if there is a general effect on elongation in every translocation step.

In addition to pausing at terminators, there exist several well-defined types of sequence-based pausing with established regulatory consequences. Before it dissociates from the transcribing core enzyme, the σ^{70} initiation factor can recognize and bind a near repeat of the −10 component of the promoter sequence early in the transcription unit, inducing a transcription pause of the order of seconds in vivo; such a paused RNAP is recognized by the phage λ gene *Q* antiterminator as it engages RNAP (111,114). High concentrations of σ^{70} can induce pausing in vitro at such sequences far from the promoter (82), but this may not occur in vivo. There is a significant occurrence of promoter-proximal sigma-dependent pauses in bacterial operons, but their regulatory significance is unknown (13,42,89).

Early in the 5′-leader RNA portions of the *his* and *trp* operons of *E. coli* and *Salmonella* (60) there is a transcription pause dependent mostly upon an RNA hairpin that forms in the emerging RNA and binds the β flap of core RNAP (138). Its function is to synchronize translation with transcription, which is required for operon regulation mediated by uncharged tRNA (59). The efficiency of pausing is determined partially by sequence elements surrounding the hairpin-coding segment (66). This pause is not backtracked; instead, there is evidence that pausing occurs because structural rearrangement of the active site prevents nucleotide addition to the RNA 3′ end (139).

A pause at the operon polarity sequence (OPS) site of *E. coli*, where the RfaH antiterminator binds and engages the elongating complex, is backtracked, so that the RNA 3′ end is unavailable until the complex isomerizes forward (5). Although there may be other important sequence elements, the major one would appear to be a GC-rich region preceding a U-rich region. This structure, similar to that at the release site of intrinsic terminators, promotes backtracking because the RNA/DNA hybrid containing GC-rich RNA is much more stable than the U-rich hybrid.

Termination

Three processes destabilize the elongation complex and release the transcript.

Intrinsic (or hairpin) termination—Intrinsic (or hairpin) termination is encoded in nucleic acid. Intrinsic terminators include most defined termination sites in bacterial transcription units, although this may be in part because they are fairly well recognized by sequence analysis. The intrinsic terminator has two essential components: a GC-rich hairpin that forms in the emerging transcript and is closed about nine nucleotides upstream of the RNA release site, and an adjacent U-rich segment that extends through most of the hybrid region. Release accompanies formation of the hairpin, an event that is believed to shorten the RNA/DNA hybrid, thus destabilizing this critical region of the complex, followed by dissociation (41,56,119,156). The upstream portion of the hairpin can be replaced experimentally by a DNA oligonucleotide added in *trans* (156), implying that the function of the hairpin formation is probably mechanical, i.e., the hairpin as a structure is not required. The weakness of the rU/dA hybrid in the terminal region is thought to facilitate its unwinding and dissociation (78). Rewinding of the upstream hybrid region is essential to efficient termination, demonstrated by the failure of artificial heteroduplex templates to support termination (116). Hybrid shortening can occur by forward translocation of the enzyme, i.e., rewinding of upstream DNA and unwinding downstream without elongation of the RNA (62,119), which also occurs in dissociation of stopped elongation complexes of bacteriophage T7 RNAP (160). Other pathways of RNA release exist; in particular, the homopolymeric terminal sequence can support slippage of the transcript past the template in RNA release by the T7 terminator (62,74). Single-molecule analysis of termination suggests an important distinction among intrinsic terminators: If the uridine-rich terminal segment is interrupted by other bases, forward translocation occurs, whereas a homopolymeric sequence allows RNA release through slippage (62). A terminator that otherwise acts by forward translocation supports (slower) RNA release, presumably by slippage, if forward translocation is prevented by an interstrand DNA crosslink (119), suggesting that both mechanisms might apply to a terminator in different conditions.

Rho-dependent termination—Rho-dependent termination depends upon the Rho termination factor, an essential (*in E. coli*) hexameric ATP-dependent RNA translocase that binds the emerging transcript and is believed to pull it out of the transcription complex; in effect, ATP provides the energy that, in the case of the intrinsic terminator, is provided by hairpin formation (21,109,128). Rho provides the natural termination mechanism for many transcription units, as detected by extensive transcription into intergenic regions when Rho is inhibited by the antibiotic bicyclomycin (18). More broadly, Rho prevents accumulation of RNA that is not being utilized, either by the translation apparatus or by incorporation into an RNA-protein complex, as occurs normally when the noncoding ribosomal RNA is bound by ribosomal protein, tRNA is bound by synthetases and translation elongation factors, and small regulatory RNAs are bound by Hfq (37). Thus, Rho is the agent of operon polarity, the interruption of downstream gene expression by a nonsense codon; polarity results from Rho binding the ribosome-free RNA and stopping transcription (3,30,110). There is evidence that the problem with free RNA is its incorporation through strand displacement into DNA to form R-loops, the deleterious consequence of which is not understood (38). R-loop formation would be facilitated by the unconstrained negative supercoiling of the *E. coli* chromosome, but in fact R-loops are deleterious in eukaryotic cells as well (47). Rho-dependent terminators are the targets of the antitermination system of ribosomal RNA transcription (23), the bacteriophage λ gene *N* antiterminator (113), and the *E. coli* tryptophanase operon antitermination control (154).

Mfd-dependent termination—The transcription repair coupling factor Mfd is an ATP-dependent DNA translocase that dissociates a transcription complex by binding both RNAP

and DNA emerging upstream from the complex (25,100,122). Mfd recognizes stalled RNAP rather than a particular genetic site, as can be demonstrated with any stalled complex in vitro. Mfd acts in DNA repair by recognizing RNAP stalled by a noncoding lesion in DNA, removing it from DNA, and mediating recruitment of the UvrABC excision repair enzymes to the site, using a domain believed to have affinity to UvrA (122).

Antitermination

Regulation of gene expression through antitermination was discovered in bacteriophage λ (113). Detailed genetic analysis of N-mediated antitermination uncovered the essential elongation proteins NusA and NusG, in addition to the involvement of other cellular proteins such as NusB and the ribosomal protein S10 (150). A common feature of antitermination by λN, λQ, *E. coli* RfaH, and the RNA-based *put* system of the λ relative HK022 is that the antiterminator binds at a genetically specified site and forms a persistent complex with RNAP that causes it to bypass terminators. Furthermore, this modification inhibits RNAP pausing (6,53,106,153), implying some basic effect on the elongation reaction. This discovery, along with the fact that both intrinsic and Rho-dependent terminators have associated pausing, suggested that antipausing can underlie antitermination, which is at least partly true (126). All four systems are studied with purified components in vitro, leading to mechanistic insights at the molecular level; these are considered in detail below.

CELLULAR MACHINERY OF ELONGATION CONTROL

Gre Proteins

Like their eukaryotic counterpart transcription factor IIS (TFIIS), the universal bacterial Gre proteins mediate activities of the active center of RNAP by binding in and near the secondary channel and projecting a finger into the region of the active center (61,98). Gre proteins (GreA and GreB of *E. coli*) were discovered, through their activity to stimulate hydrolysis of a backtracked elongation complex by the RNAP active center of a backtracked elongation complex (12,99). The reaction yields a 3′-OH primer end in the active center and a 3′-terminal oligonucleotide that diffuses away through the secondary channel. Gre enables catalysis of the reaction by providing two carboxylate residues at the inserted end that chelate an Mg^{2+} (61, 98) and complete the two Mg^{2+} set required for all reactions catalyzed by the active center, including hydrolysis (132). During chain elongation, this function is believed to be provided by the incoming NTP (132). Gre-stimulated cleavage of RNA would rescue complexes trapped in a backtracked state, although only a few specific instances of natural backtracking are known. One type of obstruction that gives rise to backtracking is the σ^{70}-dependent pause of bacteriophage λ (see below) (111). This paused complex, which presumably achieves a scrunched state as downstream DNA moves through the active center while the core framework is restrained by σ^{70}, can collapse into a Gre-sensitive structure that impedes release of enzyme from the pause both in vivo and in vitro (77). Other sorts of binding events that restrain RNAP from moving along DNA could induce backtracking and require rescue. In addition, the elongation complex might respond to collisions with barriers downstream by eventually diffusing backward into an arrested state that requires rescue by cleavage.

A specific proposal relates backtracking to proofreading of misincorporated NMP at the growing point (158). Because the mispaired base at the end provides little stabilizing energy, misincorporation favors not hydrolysis of the single mismatched terminal nucleotide, but instead backtracking, so that the active center is positioned at the previous phosphodiester bond; hydrolysis then removes a terminal dinucleotide (132). It is proposed that the second Mg^{2+} required to catalyze the hydrolysis is chelated by these two terminal nucleotides, an activity that promotes proofreading. This chelation of Mg^{2+} might be a primitive function of RNA that was usurped later by protein when the Gre factors evolved (158).

Gre proteins also promote the initial step of elongation by inhibiting abortive initiation (46), a presumably aberrant pathway in which the emerging RNA is lost and the incipient elongation complex collapses. The failure of a Gre protein altered to lack the terminal Mg^{2+}-binding carboxylates to inhibit abortive initiation (134) suggests that cleavage is required—but it does not prove it, because some other change in the environment of the active site could stabilize the scrunched intermediate in the initial elongation step and promote elongation. A possible precedent is the activity of the initiation factor DksA, a Gre-like protein that also projects into the active center through the secondary channel (105) and acts not to mediate cleavage, but instead to destabilize the open complex through interactions around the active site (104).

NusA

The highly conserved bacterial transcription elongation factor NusA, discovered over three decades ago because of its role in antitermination (31), is an intriguing protein with diverse and apparently contradictory effects on transcription elongation and termination. Its most apparent biochemical activities are to enhance pausing during transcription with purified RNAP (120), particularly at RNA hairpin-induced pauses (6), and to increase the activity of intrinsic terminators (120). Contrarily, NusA also is an essential component of the λ gene *N* antitermination complex and important for the λ gene *Q* antiterminator, both of which inhibit pausing; thus the activity of NusA is subverted to a distinct function by the antitermination proteins.

E. coli NusA is a 55-kDa monomeric RNA binding protein. Structural studies of the closely related *Mycobacterium tuberculosis* NusA either alone or in combination with RNA (10,36) reveal an elongated protein with three distinct domains: (*a*) an N-terminal RNAP binding domain; (*b*) a middle portion comprising three RNA binding globular domains, S1, KH1, and KH2; and (*c*) a C-terminal autoinhibitory domain. RNA binding is cryptic in full-length NusA (71) but revealed by deletion of its C-terminal domain (CTD) (75). A contact point of NusA to RNAP is the α-CTD, which binds the NusA CTD and potentiates RNA binding by the S1 and KH domains; cross-linking experiments suggest that RNA previously bound to the α-CTD now binds NusA (71). Deletion of the α-CTD leads to a loss of NusA functions such as enhanced pausing, Q-mediated antitermination, and Q-mediated occlusion of the RNA transcript (72,126). NusA does not require the α-CTD for its function as a cofactor of antiterminator λN (72). Binding assays and affinity chromatography show that λN binds the NusA CTD and thus presumably provides a function for which the α-CTD is needed in other NusA activities (75).

In addition to the NusA CTD binding site on the RNAP subunit α-CTD, the NusA N-terminal domain (NTD) has been suggested to have homology to σ^{70} region 2 (11) and thus to bind the same N-terminal coiled-coil of the β′ subunit of RNAP to which the σ^{70} region 2 binds; some cross-linking evidence supports this (140). Consistently, NusA binds to core RNAP in vitro but not to holoenzyme-containing σ^{70} (40). Other cross-linking experiments and RNA protection studies with functional elongation complexes suggest that NusA likely makes contacts near the RNA exit channel, around the β-flap domain (41,71,126,138); conceivably, an extended conformation of the NusA protein contacts both regions, not unlike the wide reach of σ^{70}. NusA no longer enhances pausing when the flap-tip helix of the β subunit is deleted, again suggesting an interaction with the β-flap domain (138).

The biochemical basis of pause enhancement by NusA is not understood. One view is that pausing frequently derives from RNA structure in the emerging transcript, like that of the *trp* and *his* hairpins but perhaps less extensive; NusA stimulation of the formation of such structures would increase pausing (138). The hairpin, in turn, is proposed to act through long-range allosteric changes that distort the active center and inhibit polymerization. Alternatively, NusA binding might allosterically re-shape protein structures adjacent to the nucleic acid

binding pocket, including the active center, to affect pausing; a precedent is mutations in the rifampicin binding pocket that incidentally affect pausing (29). Whatever the mechanism, this activity of NusA is fundamentally altered when an antiterminator (λQor λN) is present. For example, NusA binds reversibly to RNAP in affecting pausing and termination (120) but is believed to bind irreversibly in an antitermi-nation complex with N (8,45) or Q of bacteriophage 82 (82Q) (126). Presumably, interactions with the antiterminator and other factors provide the extra stability in the complex. 82Q actively inhibits pausing by itself, and incorporation of NusA into a complex with 82Q does not increase pausing; 82Q is dominant over NusA in the complex (153). One interpretation is that emerging RNA in a complex containing both 82Q and NusA is structured differently from RNA in a complex containing NusA alone; a further aspect of this structure is that emerging RNA is protected for about 10 nucleotides from nuclease attack in a completely NusA-dependent way, an activity that 82Q alone does not have (see below).

How does NusA enhance intrinsic termination (120,127)? The effect could be secondary to stimulation of pausing, through simple kinetic effects that provide more time for the intrinsic termination mechanisms to act; there is a competition between termination and elongation at the site of release of intrinsic terminators. However, NusA probably acts directly to stabilize the terminator hairpin: In a model system that uses a small oligonucleotide to mimic a terminator hairpin, NusA enhances RNA release from static transcription elongation complexes. Presumably, NusA stimulates formation of the DNA/RNA hybrid that substitutes for the terminator hairpin (126), consistent with previous views of NusA function (41,138).

Despite the presumption that NusA is universally involved in cellular transcription, its major known role is in antitermination of bacterial ribosomal RNA (rRNA) operons, an activity discerned both in vitro and in vivo and probably similar to the N antitermination system (23, 144). Furthermore, there is direct evidence for NusA influence on mRNA elongation in vivo from a study of response to starvation. In the stringent response, the nucleotide guanosine 3′ 5′-bisphosphate (ppGpp) accumulates to high concentration through synthesis from GTP, and one effect of ppGpp is to slow mRNA elongation. In a NusA mutant, this effect disappears, suggesting that NusA mediates a ppGpp-dependent slowing of transcription (144). Despite an obvious similarity, it is not clear how this ppGpp-dependent slowing is related to the pausing stimulated by NusA in vitro; perhaps other cellular factors are involved.

NusA is essential in wild-type *E. coli*, although in combination with other mutations a *nusA* deletion can survive (159). A recent discovery clarifies this: Deletion from *E. coli* of horizontally acquired DNA, including cryptic prophages and transposons, allows *nusA* and *nusG* deletions to survive, although they grow slowly. The single genetic locus making *nusA* essential is the cryptic prophage *Rac* (18). Furthermore, the reduced genome strain is less sensitive to the Rho inhibitor bicyclomycin, suggesting that high activity of Rho is required for cell survival because it terminates transcription of these prophages. Microarray expression analysis of the reduced genome cell reveals strikingly similar patterns of gene expression in three conditions: inhibition of Rho and deletion of either *nusA* or *nusG* (18). Because NusG stimulates Rho-dependent termination in vitro (14), the result is consistent with NusG acting as a cofactor of Rho in vivo. This result also clarifies the effect of NusA in Rho-dependent termination: Although NusA somewhat inhibits Rho function in vitro, this activity is probably irrelevant. Instead, NusA acts with Rho to stimulate termination in vivo, and an attractive model is that stimulation of pausing by NusA synchronizes Rho function with the emerging transcript by slowing RNAP and allowing Rho time to act.

NusG

NusG is a bacterial transcription elongation factor that was identified biochemically as an essential component of the phage λN-mediated antitermination complex (68) and genetically

as a suppressor of *nusA* or *nusE* mutants that impair N function (137). It is highly conserved among bacteria and archaea and is homologous to elongation factor Spt5 of eukaryotes. NusG has two known activities detectable both in vivo and in vitro: It inhibits pausing and increases the rate of elongation (17), and it enhances Rho-dependent termination (14), particularly in suboptimal conditions. NusG binds both Rho and RNAP, providing a possible link between them; however, Rho is active in vivo in the absence of NusG (18), so that this link cannot be essential. Stable NusG association with a transcription elongation complex has been detected only in the presence of either NusA or Rho. That these are RNA binding proteins suggests that NusG recognizes RNA-bound protein in the complex (68). However, NusG has no reported affinity for RNA by itself. NusG inhibits a backtracked pause but not an RNA hairpin-stabilized pause, suggesting that it acts by stabilizing the forward translocated state of the transcription complex (5,102).

The ability of NusG to stimulate Rho activity but inhibit pausing appears paradoxical, because pausing is believed to be an essential prelude to Rho activity. Most likely, NusG stimulates Rho function in some way independent of its effect on pausing. Kinetic studies revealed that NusG is required for Rho-dependent termination when RNAP is elongating rapidly but not when elongation is slowed by low NTP concentrations (14), suggesting that NusG stimulates interaction of Rho with the elongation complex. In fact, NusG enhances the rate of Rho-mediated RNA release from stalled elongation complexes (15,86). NusG also causes a promoter-proximal shift in the end points of Rho-terminated RNA and allows Rho to use shorter segments of upstream RNA than NusG can use alone, both effects indicating that NusG enhances Rho interaction with RNA (87). As discussed below, studies of the operonspecific transcription factor RfaH, whose NTD is a close paralog of NusG, suggest strongly that NusG binds the same coiled-coil structure of the RNAP β′ subunit as does σ^{70} region 2.

Rho

Several early studies uncovered the biochemical basis of Rho action. First, Rho is an RNA-dependent ATPase (73), reflecting energy input in termination that depends upon binding the transcript. Second, the amount of transcript required for termination is a substantial 60–80 nucleotides of relatively unstructured RNA (63,84). There is a distinct preference for cytidine and, to a smaller extent, uridine in the RNA, originally discovered as the high activity of polyribocytidylic acid in the ATPase assay (73). Assays with different polynucleotide activators revealed two types of RNA interaction sites, termed primary and secondary; the primary sites reflect the preference for cytidine (35,81). Despite the presence of some recurring oligonucleotide motifs in a few instances, there is no sequence-specified Rho binding site: sites of Rho action are determined mostly by the functional context of the RNA, e.g., the absence or presence of translation. Nonetheless, these sites may be well determined, as in the phage λ early operons, in the *E. coli* tryptophanase leader region (154), or presumably in the ends of a large fraction of *E. coli* operons (18). Or, the sites may be contingent upon the failure of translation, for example, after a nonsense mutation in a structural gene or accident or stress that reduces the availability of charged tRNA.

Structural analysis of Rho in complex with RNA gives a detailed picture of its function (128). The protein is a hexamer of 48 kDa subunits, each of which comprises an N-terminal RNA binding domain and a CTD with both RNA binding and ATPase activities. The primary and secondary RNA binding sites can be associated with the two domains: Primary binding to the NTDs occupies the major 5′-terminal segment of the RNA, and the secondary sites in the CTDs bind the 3′ portion of the RNA and direct it through the cavity in the center of the hexamer (128). The otherwise closed hexamer ring can exist as a lock washer variant in which the ring is separated at one monomermonomer interface, allowing RNA to enter the cavity without a free end (128).

ATP binding and hydrolysis drive a sequential binding of RNA segments that propels RNA through the hexamer in a 5′-3′ direction. In effect, the protein is an ATP-dependent RNA translocase (1,128). A widespread view is that Rho tracks along the RNA in this direction, presumably faster than RNAP emits RNA, and then effects termination when it catches up to RNAP. Although relative movement of RNA and protein are central to RNA function, it is not known that Rho moves a substantial distance from its initial binding site to release RNA; instead, the movements may reflect the act of RNA release after Rho binds initially to its site of action in the elongation complex. It is also unknown how and where Rho contacts RNAP. The only demonstrated protein interactions are between Rho and NusG (69) and between NusG and RNAP (68), so that NusG could be a natural bridge. However, Rho works both in vitro and in vivo in the absence of NusG (18). Rho may act in an untargeted mechanical fashion against RNAP (121), a notion supported by the ability of Rho to release transcripts from distantly related RNAP II of eukaryotes in vitro (26).

A unifying view of the activities of intrinsic (hairpin) terminators and Rho is that both hairpin formation and the ATP-dependent Rho translocase exert force to extract RNA from the complex (101). As for the intrinsic terminator, collapse of the upstream segment of the open transcription bubble at the site of release is necessary for efficient RNA release by Rho, i.e., the energy of DNA rewinding helps drive release (101,116). This evidence for branch migration at the back end of the transcription bubble, along with the absence of homopolymeric sequences at the release sites of Rho-dependent terminators, supports a forward translocation model for Rho function.

Mfd

Mfd stands for mutation frequency decline, reflecting its function in transcription-coupled DNA repair, a universal cellular process that removes transcribing complexes obstructed and trapped by nontemplating DNA lesions (122). In fact, any stopped transcription complex is a target of Mfd in vitro, and a nonrepair function is revealed by *mfd* mutants that facilitate transcription through transcription roadblocks in vivo (157), presumably by allowing RNAP to remain undisturbed until the block diffuses away. Mfd not only removes stopped transcription complexes, but it also recruits the DNA excision repair machinery to the site, an activity that is little understood.

Mfd is a 130-kDa multidomain protein with several well-characterized functions: an ATP-dependent DNA translocase, an RNAP binding domain, a putative UvrA binding domain, and a C-terminal regulatory domain (25). A high-resolution atomic structure of Mfd provides a basis for modeling Mfd function and its interaction with RNAP (25) and suggests how the regulatory domain may control interaction with excision repair enzymes. Mutational studies reveal the details of its interaction with the β subunit of RNAP and the molecular motions that underlie the DNA translocase activity, and show that the regulatory domain inhibits translocase activity until Mfd is bound to an elongation complex (19,130,131).

The mechanism of Mfd-mediated RNA release was revealed by the finding that Mfd induces forward translocation of RNAP in stopped complexes in vitro, as demonstrated with persistently backtracked complexes (100). Mfd simultaneously binds about 20 bp of DNA emerging upstream from the enzyme and a site on the β subunit, using the translocase activity and the energy of ATP to force the enzyme downstream. Because backtracked complexes are released if no NTP substrates are available, but are rescued into productive elongation in the presence of NTPs, release must be preceded by translocation to the fully forward position and must also occur from this position; this result indicates that a continuation of the action that induced forward movement is responsible for release (100,101). Further, as for the intrinsic terminator and Rho, RNA release is facilitated by rewinding of DNA in the upstream transcription bubble region (101). These results suggest two likely complementary models of

release: If there is a blockage that prevents further RNAP movement, collapse of the bubble within the complex from the torque imposed by the DNA translocase activity induces release, whereas failure of elongation (e.g., from a nontemplating lesion) where there is no blockage induces release by hybrid shortening through continued forward translocation with unwinding of downstream DNA.

ANTITERMINATION SYSTEMS

N Protein of Phage λ

Bacteriophage λN and λQ proteins modify *E. coli* RNAP into a termination-resistant form that allows readthrough of downstream termination signals. Antitermination was discovered through studies of the λ early promoters *pL* and *pR*, whose service to distal regions depends upon N allowing readthrough of nearby Rho-dependent terminators (113); the persistence (sometimes called processivity) of the N modification was revealed by showing that the sites of engagement and action of N are different (34). The N binding/modification sites *nutL* and *nutR* (N-utilization) (117), which are downstream of *pL* and *pR* but before the terminators, function as RNA. A complex including *nut*, N, and the *E. coli* accessory factors NusA, NusB, NusE, and NusG remains associated with RNAP after *nut* is transcribed, endowing persistence (Figure 2) (8,45). The *nut* sites consist of the *boxA* sequence and *boxB* stem loop, separated by a short spacer region (20,97) (Figure 2).

Numerous interactions construct the stable N antitermination complex: the ARM (arginine-rich motif) of the N-terminal portion of the 107-amino-acid λN protein binds to *boxB* (20,65, 67); a complex of NusB and NusE binds to *boxA* (69,79,92); the core subunit α-CTD binds to NusE (103); N binds to NusA, as well as some unknown core contact (40); and NusA binds to RNAP. Furthermore, NusG binds RNAP. There is no information about N contacts to RNAP core subunits. Whereas N alone can modify elongation complexes in vitro (107), and a complex of N and NusA alone works better (151), stable antitermination at distances from the promoter requires the complex (24,80,107).

Several of the host factor requirements, including NusA, NusB, and NusE (ribosomal protein S10), were identified genetically in screens for mutations that abolished N function (31–33). The absolute requirement of NusA for N function in vivo is shown by the failure of λ, but the ability of a λ N-independent variant, to grow on *nusA* deletion strains (18,159).

N antiterminates at both Rho-dependent and intrinsic termination sites. For a Rho-dependent site near *nutR*, the mechanism may be simply obstruction of the Rho binding site by proteins of the *nut* complex (143). However, for the persistent antitermination activity downstream there are two potential mechanisms: antipausing that speeds RNAP through the critical release sites faster than some rate-limiting step in either termination mechanism, and stabilization of the complex, for example, by blocking some step of termination. Measurements of pausing at terminators and antipausing induced by N purport to rule out antipausing as a mechanism (41,106); however, these experiments do not determine the actual kinetics at normal terminator release sites in real time. There is evidence that N-modification suppresses termination by directly preventing the formation of the hairpin at an intrinsic terminator (41). We argue below that Q protein antiterminates by both mechanisms.

Q Protein of Phage λ

The phage λ family Q proteins are antiterminators that regulate phage late gene expression by becoming become subunits of RNAP (27,156) through a pathway distinct from that of N protein: They bind to a transcription elongation complex held at a σ^{70}-dependent pause site near the promoter, specifically recognizing a site in DNA (Figure 3) (111,114,155). The paused complexes, which contain 16–25 nucleotides of RNA among the related phage, form

independently of Q and are induced by a reiteration of the −10 promoter element that binds σ^{70} region 2 in the open promoter complex. In the paused complex σ^{70} regions 2 and 4 occupy positions similar to those in the open promoter complex, even though there is no sequence like the promoter −35 element present (76). The linker between σ^{70} regions 3 and 4 is displaced in the paused complex, relative to the open complex, which is expected because emerging RNA occupies the same channel. Complexes stopped artificially at this site in conditions in which σ^{70} is not present are not modified by Q, such that σ^{70} provides an important structural or functional role in addition to stopping RNAP (155).

In the engagement complex, Q contacts a DNA site overlapping that occupied by σ^{70}-region 4, necessitating its displacement. In particular, λQ stabilizes the binding of region 4 to a DNA site adjacent to that bound by σ^{70}-region 2 through a specific protein-protein contact (91). λQ binding to the DNA in the context of the paused early elongation complex (which contains a 16-nucleotide nascent RNA) is strengthened or weakened by σ^{70} region 4 mutations that, respectively, weaken or strengthen binding of region 4 to its natural site on the β subunit flap. Thus, before λQ displaces it, σ^{70} region 4 is bound to the β flap of core RNAP in the paused complex (90). Because Qmodified complexes that proceed downstream in vitro lack σ^{70} (156), Q engagement probably is accompanied by the release of σ^{70} from the complex. The binding site of Q in the core subunits has been elusive; a search for RNAP mutations that impair Q function revealed numerous sites believed to underlie conformation changes that enable the Q modification but no plausible binding site (118). Recently a binding site has been found in the β flap (P. Deighan, C. Diez, M. Leibman, A. Hochschild, and B.E. Nickels, unpublished data), which also is the locus of σ^{70} region 4 displacement by Q and of the RNA barrier described below. Contact with the α-CTD also can be detected (B.E. Nickels and A. Hochschild, unpublished data), although Q has activity in the absence of the α-CTD.

The σ^{70}-dependent paused complex is homologous in structure to the open promoter complex, in which scrunching of DNA allows initial stages of synthesis to occur without sigma release (49,108). Thus, the λ +16 σ^{70}-dependent paused complex has a natural extension to only +12 according to the position where σ^{70} binds, implying a scrunch of four nucleotides of each DNA strand (77). Just as scrunching energy is believed to be used for the open promoter complex to break its sigma-DNA bonds, we suggest that the scrunching energy of the σ^{70}-dependent paused complex is used for escape from the pause to produce the Q-modified complex. In this case, scrunching energy may enable breaking bonds between DNA and both σ^{70}-region 2 and Q in order to allow escape. (A variant model is that only one monomer of a Q dimer binds DNA, and only the second monomer binds and travels with RNAP; in this case, scrunching energy might be used to separate the Q subunits). Q binding initiates the escape process, because addition of Q to a preformed paused complex accelerates escape. An interesting speculation is that in the initial stage of escape, scrunching continues beyond the usual stable extension (e.g., +16), storing more energy. A possible by-product of such extended scrunching is a backtracked λQ-dependent pause at about +25 (39). Collapse at the +25 site into a backtracked state could be analogous to collapse of the +16 complex when it extends (in the absence of Q) to +17, and to collapse of the open promoter complex in the process of abortive initiation.

A final element of the Q-bound paused complex is NusA. NusA stimulates the activity of Q (even though Q is distinctly active in its absence) (39,152) and stabilizes the Q-bound paused complex against exonuclease digestion (155). For 82Q from the λ relative phage 82, but likely also for λQ, the modified complex that eventually escapes the pause site has distinct properties if NusA is present during modification (see below). Thus, NusA also can be present at the σ^{70}-dependent paused complex. Because the NusA-dependent effect also depends on the RNAP core subunit α-CTD— a binding site of NusA—both NusA and the α-CTD can be functional elements of the Qmodified paused complex (Figure 3).

The properties of the 82Q-modified elongation complex provide a new view of how an antiterminator can modify RNAP, as well as a potential resolution of the roles of NusA and antipausing in antitermination (Figure 4). Although NusA stimulates antitermination, Q antiterminates without it, and in fact Q is active in vivo in a *nusA* deletion strain. What NusA contributes was revealed by nuclease probing of RNA emerging from the 82Q-modified complex: If NusA is present, the usual RNA emergence at about 14 nucleotides from the growing end is extended to about 25 nucleotides, implying that NusA and 82Q construct a barrier where the hairpin of an intrinsic terminator forms (126). Static complexes modified by 82Q in the presence of NusA but not in its absence resist both an oligonucleotide-mediated release reaction that models the intrinsic terminator (126,156) and the activity of Rho (126); the latter suggests either that Rho has an obligatory target site in the region where RNA emerges, or that the Q-modified complex is strengthened enough to resist the force of the Rho translocase.

If NusA is not present, antitermination still occurs, but in this case there is evidence that antipausing is the mechanism: Restricting elongation rate at the critical release site of an intrinsic terminator by reducing the substrate NTP concentration impairs antitermination in the absence of NusA, but not in its presence (126). The role of these two different modes of antitermination in vivo remains to be understood.

Put RNA of HK022

The phage λ relative HK022 also regulates expression of its early and late genes by antitermination. Like λN and its Nut sites, the Put system requires two RNA sites, PutL and PutR (polymerase utilization, left and right), encoded downstream of the early promoters *pL* and *pR*. The novelty of the Put system is that these nascent RNAs alone are the antiterminators (22,53,96,124): Mutational analysis, structural probing by RNase cleavage, and prediction methods show that PutL and PutR fold into a pair of stem loops that bind to and modify RNAP to a termination-resistant form (Figure 5) (7,53). The downstream or proximal 3′ loop of the Put RNA interacts directly with RNAP, whereas the distal 5′ loop facilitates this interaction, without making detectable contacts with the elongation complex itself (124). Put RNA interacts with RNAP through the zinc finger motif of the β′ subunit, and mutations in this motif eliminate *put* function (53,55,123). Put-modified elongation complexes are similar to λN-modified or Qmodified complexes: They read through both Rho-dependent and intrinsic terminators, and they display an increased elongation rate (53,124). Presumably this effect on elongation rate is equivalent to antipausing, meaning that the Put modification could act by affecting the elongation/release competition at a terminator, similarly to λQ or N. Put also of course could directly block emerging RNA, similarly to λQ and possibly λN.

The PutL modification of RNAP strongly prevents pausing at a uridine-rich, backtracked pause site just downstream of PutL itself. This mechanism, however, is unique: Formation and binding of the PutL structure to RNAP constrain the transcript such that its retraction into RNAP to produce a backtracked complex close to PutL is prevented (T. Velikodvorskaya, N. Komissarova, R. Sen, R. King, S. Banik-Maiti, and R. Weisberg, unpublished data). Moving the pause sequence a few base pairs downstream prevents this PutL effect on pausing. Because antitermination works at any distance, and the elongation effect persists for long distances downstream, the processive effects of Put must have a different basis, presumably like that of other antiterminators.

Nun Protein of Phage HK022

The λ-related phage HK022 mounts a striking assault in the evolutionary wars: It expresses a protein that binds RNA similar to λN, but with the opposite activity. Nun binding to Nut sites causes termination, not antitermination. Furthermore, Nun binding blocks translation of N

protein mRNA (52). The HK022 lysogen constitutively expresses Nun, which arrests transcribing RNAP and destroys the transcription program of a sensitive phage (54,112), no doubt acting to prevent superinfection by competing phages (20,96,112,115). In a purified system with RNAP and λ DNA, Nun alone can cause transcription arrest (48), but NusA, NusB, NusE, and NusG enhance this arrest activity in vitro (115). Correspondingly, several of the *nus* mutations that interfere with N-mediated anititermination also disrupt Nun function in vivo. The requirements at *nutL* and *nutR* differ: NusA and NusG are not required for Nun-mediated arrest at *nutL* (16,51,147). Despite this difference, the set of Nus factors required by N also is recruited by Nun to construct a particle around the *nut* site.

The N-terminal segments (ARMs) of N and Nun are similar and contact *nut* identically (20, 28,136). The N-terminal portions of N and Nun can be interchanged without loss of function in either case, implying that the termination function of Nun resides in the C terminus (43). In fact, the C terminus of Nun contains residues responsible for interaction with both RNAP and the DNA template, contacting RNAP in a Zn^{2}+-dependent manner through a cluster of histidine residues (148,149). The C terminus of Nun also contains residues responsible for its interaction with the DNA template just downstream of the transcription bubble: A pair of charged residues (K106 and K107) binds the phosphate backbone of the DNA, and the penultimate residue (W108) is proposed to intercalate into the DNA strands (50,149), causing the elongation complex to arrest just downstream of the *nut* site (149). In vivo, arrested transcripts are terminated and released, whereas in a minimal in vitro system the transcripts remain associated with the elongation complex (48,112,129). The minimal system lacks the *E. coli* Mfd protein, which releases stalled transcription elongation complexes (100,122) and facilitates the release of Nun-arrested complexes both in vivo and in vitro (146).

E coli RfaH Protein

E. coli RfaH, a regulator of virulence genes, is an antiterminator that inhibits transcription pausing (6), an activity that probably underlies its antitermination mechanism. Like λQ, RfaH engages RNAP at a natural transcription pause site (OPS), although this pause is induced by sequence elements that affect RNAP core subunits rather than σ^{70}; the paused complex at OPS is stabilized by backtracking (6). One underlying similarity is that both σ^{70} and RfaH recognize the nontemplate DNA strand in the paused complex. The mechanism of engagement is well understood: RfaH has an NTD similar to the close paralog NusG that binds RNAP and DNA, and a distinct CTD that sequesters the RNAP binding site (9). Binding of RfaH to OPS site DNA in the paused complex induces a rearrangement that exposes its RNAP binding surface, which then associates with RNAP and allows RfaH to travel along with the elongation complex.

As this pathway would predict, the isolated RNAP binding domain of RfaH associates with RNAP independently of the OPS pause site. The binding site of RfaH on RNAP is the β′ subunit coiled coil that also is the binding site of σ^{70} region 2 (9). Mutations that block RfaH binding and function without interfering with σ^{70} function can be found. An important implication of this binding site is that the close paralog NusG likely binds to the β′ coiled coil. A further implication is that because RfaH, σ^{70} region 2, and NusG compete for the same site, the ability of σ^{70} region 2 to rebind during elongation after its initial dissociation and to induce downstream pauses by the same mechanism as the promoter proximal pause where λQ protein engages would depend upon its outcompeting NusG or RfaH. In vitro, RfaH wins this competition, suggesting that σ^{70}-dependent pausing in downstream elongation might not occur through σ^{70} rebinding (125).

E. coli Ribosomal RNA Antitermination

The ribosomal RNA operons of *E. coli* include an antitermination system similar to that of λN protein, in that the same accessory factors, NusA, NusB, NusE, and NusG, appear to be

involved (23,133). Thus, mutations in *nusB* and *nusG* inhibit antitermination in ribosomal operons. Furthermore, there is a defined site similar to *λ nut*, including an essential *boxA* sequence (70). However, there is no known analogue of N itself, so either the analog has not been found, or, more likely, the system operates differently.

The existence of the ribosomal RNA antitermination system was implied by the discovery that insertion sequences in the ribosomal operons are not polar, i.e., do not support the expected Rho-dependent termination (83). The system is active in antiterminating at Rho-dependent terminators both in vitro and in vivo with a reporter system (4,133), although it has not been reconstituted with entirely purified components, unlike the other antitermination systems. Clear evidence for the function of the ribosomal system in vivo derives from measurements of elongation rates of either ribosomal RNA synthesis or mRNA synthesis in constructs to which the ribosomal antitermination site is fused: These rates are about twice as great in the presence of antitermination (144). Presumably this greater speed reflects the absence of pausing, which also underlies antitermination; however, pausing is generally measured in vitro, and there is no precise correlation between the in vivo and in vitro phenomena. A biological rationale for antitermi-nation in transcription of noncoding RNA is clear: Ribosomes are not present to inhibit the activity of Rho. Nonetheless, the system is not essential, as shown, for example, by the viability of *nusA* deletion strains (18). Ribosomal proteins assembling on the emerging RNA might also inhibit Rho activity, at least sufficiently for viability.

SUMMARY POINTS

1. 1. Transcription elongation is highly regulated through encoded signals recognized by regulatory factors and by RNAP itself.
2. 2. Transcription is terminated by intrinsic terminators, by Rho, and by the release factor Mfd, which removes RNAP stalled by DNA damage.
3. 3. Regulation of transcription elongation frequently is mediated through transcription pausing, the property of RNAP to stay at a single template site for much longer than the average elongation time. Pausing is a first step of transcription termination.
4. 4. The cellular proteins NusA and NusG modulate pausing and are components of antitermination systems.
5. 5. Antiterminators are operon-specific regulators consisting of either protein or RNA.
6. 6. Antitermination occurs through both antipausing and direct shielding of emerging RNA of the elongation complex.

FUTURE ISSUES

1. 1. How do transcription elongation regulatory proteins contact RNAP core subunits?
2. 2. What are the relative roles of antipausing and complex stabilization in antitermination in vivo?
3. 3. What is the molecular mechanism by which antiterminators inhibit pausing?
4. 4. What is the atomic structure of an elongation complex modified by an antiterminator?

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LITERATURE CITED

1. Adelman JL, Jeong YJ, Liao JC, Patel G, Kim DE, et al. Mechanochemistry of transcription termination factor Rho. Mol. Cell 2006;22:611–21. [PubMed: 16762834]
2. Adelman K, La Porta A, Santangelo TJ, Lis JT, Roberts JW, Wang MD. Single molecule analysis of RNA polymerase elongation reveals uniform kinetic behavior. Proc. Natl. Acad. Sci. USA 2002;99:13538–43. [PubMed: 12370445]
3. Adhya S, Gottesman M, De Crombrugghe B. Release of polarity in *Escherichia coli* by gene N of phage lambda: termination and antitermination of transcription. Proc. Natl. Acad. Sci. USA 1974;71:2534–38. [PubMed: 4601822]
4. Albrechtsen B, Squires CL, Li S, Squires C. Antitermination of characterized transcriptional terminators by the *Escherichia coli* rrnG leader region. J. Mol. Biol 1990;213:123–34. [PubMed: 2187097]
5. Artsimovitch I, Landick R. Pausing by bacterial RNA polymerase is mediated by mechanistically distinct classes of signals. Proc. Natl. Acad. Sci. USA 2000;97:7090–95. [PubMed: 10860976]
6. Artsimovitch I, Landick R. The transcriptional regulator RfaH stimulates RNA chain synthesis after recruitment to elongation complexes by the exposed nontemplate DNA strand. Cell 2002;109:193–203. [PubMed: 12007406]
7. Banik-Maiti S, King RA, Weisberg RA. The antiterminator RNA of phage HK022. J. Mol. Biol 1997;272:677–87. [PubMed: 9368650]
8. Barik S, Ghosh B, Whalen W, Lazinski D, Das A. An antitermination protein engages the elongating transcription apparatus at a promoter-proximal recognition site. Cell 1987;50:885–99. [PubMed: 3040263]
9. Belogurov GA, Vassylyeva MN, Svetlov V, Klyuyev S, Grishin NV, et al. Structural basis for converting a general transcription factor into an operon-specific virulence regulator. Mol. Cell 2007;26:117–29. [PubMed: 17434131]
10. Beuth B, Pennell S, Arnvig KB, Martin SR, Taylor IA. Structure of a *Mycobacterium tuberculosis* NusA-RNA complex. EMBO J 2005;24:3576–87. [PubMed: 16193062]
11. Borukhov S, Lee J, Laptenko O. Bacterial transcription elongation factors: new insights into molecular mechanism of action. Mol. Microbiol 2005;55:1315–24. [PubMed: 15720542]
12. Borukhov S, Polyakov A, Nikiforov V, Goldfarb A. GreA protein: a transcription elongation factor from *Escherichia coli*. Proc. Natl. Acad. Sci. USA 1992;89:8899–902. [PubMed: 1384037]
13. Brodolin K, Zenkin N, Mustaev A, Mamaeva D, Heumann H. The sigma 70 subunit of RNA polymerase induces lacUV5 promoter-proximal pausing of transcription. Nat. Struct. Mol. Biol 2004;11:551–57. [PubMed: 15122346]
14. Burns CM, Nowatzke WL, Richardson JP. Activation of Rho-dependent transcription termination by NusG. Dependence on terminator location and acceleration of RNA release. J. Biol. Chem 1999;274:5245–51. [PubMed: 9988775]
15. Burns CM, Richardson LV, Richardson JP. Combinatorial effects of NusA and NusG on transcription elongation and Rho-dependent termination in *Escherichia coli*. J. Mol. Biol 1998;278:307–16. [PubMed: 9571053]
16. Burova E, Hung SC, Chen J, Court DL, Zhou JG, et al. *Escherichia coli* nusG mutations that block transcription termination by coliphage HK022 Nun protein. Mol. Microbiol 1999;31:1783–93. [PubMed: 10209750]
17. Burova E, Hung SC, Sagitov V, Stitt BL, Gottesman ME. *Escherichia coli* NusG protein stimulates transcription elongation rates in vivo and in vitro. J. Bacteriol 1995;177:1388–92. [PubMed: 7868616]

18. Cardinale CJ, Washburn RS, Tadigotla VR, Brown LM, Gottesman ME, Nudler E. Termination factor Rho and its cofactors NusA and NusG silence foreign DNA in *E. coli*. Science. 2008 In press.
19. Chambers AL, Smith AJ, Savery NJ. A DNA translocation motif in the bacterial transcription–repair coupling factor, Mfd. Nucleic Acids Res 2003;31:6409–18. [PubMed: 14602898]
20. Chattopadhyay S, Garcia-Mena J, DeVito J, Wolska K, Das A. Bipartite function of a small RNA hairpin in transcription antitermination in bacteriophage lambda. Proc. Natl. Acad. Sci. USA 1995;92:4061–65. [PubMed: 7732031]
21. Ciampi MS. Rho-dependent terminators and transcription termination. Microbiology 2006;152:2515–28. [PubMed: 16946247]
22. Clerget M, Jin DJ, Weisberg RA. A zinc-binding region in the beta' subunit of RNA polymerase is involved in antitermination of early transcription of phage HK022. J. Mol. Biol 1995;248:768–80. [PubMed: 7752239]
23. Condon C, Squires C, Squires CL. Control of rRNA transcription in *Escherichia coli*. Microbiol. Rev 1995;59:623–45. [PubMed: 8531889]
24. Das A, Wolska K. Transcription antitermination in vitro by lambda N gene product: requirement for a phage nut site and the products of host nusA, nusB, and nusE genes. Cell 1984;38:165–73. [PubMed: 6088061]
25. Deaconescu AM, Chambers AL, Smith AJ, Nickels BE, Hochschild A, et al. Structural basis for bacterial transcription-coupled DNA repair. Cell 2006;124:507–20. [PubMed: 16469698]
26. Dedrick RL, Kane CM, Chamberlin MJ. Purified RNA polymerase II recognizes specific termination sites during transcription in vitro. J. Biol. Chem 1987;262:9098–108. [PubMed: 3036847]
27. Deighan P, Hochschild A. The bacteriophage lambdaQ antiterminator protein regulates late gene expression as a stable component of the transcription elongation complex. Mol. Microbiol 2007;63:911–20. [PubMed: 17302807]
28. Faber C, Scharpf M, Becker T, Sticht H, Rosch P. The structure of the coliphage HK022 Nun protein-lambda-phage boxB RNA complex. Implications for the mechanism of transcription termination. J. Biol. Chem 2001;276:32064–70. [PubMed: 11356847]
29. Fisher RF, Yanofsky C. Mutations of the beta subunit of RNA polymerase alter both transcription pausing and transcription termination in the *trp* operon leader region in vitro. J. Biol. Chem 1983;258:8146–50. [PubMed: 6345540]
30. Franklin NC. Altered reading of genetic signals fused to the *N* operon of bacteriophage lambda: genetic evidence for modification of polymerase by the protein product of the N gene. J. Mol. Biol 1974;89:33–48. [PubMed: 4613856]
31. Friedman DI, Baron LS. Genetic characterization of a bacterial locus involved in the activity of the N function of phage lambda. Virology 1974;58:141–48. [PubMed: 4595374]
32. Friedman DI, Baumann M, Baron LS. Cooperative effects of bacterial mutations affecting lambda N gene expression. I. Isolation and characterization of a *nusB* mutant. Virology 1976;73:119–27. [PubMed: 785802]
33. Friedman DI, Schauer AT, Baumann MR, Baron LS, Adhya SL. Evidence that ribosomal protein S10 participates in control of transcription termination. Proc. Natl. Acad. Sci. USA 1981;78:1115–18. [PubMed: 6453343]
34. Friedman DI, Wilgus GS, Mural RJ. Gene N regulator function of phage lambda immun21: evidence that a site of N action differs from a site of N recognition. J. Mol. Biol 1973;81:505–16. [PubMed: 4778807]
35. Galluppi GR, Richardson JP. ATP-induced changes in the binding of RNA synthesis termination protein Rho to RNA. J. Mol. Biol 1980;138:513–39. [PubMed: 6157829]
36. Gopal B, Haire LF, Gamblin SJ, Dodson EJ, Lane AN, et al. Crystal structure of the transcription elongation/antitermination factor NusA from *Mycobacterium tuberculosis* at 1.7 A resolution. J. Mol. Biol 2001;314:1087–95. [PubMed: 11743725]
37. Gottesman S. The small RNA regulators of *Escherichia coli*: roles and mechanisms. Annu. Rev. Microbiol 2004;58:303–28. [PubMed: 15487940]
38. Gowrishankar J, Harinarayanan R. Why is transcription coupled to translation in bacteria? Mol. Microbiol 2004;54:598–603. [PubMed: 15491353]

39. Grayhack EJ, Yang XJ, Lau LF, Roberts JW. Phage lambda gene *Q* antiterminator recognizes RNA polymerase near the promoter and accelerates it through a pause site. Cell 1985;42:259–69. [PubMed: 2990726]
40. Greenblatt J, McLimont M, Hanly S. Termination of transcription by nusA gene protein of *Escherichia coli*. Nature 1981;292:215–20. [PubMed: 6265785]
41. Gusarov I, Nudler E. Control of intrinsic transcription termination by N and NusA: the basic mechanisms. Cell 2001;107:437–49. [PubMed: 11719185]
42. Hatoum A, Roberts J. Prevalence of RNA polymerase stalling at *Escherichia coli* promoters after open complex formation. Mol. Microbiol 2008;68:17–28. [PubMed: 18333883]
43. Henthorn KS, Friedman DI. Identification of functional regions of the Nun transcription termination protein of phage HK022 and the N antitermination protein of phage lambda using hybrid nun-N genes. J. Mol. Biol 1996;257:9–20. [PubMed: 8632463]
44. Herbert KM, La Porta A, Wong BJ, Mooney RA, Neuman KC, et al. Sequence-resolved detection of pausing by single RNA polymerase molecules. Cell 2006;125:1083–94. [PubMed: 16777599]
45. Horwitz RJ, Li J, Greenblatt J. An elongation control particle containing the N gene transcriptional antitermination protein of bacteriophage lambda. Cell 1987;51:631–41. [PubMed: 2445491]
46. Hsu LM, Vo NV, Chamberlin MJ. *Escherichia coli* transcript cleavage factors GreA and GreB stimulate promoter escape and gene expression in vivo and in vitro. Proc. Natl. Acad. Sci. USA 1995;92:11588–92. [PubMed: 8524809]
47. Huertas P, Aguilera A. Cotranscriptionally formed DNA:RNA hybrids mediate transcription elongation impairment and transcription-associated recombination. Mol. Cell 2003;12:711–21. [PubMed: 14527416]
48. Hung SC, Gottesman ME. Phage HK022 Nun protein arrests transcription on phage lambda DNA in vitro and competes with the phage lambda N antitermination protein. J. Mol. Biol 1995;247:428–42. [PubMed: 7714899]
49. Kapanidis AN, Margeat E, Ho SO, Kortkhonjia E, Weiss S, Ebright RH. Initial transcription by RNA polymerase proceeds through a DNA-scrunching mechanism. Science 2006;314:1144–47. [PubMed: 17110578]
50. Kim HC, Gottesman ME. Transcription termination by phage HK022 Nun is facilitated by COOH-terminal lysine residues. J. Biol. Chem 2004;279:13412–17. [PubMed: 14742436]
51. Kim HC, Washburn RS, Gottesman ME. Role of E. coli NusA in phage HK022 Nun-mediated transcription termination. J. Mol. Biol 2006;359:10–21. [PubMed: 16631197]
52. Kim HC, Zhou JG, Wilson HR, Mogilnitskiy G, Court DL, Gottesman ME. Phage HK022 Nun protein represses translation of phage lambda N (transcription termination/translation repression). Proc. Natl. Acad. Sci. USA 2003;100:5308–12. [PubMed: 12684530]
53. King RA, Banik-Maiti S, Jin DJ, Weisberg RA. Transcripts that increase the processivity and elongation rate of RNA polymerase. Cell 1996;87:893–903. [PubMed: 8945516]
54. King RA, Madsen PL, Weisberg RA. Constitutive expression of a transcription termination factor by a repressed prophage: promoters for transcribing the phage HK022 nun gene. J. Bacteriol 2000;182:456–62. [PubMed: 10629193]
55. King RA, Markov D, Sen R, Severinov K, Weisberg RA. A conserved zinc binding domain in the largest subunit of DNA-dependent RNA polymerase modulates intrinsic transcription termination and antitermination but does not stabilize the elongation complex. J. Mol. Biol 2004;342:1143–54. [PubMed: 15351641]
56. Komissarova N, Becker J, Solter S, Kireeva M, Kashlev M. Shortening of RNA:DNA hybrid in the elongation complex of RNA polymerase is a prerequisite for transcription termination. Mol. Cell 2002;10:1151–62. [PubMed: 12453422]
57. Komissarova N, Kashlev M. RNA polymerase switches between inactivated and activated states by translocating back and forth along the DNA and the RNA. J. Biol. Chem 1997;272:15329–38. [PubMed: 9182561]
58. Korzheva N, Mustaev A, Kozlov M, Malhotra A, Nikiforov V, et al. A structural model of transcription elongation. Science 2000;289:619–25. [PubMed: 10915625]

59. Landick R, Carey J, Yanofsky C. Translation activates the paused transcription complex and restores transcription of the *trp* operon leader region. Proc. Natl. Acad. Sci. USA 1985;82:4663–67. [PubMed: 2991886]
60. Landick, R.; Turnbough, J.; Yanofsky, C. Transcription attenuation. In Escherichia coli *and* Salmonella:. In: Neidhardt, F.; Curtiss, R.; Ingraham, JL.; Lin, ECC.; Low, KB., et al., editors. Cellular and Molecular Biology. ASM Press; Washington, DC: 1996. p. 1263-86.
61. Laptenko O, Lee J, Lomakin I, Borukhov S. Transcript cleavage factors GreA and GreB act as transient catalytic components of RNA polymerase. EMBO J 2003;22:6322–34. [PubMed: 14633991]
62. Larson MH, Greenleaf WJ, Landick R, Block SM. Applied force reveals mechanistic and energetic details of transcription termination. Cell 2008;132:971–82. [PubMed: 18358810]
63. Lau LF, Roberts JW. Rho-dependent transcription termination at lambda R1 requires upstream sequences. J. Biol. Chem 1985;260:574–84. [PubMed: 2981220]
64. Lau LF, Roberts JW, Wu R. RNA polymerase pausing and transcript release at the lambda tR1 terminator in vitro. J. Biol. Chem 1983;258:9391–97. [PubMed: 6308007]
65. Lazinski D, Grzadzielska E, Das A. Sequence-specific recognition of RNA hairpins by bacteriophage antiterminators requires a conserved arginine-rich motif. Cell 1989;59:207–18. [PubMed: 2477156]
66. Lee DN, Phung L, Stewart J, Landick R. Transcription pausing by *Escherichia coli* RNA polymerase is modulated by downstream DNA sequences. J. Biol. Chem 1990;265:15145–53. [PubMed: 1697586]
67. Legault P, Li J, Mogridge J, Kay LE, Greenblatt J. NMR structure of the bacteriophage lambda N peptide/boxB RNA complex: recognition of a GNRA fold by an arginine-rich motif. Cell 1998;93:289–99. [PubMed: 9568720]
68. Li J, Horwitz R, McCracken S, Greenblatt J. NusG, a new *Escherichia coli* elongation factor involved in transcriptional antitermination by the N protein of phage lambda. J. Biol. Chem 1992;267:6012–19. [PubMed: 1532577]
69. Li J, Mason SW, Greenblatt J. Elongation factor NusG interacts with termination factor Rho to regulate termination and antitermination of transcription. Genes Dev 1993;7:161–72. [PubMed: 8422985]
70. Li SC, Squires CL, Squires C. Antitermination of *E. coli* rRNA transcription is caused by a control region segment containing lambda nut-like sequences. Cell 1984;38:851–60. [PubMed: 6091902]
71. Liu K, Hanna MM. NusA interferes with interactions between the nascent RNA and the C-terminal domain of the alpha subunit of RNA polymerase in *Escherichia coli* transcription complexes. Proc. Natl. Acad. Sci. USA 1995;92:5012–16. [PubMed: 7539140]
72. Liu K, Zhang Y, Severinov K, Das A, Hanna MM. Role of *Escherichia coli* RNA polymerase alpha subunit in modulation of pausing, termination and antitermination by the transcription elongation factor NusA. EMBO J 1996;15:150–61. [PubMed: 8598198]
73. Lowery-Goldhammer C, Richardson JP. An RNA-dependent nucleoside triphosphate phosphohydrolase (ATPase) associated with Rho termination factor. Proc. Natl. Acad. Sci. USA 1974;71:2003–7. [PubMed: 4365581]
74. Macdonald LE, Zhou Y, McAllister WT. Termination and slippage by bacteriophage T7 RNA polymerase. J. Mol. Biol 1993;232:1030–47. [PubMed: 8371265]
75. Mah TF, Li J, Davidson AR, Greenblatt J. Functional importance of regions in *Escherichia coli* elongation factor NusA that interact with RNA polymerase, the bacteriophage lambda N protein and RNA. Mol. Microbiol 1999;34:523–37. [PubMed: 10564494]
76. Marr MT, Datwyler SA, Meares CF, Roberts JW. Restructuring of an RNA polymerase holoenzyme elongation complex by lambdoid phage Q proteins. Proc. Natl. Acad. Sci. USA 2001;98:8972–78. [PubMed: 11481468]
77. Marr MT, Roberts JW. Function of transcription cleavage factors GreA and GreB at a regulatory pause site. Mol. Cell 2000;6:1275–85. [PubMed: 11163202]
78. Martin FH, Tinoco I Jr. DNA-RNA hybrid duplexes containing oligo(dA:rU) sequences are exceptionally unstable and may facilitate termination of transcription. Nucleic Acids Res 1980;8:2295–99. [PubMed: 6159577]

79. Mason SW, Li J, Greenblatt J. Direct interaction between two *Escherichia coli* transcription antitermination factors, NusB and ribosomal protein S10. J. Mol. Biol 1992;223:55–66. [PubMed: 1731086]
80. Mason SW, Li J, Greenblatt J. Host factor requirements for processive antitermination of transcription and suppression of pausing by the N protein of bacteriophage lambda. J. Biol. Chem 1992;267:19418–26. [PubMed: 1388170]
81. McSwiggen JA, Bear DG, von Hippel PH. Interactions of *Escherichia coli* transcription termination factor Rho with RNA. I. Binding stoichiometries and free energies. J. Mol. Biol 1988;199:609–22. [PubMed: 2451028]
82. Mooney RA, Landick R. Tethering sigma70 to RNA polymerase reveals high in vivo activity of sigma factors and sigma70-dependent pausing at promoter-distal locations. Genes Dev 2003;17:2839–51. [PubMed: 14630944]
83. Morgan EA. Insertions of Tn 10 into an *E. coli* ribosomal RNA operon are incompletely polar. Cell 1980;21:257–65. [PubMed: 6157478]
84. Morgan WD, Bear DG, Litchman BL, von Hippel PH. RNA sequence and secondary structure requirements for Rho-dependent transcription termination. Nucleic Acids Res 1985;13:3739–54. [PubMed: 2409526]
85. Morgan WD, Bear DG, von Hippel PH. Rho-dependent termination of transcription. II. Kinetics of mRNA elongation during transcription from the bacteriophage lambda PR promoter. J. Biol. Chem 1983;258:9565–74. [PubMed: 6223930]
86. Nehrke KW, Platt T. A quaternary transcription termination complex. Reciprocal stabilization by Rho factor and NusG protein. J. Mol. Biol 1994;243:830–39. [PubMed: 7525972]
87. Nehrke KW, Zalatan F, Platt T. NusG alters Rho-dependent termination of transcription in vitro independent of kinetic coupling. Gene Expr 1993;3:119–33. [PubMed: 7505669]
88. Neuman KC, Abbondanzieri EA, Landick R, Gelles J, Block SM. Ubiquitous transcriptional pausing is independent of RNA polymerase backtracking. Cell 2003;115:437–47. [PubMed: 14622598]
89. Nickels BE, Mukhopadhyay J, Garrity SJ, Ebright RH, Hochschild A. The sigma 70 subunit of RNA polymerase mediates a promoter-proximal pause at the lac promoter. Nat. Struct. Mol. Biol 2004;11:544–50. [PubMed: 15122345]
90. Nickels BE, Roberts CW, Roberts JW, Hochschild A. RNA-mediated destabilization of the sigma (70) region 4/beta flap interaction facilitates engagement of RNA polymerase by the Q antiterminator. Mol. Cell 2006;24:457–68. [PubMed: 17081994]
91. Nickels BE, Roberts CW, Sun H, Roberts JW, Hochschild A. The sigma(70) subunit of RNA polymerase is contacted by the (lambda)Q antiterminator during early elongation. Mol. Cell 2002;10:611–22. [PubMed: 12408828]
92. Nodwell JR, Greenblatt J. Recognition of boxA antiterminator RNA by the *E. coli* antitermination factors NusB and ribosomal protein S10. Cell 1993;72:261–68. [PubMed: 7678781]
93. Nudler E, Avetissova E, Markovtsov V, Goldfarb A. Transcription processivity: protein-DNA interactions holding together the elongation complex. Science 1996;273:211–17. [PubMed: 8662499]
94. Nudler E, Gottesman ME. Transcription termination and antitermination in *E. coli*. Genes Cells 2002;7:755–68. [PubMed: 12167155]
95. Nudler E, Mustaev A, Lukhtanov E, Goldfarb A. The RNA-DNA hybrid maintains the register of transcription by preventing backtracking of RNA polymerase. Cell 1997;89:33–41. [PubMed: 9094712]
96. Oberto J, Clerget M, Ditto M, Cam K, Weisberg RA. Antitermination of early transcription in phage HK022. Absence of a phage-encoded antitermination factor. J. Mol. Biol 1993;229:368–81. [PubMed: 8429552]
97. Olson ER, Flamm EL, Friedman DI. Analysis of nutR: a region of phage lambda required for antitermination of transcription. Cell 1982;31:61–70. [PubMed: 6218883]
98. Opalka N, Chlenov M, Chacon P, Rice WJ, Wriggers W, Darst SA. Structure and function of the transcription elongation factor GreB bound to bacterial RNA polymerase. Cell 2003;114:335–45. [PubMed: 12914698]

99. Orlova M, Newlands J, Das A, Goldfarb A, Borukhov S. Intrinsic transcript cleavage activity of RNA polymerase. Proc. Natl. Acad. Sci. USA 1995;92:4596–600. [PubMed: 7538676]
100. Park JS, Marr MT, Roberts JW. *E. coli* Transcription repair coupling factor (Mfd protein) rescues arrested complexes by promoting forward translocation. Cell 2002;109:757–67. [PubMed: 12086674]
101. Park JS, Roberts JW. Role of DNA bubble rewinding in enzymatic transcription termination. Proc. Natl. Acad. Sci. USA 2006;103:4870–75. [PubMed: 16551743]
102. Pasman Z, von Hippel PH. Regulation of Rho-dependent transcription termination by NusG is specific to the *Escherichia coli* elongation complex. Biochemistry 2000;39:5573–85. [PubMed: 10820031]
103. Patterson TA, Zhang Z, Baker T, Johnson LL, Friedman DI, Court DL. Bacteriophage lambda N-dependent transcription antitermination. Competition for an RNA site may regulate antitermination. J. Mol. Biol 1994;236:217–28. [PubMed: 8107107]
104. Paul BJ, Barker MM, Ross W, Schneider DA, Webb C, et al. DksA: a critical component of the transcription initiation machinery that potentiates the regulation of rRNA promoters by ppGpp and the initiating NTP. Cell 2004;118:311–22. [PubMed: 15294157]
105. Perederina A, Svetlov V, Vassylyeva MN, Tahirov TH, Yokoyama S, et al. Regulation through the secondary channel–structural framework for ppGpp-DksA synergism during transcription. Cell 2004;118:297–309. [PubMed: 15294156]
106. Rees WA, Weitzel SE, Das A, von Hippel PH. Regulation of the elongation-termination decision at intrinsic terminators by antitermination protein N of phage lambda. J. Mol. Biol 1997;273:797–813. [PubMed: 9367773]
107. Rees WA, Weitzel SE, Yager TD, Das A, von Hippel PH. Bacteriophage lambda N protein alone can induce transcription antitermination in vitro. Proc. Natl. Acad. Sci. USA 1996;93:342–46. [PubMed: 8552635]
108. Revyakin A, Liu C, Ebright RH, Strick TR. Abortive initiation and productive initiation by RNA polymerase involve DNA scrunching. Science 2006;314:1139–43. [PubMed: 17110577]
109. Richardson, JP.; Greenblatt, J. Control of RNA chain elongation and termination. In: Neidhardt, FC.; Curtiss, R.; Ingraham, JL.; Lin, ECC.; Low, KB., et al., editors. In Escherichia coli *and* Salmonella: Cellular and Molecular Biology. ASM Press; Washington, DC: 1996. p. 822-48.
110. Richardson JP, Grimley C, Lowery C. Transcription termination factor Rho activity is altered in *Escherichia coli* strains with suA gene mutations. Proc. Natl. Acad. Sci. USA 1975;72:1725–28. [PubMed: 1098042]
111. Ring BZ, Yarnell WS, Roberts JW. Function of *E. coli* RNA polymerase sigma factor sigma 70 in promoter-proximal pausing. Cell 1996;86:485–93. [PubMed: 8756730]
112. Robert J, Sloan SB, Weisberg RA, Gottesman ME, Robledo R, Harbrecht D. The remarkable specificity of a new transcription termination factor suggests that the mechanisms of termination and antitermination are similar. Cell 1987;51:483–92. [PubMed: 2822258]
113. Roberts JW. Termination factor for RNA synthesis. Nature 1969;224:1168–74. [PubMed: 4902144]
114. Roberts JW, Yarnell W, Bartlett E, Guo J, Marr M, et al. Antitermination by bacteriophage lambda Q protein. Cold Spring Harb. Symp. Quant. Biol 1998;63:319–25. [PubMed: 10384296]
115. Robledo R, Atkinson BL, Gottesman ME. *Escherichia coli* mutations that block transcription termination by phage HK022 Nun protein. J. Mol. Biol 1991;220:613–19. [PubMed: 1831236]
116. Ryder AM, Roberts JW. Role of the nontemplate strand of the elongation bubble in intrinsic transcription termination. J. Mol. Biol 2003;334:205–13. [PubMed: 14607113]
117. Salstrom JS, Szybalski W. Coliphage lambdanutL-: a unique class of mutants defective in the site of gene N product utilization for antitermination of leftward transcription. J. Mol. Biol 1978;124:195–221. [PubMed: 361968]
118. Santangelo TJ, Mooney RA, Landick R, Roberts JW. RNA polymerase mutations that impair conversion to a termination-resistant complex by Q antiterminator proteins. Genes Dev 2003;17:1281–92. [PubMed: 12756229]
119. Santangelo TJ, Roberts JW. Forward translocation is the natural pathway of RNA release at an intrinsic terminator. Mol. Cell 2004;14:117–26. [PubMed: 15068808]

120. Schmidt MC, Chamberlin MJ. nusA protein of *Escherichia coli* is an efficient transcription termination factor for certain terminator sites. J. Mol. Biol 1987;195:809–18. [PubMed: 2821282]
121. Schwartz A, Margeat E, Rahmouni AR, Boudvillain M. Transcription termination factor Rho can displace streptavidin from biotinylated RNA. J. Biol. Chem 2007;282:31469–76. [PubMed: 17724015]
122. Selby CP, Sancar A. Molecular mechanism of transcription-repair coupling. Science 1993;260:53–58. [PubMed: 8465200]
123. Sen R, King RA, Mzhavia N, Madsen PL, Weisberg RA. Sequence-specific interaction of nascent antiterminator RNA with the zinc-finger motif of *Escherichia coli* RNA polymerase. Mol. Microbiol 2002;46:215–22. [PubMed: 12366844]
124. Sen R, King RA, Weisberg RA. Modification of the properties of elongating RNA polymerase by persistent association with nascent antiterminator RNA. Mol. Cell 2001;7:993–1001. [PubMed: 11389846]
125. Sevostyanova A, Svetlov V, Vassylyev DG, Artsimovitch I. The elongation factor RfaH and the initiation factor sigma bind to the same site on the transcription elongation complex. Proc. Natl. Acad. Sci. USA 2008;105:865–70. [PubMed: 18195372]
126. Shankar S, Hatoum A, Roberts JW. A transcription antiterminator constructs a NusA-dependent shield to the emerging transcript. Mol. Cell 2007;27:914–27. [PubMed: 17889665]
127. Sigmund CD, Morgan EA. NusA protein affects transcriptional pausing and termination in vitro by binding to different sites on the transcription complex. Biochemistry 1988;27:5622–27. [PubMed: 2846044]
128. Skordalakes E, Berger JM. Structural insights into RNA-dependent ring closure and ATPase activation by the Rho termination factor. Cell 2006;127:553–64. [PubMed: 17081977]
129. Sloan SB, Weisberg RA. Use of a gene encoding a suppressor tRNA as a reporter of transcription: analyzing the action of the Nun protein of bacteriophage HK022. Proc. Natl. Acad. Sci. USA 1993;90:9842–46. [PubMed: 8234323]
130. Smith AJ, Savery NJ. RNA polymerase mutants defective in the initiation of transcription-coupled DNA repair. Nucleic Acids Res 2005;33:755–64. [PubMed: 15687384]
131. Smith AJ, Szczelkun MD, Savery NJ. Controlling the motor activity of a transcription-repair coupling factor: autoinhibition and the role of RNA polymerase. Nucleic Acids Res 2007;35:1802–11. [PubMed: 17329375]
132. Sosunov V, Sosunova E, Mustaev A, Bass I, Nikiforov V, Goldfarb A. Unified two-metal mechanism of RNA synthesis and degradation by RNA polymerase. EMBO J 2003;22:2234–44. [PubMed: 12727889]
133. Squires CL, Greenblatt J, Li J, Condon C, Squires CL. Ribosomal RNA antitermination in vitro: requirement for Nus factors and one or more unidentified cellular components. Proc. Natl. Acad. Sci. USA 1993;90:970–74. [PubMed: 8430111]
134. Stepanova E, Lee J, Ozerova M, Semenova E, Datsenko K, et al. Analysis of promoter targets for *Escherichia coli* transcription elongation factor GreA in vivo and in vitro. J. Bacteriol 2007;189:8772–85. [PubMed: 17766423]
135. Straney DC, Crothers DM. A stressed intermediate in the formation of stably initiated RNA chains at the *Escherichia coli* lac UV5 promoter. J. Mol. Biol 1987;193:267–78. [PubMed: 2439694]
136. Stuart AC, Gottesman ME, Palmer AG 3rd. The N-terminus is unstructured, but not dynamically disordered, in the complex between HK022 Nun protein and lambda-phage BoxB RNA hairpin. FEBS Lett 2003;553:95–98. [PubMed: 14550553]
137. Sullivan SL, Ward DF, Gottesman ME. Effect of *Escherichia coli* nusG function on lambda N-mediated transcription antitermination. J. Bacteriol 1992;174:1339–44. [PubMed: 1531224]
138. Toulokhonov I, Artsimovitch I, Landick R. Allosteric control of RNA polymerase by a site that contacts nascent RNA hairpins. Science 2001;292:730–33. [PubMed: 11326100]
139. Toulokhonov I, Zhang J, Palangat M, Landick R. A central role of the RNA polymerase trigger loop in active-site rearrangement during transcriptional pausing. Mol. Cell 2007;27:406–19. [PubMed: 17679091]

140. Traviglia SL, Datwyler SA, Yan D, Ishihama A, Meares CF. Targeted protein footprinting: where different transcription factors bind to RNA polymerase. Biochemistry 1999;38:15774–78. [PubMed: 10625443]
141. Vassylyev DG, Vassylyeva MN, Perederina A, Tahirov TH, Artsimovitch I. Structural basis for transcription elongation by bacterial RNA polymerase. Nature 2007;448:157–62. [PubMed: 17581590]
142. Vassylyev DG, Vassylyeva MN, Zhang J, Palangat M, Artsimovitch I, Landick R. Structural basis for substrate loading in bacterial RNA polymerase. Nature 2007;448:163–68. [PubMed: 17581591]
143. Vieu E, Rahmouni AR. Dual role of boxB RNA motif in the mechanisms of termination/antitermination at the lambda tR1 terminator revealed in vivo. J. Mol. Biol 2004;339:1077–87. [PubMed: 15178249]
144. Vogel U, Jensen KF. NusA is required for ribosomal antitermination and for modulation of the transcription elongation rate of both antiterminated RNA and mRNA. J. Biol. Chem 1997;272:12265–71. [PubMed: 9139668]
145. Wagner LA, Weiss RB, Driscoll R, Dunn DS, Gesteland RF. Transcriptional slippage occurs during elongation at runs of adenine or thymine in *Escherichia coli*. Nucleic Acids Res 1990;18:3529–35. [PubMed: 2194164]
146. Washburn RS, Wang Y, Gottesman ME. Role of *E. coli* transcription-repair coupling factor Mfd in Nun-mediated transcription termination. J. Mol. Biol 2003;329:655–62. [PubMed: 12787667]
147. Watnick RS, Gottesman ME. *Escherichia coli* NusA is required for efficient RNA binding by phage HK022 nun protein. Proc. Natl. Acad. Sci. USA 1998;95:1546–51. [PubMed: 9465052]
148. Watnick RS, Gottesman ME. Binding of transcription termination protein nun to nascent RNA and template DNA. Science 1999;286:2337–39. [PubMed: 10600743]
149. Watnick RS, Herring SC, Palmer AG 3rd, Gottesman ME. The carboxyl terminus of phage HK022 Nun includes a novel zinc-binding motif and a tryptophan required for transcription termination. Genes Dev 2000;14:731–39. [PubMed: 10733532]
150. Weisberg RA, Gottesman ME. Processive antitermination. J. Bacteriol 1999;181:359–67. [PubMed: 9882646]
151. Whalen W, Ghosh B, Das A. NusA protein is necessary and sufficient in vitro for phage lambda N gene product to suppress a Rho-independent terminator placed downstream of nutL. Proc. Natl. Acad. Sci. USA 1988;85:2494–98. [PubMed: 2965813]
152. Yang XJ, Hart CM, Grayhack EJ, Roberts JW. Transcription antitermination by phage lambda gene *Q* protein requires a DNA segment spanning the RNA start site. Genes Dev 1987;1:217–26. [PubMed: 2960589]
153. Yang XJ, Roberts JW. Gene *Q* antiterminator proteins of *Escherichia coli* phages 82 and lambda suppress pausing by RNA polymerase at a Rho-dependent terminator and at other sites. Proc. Natl. Acad. Sci. USA 1989;86:5301–5. [PubMed: 2526335]
154. Yanofsky C. RNA-based regulation of genes of tryptophan synthesis and degradation, in bacteria. Rna 2007;13:1141–54. [PubMed: 17601995]
155. Yarnell WS, Roberts JW. The phage lambda gene *Q* transcription antiterminator binds DNA in the late gene promoter as it modifies RNA polymerase. Cell 1992;69:1181–89. [PubMed: 1535556]
156. Yarnell WS, Roberts JW. Mechanism of intrinsic transcription termination and antitermination. Science 1999;284:611–15. [PubMed: 10213678]
157. Zalieckas JM, Wray LV Jr, Ferson AE, Fisher SH. Transcription-repair coupling factor is involved in carbon catabolite repression of the *Bacillus subtilis hut* and *gnt* operons. Mol. Microbiol 1998;27:1031–38. [PubMed: 9535092]
158. Zenkin N, Yuzenkova Y, Severinov K. Transcript-assisted transcriptional proofreading. Science 2006;313:518–20. [PubMed: 16873663]
159. Zheng C, Friedman DI. Reduced Rho-dependent transcription termination permits NusA-independent growth of *Escherichia coli*. Proc. Natl. Acad. Sci. USA 1994;91:7543–47. [PubMed: 8052617]
160. Zhou Y, Navaroli DM, Enuameh MS, Martin CT. Dissociation of halted T7 RNA polymerase elongation complexes proceeds via a forward-translocation mechanism. Proc. Natl. Acad. Sci. USA 2007;104:10352–57. [PubMed: 17553968]

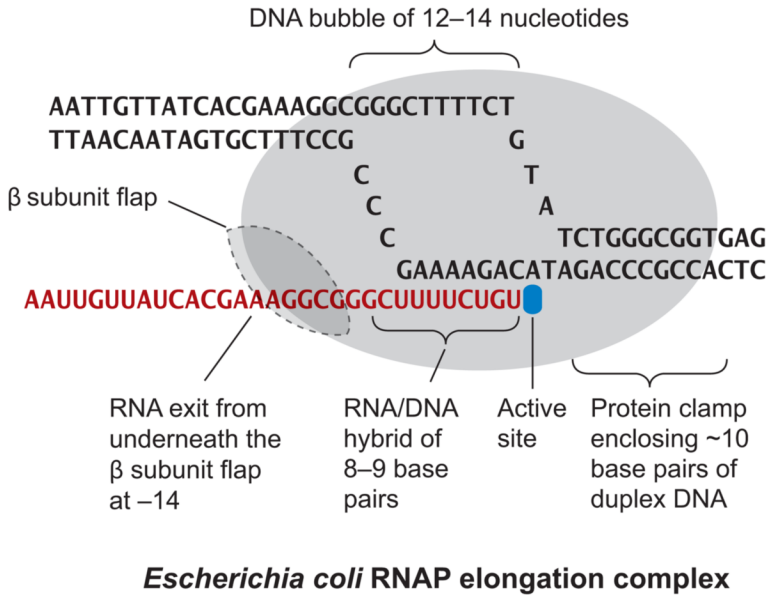

Figure 1.
Structure of the transcription elongation complex.

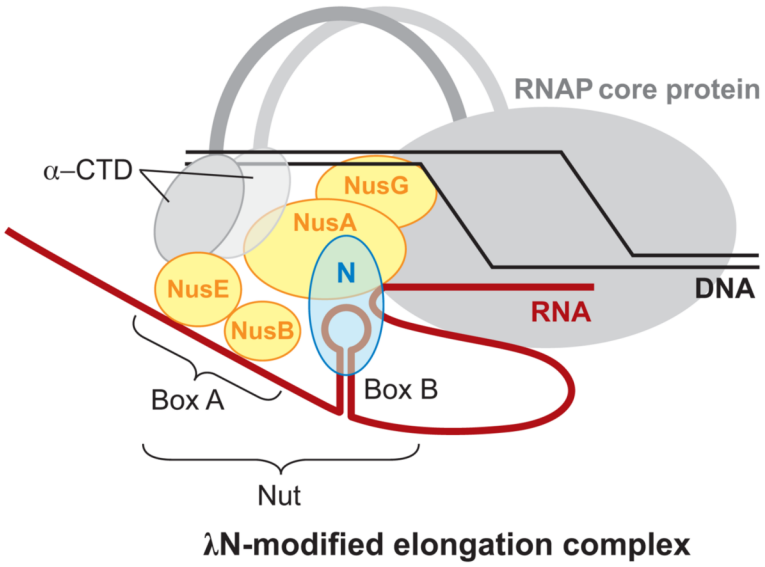

Figure 2.

Structure of the λN-modified elongation complex. Bacterial accessory proteins are shown in orange (NusA, NusB, NusE, NusG), RNAP core protein in gray, RNA in red, and DNA in black. Contacts between most accessory proteins and the RNAP core protein are based on known interactions, but the overall configuration is arbitrary. No contact of NusG with other accessory proteins is known, although NusG binds to the RNAP core.

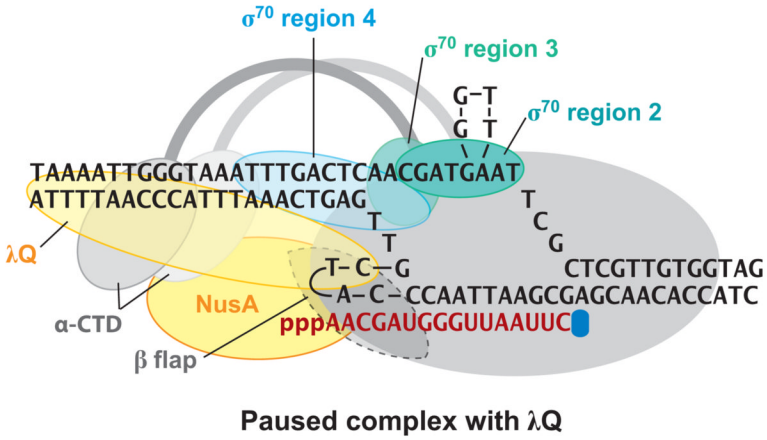

Figure 3.
The promoter-proximal σ^{70}-dependent paused complex of the λ late gene promoter, with the antiterminator λQ bound. As described in the text, the DNA is shown scrunched by four nucleotides (49), allowing synthesis to +16.

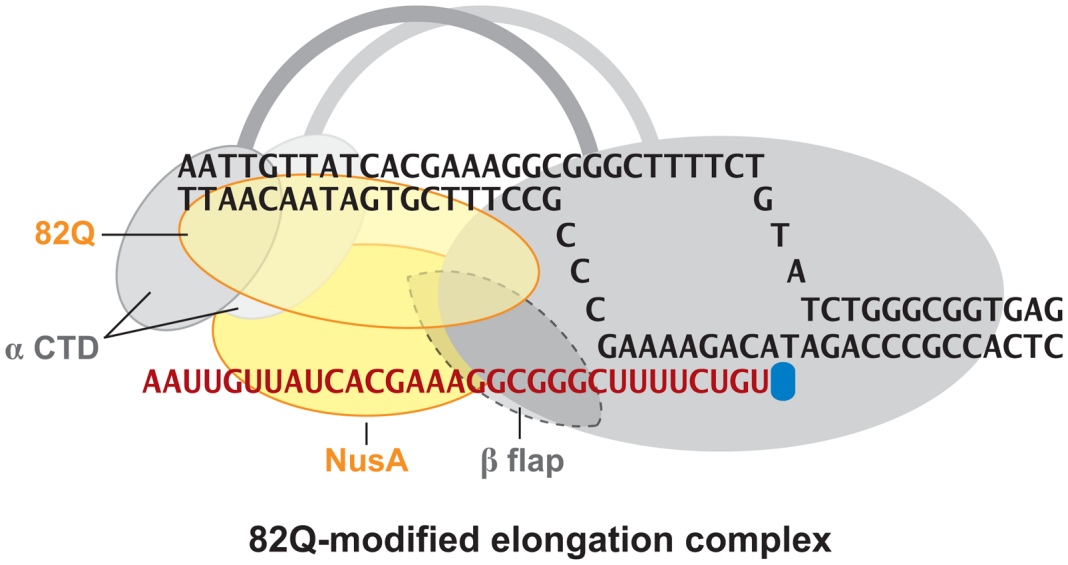

Figure 4.
The 82Q-modified elongation complex. NusA and 82Q are shown obscuring about 10 nucleotides of emerging RNA to represent the protection of this segment from nuclease digestion, but the actual relative configuration of RNA and proteins is unknown.

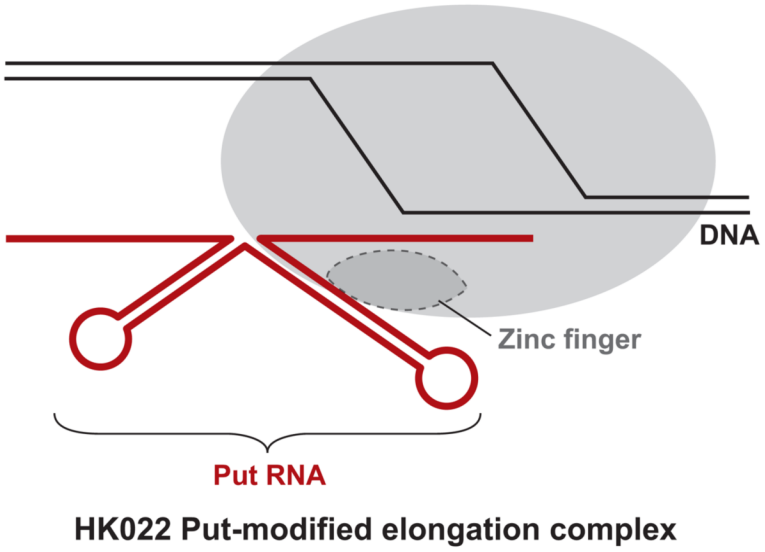

Figure 5.
The phage HK022 Put-modified elongation complex. The RNA secondary structure is indicated by two hairpin stems and loops, and the length of RNA is shown as it might be when Put first binds as it emerges from RNAP.