Published in final edited form as:

Trends Biochem Sci. 2016 August ; 41(8): 690–699. doi:10.1016/j.tibs.2016.05.012.

Learning from the Leaders: Gene Regulation by the Transcription Termination Factor Rho

Michelle A. Kriner^{1,2,^}, Anastasia Sevostyanova^{3,^}, and Eduardo A. Groisman^{1,2,*}

¹Department of Microbial Pathogenesis, Yale University School of Medicine, New Haven, CT 06536

²Microbial Sciences Institute, Yale University, West Haven, CT 06516

³Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06511

Abstract

The RNA helicase Rho triggers 20–30% of transcription termination events in bacteria. While Rho is associated with most transcription elongation complexes, it only promotes termination of a subset. Recent studies of individual Rho-dependent terminators located within the 5' leader regions of bacterial mRNAs have identified novel mechanisms that govern Rho target specificity and revealed unanticipated physiological functions for Rho. In particular, the multistep nature of Rho-dependent termination enables regulatory input from determinants beyond the sequence of the Rho loading site and allows a given Rho-dependent terminator to respond to multiple signals. Further, the unique position of Rho as a sensor of cellular translation has been exploited to regulate transcription of genes required for protein synthesis, including those specifying Mg²⁺ transporters.

Broadening the scope of Rho function

Accurate gene expression requires termination of transcription at the proper place and time. In bacteria, transcription termination is either intrinsic (i.e., directly encoded by the template DNA), or dependent on the RNA helicase Rho [1]. Rho mediates at least 20% of bacterial transcription termination events [2, 3]. Rho performs a number of functions on a genome-wide basis that serve to maintain integrity of the genome and gene expression as a whole. For example, Rho suppresses antisense transcription [3], prevents synthesis of aberrant and/or unnecessary transcripts when translation is impaired [4] and resolves conflicts between transcription and replication machineries [5].

Recently, the importance of Rho in implementing gene-specific regulatory decisions has become a subject of intense interest. Rather than promoting constitutive transcription

*Correspondence: eduardo.groisman@yale.edu (E.A. Groisman), Tel. (+1) 203-737-7940; Fax (+1) 203-737-2630.

[^]These authors contributed equally to this work

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

termination at the end of genes or operons, regulated Rho-dependent terminators operate with tunable efficiency based on the presence of specific cytoplasmic signals. Because the molecular features of Rho-dependent terminators were defined by studying a handful of examples, it remains an open question whether there are structural and/or mechanistic differences between constitutive and regulated terminators. Here, we review how studies of individual Rho-dependent terminators within 5' leader regions have generated insights about the roles of Rho in gene regulation and cellular physiology, particularly as a sensor of the translational state of the cell.

Identification of Rho-dependent terminators

Intrinsic terminators consist of an RNA hairpin followed by a U-tract and are often identifiable using bioinformatic approaches. By contrast, Rho-dependent terminators have so far proven impossible to predict computationally [6]. As a result, discovery of Rho-dependent terminators is a slow process, with new examples largely being identified on a case-by-case basis only after isolation of suppressor mutations in *rho* or when transcriptional regulation is observed in the absence of accompanying intrinsic termination signals [7, 8].

Over the past decade, high-throughput techniques have led to a significantly improved picture of where in the genome Rho acts. For example, measurement of changes in mRNA abundance [3, 9] and RNAP occupancy [2] upon treatment with the Rho-specific inhibitor **bicyclomycin** (BCM) (see Glossary) identified transcripts and genomic locations of bicyclomycin sensitivity, respectively, with high resolution. Whereas these approaches revealed that Rho-dependent termination impacts specific subsets of genes, they did not distinguish direct effects of Rho from those mediated by targets of Rho action. A chromatin immunoprecipitation-microarray hybridization (ChIP-chip) assay examining Rho association with DNA indicated that Rho is actually associated with most transcription elongation complexes (TEC) [10], even though Rho-dependent termination occurs in only a fraction of these TEC [2]. As a result, how Rho selects its targets remains largely unknown. Studies of individual Rho-dependent terminators can complement genome-wide approaches by identifying molecular determinants required for the specificity of Rho-RNA interactions.

The process of Rho-dependent transcription termination

Rho-dependent transcription termination involves a complex series of events (Fig. 1). Initial RNA binding occurs at the primary binding site (PBS) of Rho, which is located on an exposed face of the ring-shaped hexamer formed by the N-terminal domains of each Rho monomer (Fig. 1) [11–13]. Substrate specificity is determined by clefts (one per Rho monomer) that are only big enough to accommodate pyrimidines and exhibit a preference for YC dinucleotides [14]. The distance between clefts necessitates a spacer of at least ~12–14 RNA residues between recognized YC dinucleotides [15, 16]. As a result of these requirements, targeted mRNAs tend to be rich in pyrimidines, especially cytidines, within a 60–90 nt window designated a **Rho utilization (*rut*) site** [17, 18]. It is generally assumed that *rut* sites need to be free of RNA secondary structures [19], although it is feasible that structured regions between sites of direct Rho-RNA binding could be “looped out” [20]. In

addition, Rho cannot access actively translated transcripts because ribosomes block access to *rut* sites [4].

The Rho hexamer is thought to initially bind its substrates in a “lock-washer” conformation in which the ring is open wide enough to accommodate single-stranded RNA (Fig. 1) [21]. The inward tilt of the crown-like PBS naturally directs the 3' portion of a bound RNA into the central channel of Rho [21, 22], where it contacts a secondary binding site (SBS) (Fig. 1) [23–25]. This RNA-SBS interaction is thought to cause closure of the ring [25, 26] and activate the ATPase activity of the protein [12, 25], which, in turn, allows Rho to act as a helicase and translocate along the RNA in a 5' to 3' direction (Fig. 1) [27]. Recent results have challenged the conventional model that a translocating Rho molecule “chases” RNAP along the transcript. Instead, Rho may stably associate with RNAP throughout the transcription cycle, although studies do not agree whether this interaction is independent of the nascent RNA [10, 28] or occurs after PBS-*rut* site recognition [29].

Multiple mechanisms have been proposed regarding how the Rho SBS couples ATP hydrolysis to translocation. Crystallographic evidence suggests an “escort” model in which a single Rho protomer remains bound to a given RNA nucleotide as it moves through the pore along an asymmetrical “staircase” path in which each ATP hydrolysis event is predicted to move Rho forward one base by tugging the RNA into the channel [30, 31]. However, use of a nucleotide analog interference mapping technique suggested that Rho movement along an RNA instead occurs in leaps about 7-nt long [32]. It has been established that the RNA remains bound to the PBS throughout, resulting in a tethered tracking model of translocation [33–36].

Once a translocating Rho molecule nears the point at which the 3' end of the RNA emerges from RNAP, it can trigger transcription termination (Fig. 1). In order for Rho to have a sufficient time window in which to “catch” RNAP, the transcription elongation complex must pause. As a result of the kinetic coupling between Rho and RNAP, all sites of Rho-dependent termination are thought to be RNAP pause sites [6, 37, 38]. In addition, the window in which Rho can act is limited by translation; if a ribosome loads in between Rho and RNAP, then Rho will not be able to trigger transcription termination.

It is generally agreed that ATP hydrolysis by Rho generates a sufficient force to dislodge RNAP from the DNA template [39]. However, the mechanism by which this happens remains a topic of debate. For example, one model posits that Rho causes shearing of the RNA-DNA hybrid by pulling on the RNA [40]. Another model postulates that Rho allosterically triggers conformational changes in RNAP that inactivate the RNAP active site without displacing the RNA 3' end [28]. Alternatively, Rho may exert a pushing force that causes RNAP to hyper-translocate along the DNA without continuing RNA synthesis [41]. None of the proposed models can yet account for all experimental observations [6].

In summary, in order to trigger transcription termination, Rho must: (i) recognize and bind a substrate RNA via its PBS, (ii) thread the RNA into its central channel to begin translocating in a 5' to 3' direction, (iii) catch a paused transcription elongation complex, and (iv) catalyze release of the nascent RNA and template DNA from RNAP (Fig. 1). Many molecular details

of this process remain to be fully elucidated (see Outstanding Questions). As discussed in later sections, the multi-step nature of Rho-dependent transcription termination allows regulation of this process at multiple points, thus offering a useful platform for gene control.

Genome-wide functions of Rho

The *rho* gene is essential in many bacteria, including *Escherichia coli* [42] and *Salmonella* [43], indicating that Rho plays important and non-redundant roles in the cell. Indeed, Rho has been found to execute several functions on a genome wide scale that maintain genome integrity and fidelity of gene expression. We briefly summarize these functions and refer readers to excellent reviews on this topic [6, 44, 45].

Early studies identified Rho as the mediator of **transcriptional polarity**, the phenomenon by which a nonsense mutation in an early gene within a multicistronic operon reduces transcription of downstream genes within the same operon (Fig. 2B) [46–50]. Today, transcriptional polarity is more broadly defined as the process by which Rho terminates transcription of mRNAs genome-wide when **transcriptional-translational coupling** is disrupted [4]. The ability of Rho to implement polarity centers on the notion that the presence of translating ribosomes on a given RNA normally blocks Rho access to cryptic *rut* sites (Fig. 2A). This situation is unique to bacteria because transcription and translation occur at the same time and location, in contrast to eukaryotes in which transcription and translation are physically separated by the nuclear membrane. Moreover, recent evidence suggests that the leading ribosome actually contacts RNA polymerase (RNAP) during coupled transcription-translation via the NusG:NusE protein complex [51, 52]. Because interaction between Rho and NusG is required for a significant portion of Rho-dependent termination *in vivo* [53], liberation of NusG from the NusG:NusE complex when transcription and translation become uncoupled should also contribute to transcriptional polarity.

Beyond its role as the arbiter of polarity, Rho is responsible for silencing expression of prophages and other horizontally acquired DNA, likely because these sequences often lack intrinsic termination signals [9]. In addition, Rho (with help from the transcription elongation factor NusG and the histone-like protein H-NS) prevents excessive antisense transcription from occurring, which would otherwise interfere with normal transcription [3]. Aside from its role in maintaining transcriptome quality, proper termination of transcription by Rho preserves genome integrity by preventing the formation of **R-loops**, which are RNA-DNA hybrids generated when untranslated nascent RNA re-anneals to upstream DNA [54], and by resolving collisions between transcription and replication machineries [5].

Gene-specific Rho-dependent terminators

Beyond its roles in maintaining integrity of gene expression genome-wide, Rho action at particular sites controls transcription of individual genes or operons. The function of a gene-specific Rho-dependent terminator can be influenced by intracellular conditions, and recent studies highlight that this property provides the basis for many gene regulatory mechanisms.

We divide our discussion of these terminators based on their location within a given genomic locus.

Operonic and intergenic

Rho is required for normal expression of many genes. For instance, Rho-dependent termination signals encoded at the end of transcription units constitutively trigger transcription termination, such as in the *trpEDCBA* operon [55] and *tyrT* locus in *E. coli* [56]. Indeed, most Rho-dependent terminators are located at gene ends [3]. RNA 3' ends generated by Rho-dependent termination may be important for proper RNA turnover; for example, the *slrA* transcript in *B. subtilis* can only be degraded by polynucleotide phosphorylase when the 3' end lacks a strong structured element such as an intrinsic terminator [57].

Individual Rho-dependent terminators can also serve as platforms for gene regulation. Unlike their constitutive counterparts, the efficiency of regulated Rho-dependent terminators is controlled by cytoplasmic signal(s) sensed by a variety of factors [8, 43, 58, 59]. In the case of the otherwise constitutive *tR1* terminator located within the major rightward operon of bacteriophage λ , Rho-dependent termination is abolished by binding of the phage *N* protein to an RNA hairpin located within the *rut* site [60, 61]. *N*-binding prompts assembly of a multiprotein anti-termination complex that allows RNAP to continue past the Rho-dependent terminator [62], thus facilitating sequential expression of phage genes [63].

Alternatively, regulator binding to an mRNA can expose a *rut* site that would normally be occluded by translating ribosomes. For example, binding of the sRNA ChiX to the *chiP* translation start site of the *chiPQ* operon in *Salmonella* induces Rho-dependent termination prior to the *chiQ* coding region [8]. ChiX is constitutively expressed and thus prevents expression of *chiPQ*, which encodes an uptake system for chitin-derived saccharides, in the absence of its substrates. Repression is relieved upon expression of a chitooligosaccharide-induced transcript that can base pair with ChiX and promote its degradation [64]. This mechanism contributes to cellular economy by avoiding unnecessary transcription of the full operon under conditions in which it will not be translated.

Within 5' leader regions

Regulated Rho-dependent terminators are often located within 5' leader regions of bacterial mRNA transcripts. In general, the signals sensed by these leaders—including proteins, ions and small molecules—do not occlude *rut* sites on their own; instead, they induce changes in RNA folding or ribosome translocation that, in turn, influence *rut* site accessibility. For example, the RNA-binding protein CsrA unfolds an RNA structure within the *E. coli* *pgaABCD* leader that normally sequesters a *rut* site (Fig. 3A) [58]. This mechanism links expression of *pgaABCD*, an operon involved in the biosynthesis and export of a polysaccharide adhesin associated with biofilm formation, to the carbon metabolism-related signals that control CsrA [58].

The efficiency of Rho-dependent termination upstream of the structural genes in the *E. coli* tryptophanase operon *tnaCAB* is controlled by the availability of L-tryptophan [65]. When L-tryptophan levels are above a certain threshold, interactions between L-tryptophan, the

ribosome translating the *tnaC* open reading frame (ORF), and the TnaC peptide itself cause the ribosome to stall within *tnaC*, thereby occluding a *rut* site. By contrast, when L-tryptophan levels are low (thus obviating the need for tryptophanase expression), ribosomes complete translation of *tnaC* and then dissociate from the transcript, leaving the *rut* site accessible to Rho [66, 67].

Binding of the small molecule flavin mononucleotide to the *E. coli ribB* leader or Mg^{2+} to the *Salmonella mgtA* leader both favor adoption of RNA conformations that increase accessibility of a *rut* site to Rho (Fig. 3B) [43]. In turn, Rho promotes transcription termination within the respective leader regions, thereby preventing expression of proteins involved in the biosynthesis and uptake of nutrients when they are abundant in the cytoplasm. Riboswitch regulation of Rho-dependent termination may be widespread given that many known riboswitch aptamers are not associated with recognizable intrinsic terminators [43]. In the case of the *mgtA* leader, efficient translation of a short ORF within the leader, termed *mgtL*, is also expected to favor the RNA conformation that exposes the *rut* site (Fig. 2C and 3B) [43, 68]. Because *mgtL* harbors three conserved proline codons, translational efficiency is influenced by the availability of proline-charged tRNA^{Pro} [68].

mRNA leaders can regulate steps of Rho-dependent termination beyond *rut* site accessibility

All of the aforementioned examples of regulatory Rho-dependent termination involve modulation of *rut* site accessibility. However, it has recently been shown that cytoplasmic signals can regulate additional steps of Rho-dependent termination. For example, the *mgtCBR* leader RNA in *Salmonella* can adopt two mutually exclusive conformations that expose or sequester a Rho-antagonizing RNA element (RARE) that prevents Rho from forming a translocation-competent complex (Fig. 3C) [69]. RARE does not hinder Rho binding to the *rut* site within the *mgtCBR* leader; instead, it promotes formation of an inactive complex that could represent a trapped intermediate in the Rho recruitment pathway [69]. Discovery of RARE signifies that the specificity of Rho, and potentially other nucleic acid-binding proteins, is defined not only by sequences that mediate their recruitment but also by sequences that antagonize their activity.

In addition to modulating *rut* site accessibility (Fig. 3B), the conformation of the *mgtA* leader RNA also influences RNAP pausing at the site of Rho-dependent termination (Fig. 3D). Specifically, pausing is hyper-stabilized by the same RNA structure that exposes the *rut* site [70], thus enhancing the ability of this conformation to promote Rho-dependent termination (Fig. 3B and D). This result further indicates that a given RNA structure can control two features of a Rho-dependent terminator (Rho-RNA binding and RNAP pausing), potentially allowing integration of multiple regulatory signals. Curiously, there is a single site of Rho-dependent termination in the *mgtA* leader; typically, Rho can terminate at any of a number of RNAP pause sites within a “termination zone” [71, 72]. Compared to modulation of *rut* site accessibility, regulation of RNAP pausing efficiency or the ability of the Rho-RNA complex to adopt a translocation-competent form is likely to have more subtle effects on the efficiency of transcription termination.

Control of these later steps also allows determinants beyond the nucleotide sequence of the *rut* site to influence the specificity of Rho-RNA interactions. Moreover, regulation of multiple steps by a single RNA enables fine-tuning of termination efficiency in response to multiple physiological signals. Interestingly, a recent study found that Rho SBS mutants, but not PBS mutants, are defective for genome-wide Rho-dependent termination, which suggests that the primary binding function of Rho is largely dispensable *in vivo* [73]. Together, these results suggest that regulation of steps other than *rut* site recognition is likely to be widespread.

A physiological link between Rho and Mg²⁺ homeostasis

It was recently established that Rho-dependent transcription terminators reside within the 5' leader regions of all three Mg²⁺ transporter genes in *Salmonella*—*mgtA*, *mgtB* and *corA* [43, 59, 69]. Given the small number of regulated terminators that have been identified to date, this result is conspicuous. One possible advantage to employing Rho, rather than an intrinsic terminator, to regulate Mg²⁺ uptake is that the efficiency of intrinsic termination increases in low Mg²⁺ conditions [74]; if the Mg²⁺ uptake genes were regulated by intrinsic terminator-based attenuation mechanisms, then the cell would risk shutting down transporter expression in low Mg²⁺. By contrast, Mg²⁺ is required for ATP binding and hydrolysis by Rho *in vitro* [75, 76], although it is not clear whether fluctuations in intracellular Mg²⁺ would significantly affect Rho activity *in vivo* given that (i) such fluctuations are small [77] and (ii) ATP is typically present in millimolar quantities [78], far higher than the low micromolar affinity of Rho for ATP [79].

As discussed earlier, the efficiency of Rho-dependent termination is naturally determined by the relative rates of many processes, including Rho ATP hydrolysis and translocation, RNA chain elongation by RNAP and translation by ribosomes. In certain cases, such as the *Salmonella* Mg²⁺ transporter genes, RNA folding dynamics as well as additional Rho-RNA and RNA-RNAP interactions also influence termination efficiency. Notably, Mg²⁺ levels should influence all of these processes. For example, Mg²⁺ is essential to electrostatic and structural stabilization of nucleic acids and nucleotides, and participates directly or indirectly in catalysis of many enzymes, including RNAP and ribosomes [80]. Thus, Rho has a unique ability to integrate many signals related to Mg²⁺ homeostasis itself. While these factors are expected to influence overall Rho function, regulation of Mg²⁺ transport genes by Rho may represent a mechanism for balancing Mg²⁺ levels according to the needs of processes essential for proper gene expression.

Rho as a sensor of translational status

The efficiency of Rho-dependent termination within the 5' leaders of all three Mg²⁺ transporter genes in *Salmonella* is influenced by translation of a short ORF located near the *rut* site [43, 59, 69]. Strikingly, translation of the adjacent ORF stimulates Rho action by promoting formation of secondary structures that favor *rut* site access or Rho activity (Fig. 2C). This scenario is opposite of canonical transcriptional polarity, in which Rho prompts transcription termination of poorly translated mRNAs (Fig. 2B).

The phenomenon of “reverse polarity” may aid *Salmonella* in coping with translational stressors. For the vast majority of genes, impaired translation would favor termination of RNA synthesis by Rho, whereas impaired translation leads to elevated transcription of the Mg^{2+} uptake genes (Fig. 2C). Increased Mg^{2+} uptake may aid in restoring optimal protein synthesis because Mg^{2+} acts as a structural component of ribosomes, promotes assembly of initiation complexes, and (as Mg^{2+} -ATP) supplies energy for charging of tRNAs [81]. In accordance with this notion, ribosomes and ATP together chelate the majority of Mg^{2+} in the cell [81], indicating that sufficient intracellular Mg^{2+} pools are essential for continuous translation. Indeed, Mg^{2+} starvation of *E. coli* leads to translational arrest and, ultimately, to depletion of the ribosome pool [82].

A decrease in intracellular Mg^{2+} levels should decrease the translation efficiency of all coding regions in the cell, including short ORFs within leader regions. As a result, regulation of Mg^{2+} uptake genes by Rho could be interpreted as a means of specifically sensing that Mg^{2+} levels have decreased enough to negatively impact translation, thus indicating that increased expression of Mg^{2+} transporters is necessary. Moreover, the ability of Mg^{2+} transporter leader mRNAs to reverse the coupling of transcription and translation signifies that the relationship between Rho-dependent termination and translation is more nuanced than previously thought; Rho can be used to sense subtle changes in the rate and/or efficiency of translation, not just the lack of translation *per se*.

Rho was recently implicated in regulation of the *Salmonella tufB* gene, which encodes an allele of the translation elongation factor EF-Tu [83]. As with the Mg^{2+} transporter genes, the proposed mechanism results in an inverse coupling of transcription with translational efficiency. Taken together, these observations suggest that surveillance of nascent transcripts by Rho helps the cell cope with compromised translation in two ways: transcriptional polarity prevents accumulation of truncated, potentially toxic, proteins (Fig. 2B), while reverse polarity in specific gene leaders promotes expression of proteins that can combat translational stress (Fig. 2C).

CONCLUDING REMARKS

We propose that the unique dependence of Rho-dependent termination on multiple intricately timed steps facilitates a regulatory structure in which termination efficiency can be tightly controlled at several regulatory checkpoints in response to multiple physiological signals. In addition, the tendency of Rho to target growing RNAs that are not protected by ribosomes positions it as a sensor of translational status of the cell. We now understand that, beyond genome-wide suppression of dysfunctional and unnecessary transcripts, this ability is actively employed to activate transcription of particular genes in response to translational stress.

GLOSSARY

Bicyclomycin

a small molecule that inhibits Rho activity

R-loop

a nucleic acid structure that forms when a nascent RNA re-anneals to the template strand, thus disrupting the DNA duplex. Unrepaired R-loops can cause DNA breaks, leading to genome instability

***rut* site**

Rho-utilization site; a sequence within a nascent RNA that can be specifically recognized by the primary binding surface of Rho

Transcriptional-translational coupling

the process whereby the first ribosome begins translating a nascent RNA message while it is still being transcribed by RNAP

Transcriptional polarity

the phenomenon in which disrupted translation of an upstream ORF leads to premature transcription termination of downstream gene(s) in the same operon

References

1. Roberts JW. Termination factor for RNA synthesis. *Nature*. 1969; 224:1168–1174. [PubMed: 4902144]
2. Peters JM, et al. Rho directs widespread termination of intragenic and stable RNA transcription. *Proc Natl Acad Sci U S A*. 2009; 106:15406–15411. [PubMed: 19706412]
3. Peters JM, et al. Rho and NusG suppress pervasive antisense transcription in *Escherichia coli*. *Genes Dev*. 2012; 26:2621–2633. [PubMed: 23207917]
4. Richardson JP. Preventing the synthesis of unused transcripts by Rho factor. *Cell*. 1991; 64:1047–1049. [PubMed: 2004415]
5. Washburn RS, Gottesman ME. Transcription termination maintains chromosome integrity. *Proc Natl Acad Sci U S A*. 2011; 108:792–797. [PubMed: 21183718]
6. Peters JM, et al. Bacterial transcription terminators: the RNA 3'-end chronicles. *J Mol Biol*. 2011; 412:793–813. [PubMed: 21439297]
7. Stewart V, Yanofsky C. Evidence for transcription antitermination control of tryptophanase operon expression in *Escherichia coli* K-12. *J Bacteriol*. 1985; 164:731–740. [PubMed: 3902796]
8. Bossi L, et al. A role for Rho-dependent polarity in gene regulation by a noncoding small RNA. *Genes Dev*. 2012; 26:1864–1873. [PubMed: 22895254]
9. Cardinale CJ, et al. Termination factor Rho and its cofactors NusA and NusG silence foreign DNA in *E. coli*. *Science*. 2008; 320:935–938. [PubMed: 18487194]
10. Mooney RA, et al. Regulator trafficking on bacterial transcription units in vivo. *Mol Cell*. 2009; 33:97–108. [PubMed: 19150431]
11. Galluppi GR, Richardson JP. ATP-induced changes in the binding of RNA synthesis termination protein Rho to RNA. *J Mol Biol*. 1980; 138:513–539. [PubMed: 6157829]
12. Dombroski AJ, Platt T. Structure of rho factor: an RNA-binding domain and a separate region with strong similarity to proven ATP-binding domains. *Proc Natl Acad Sci U S A*. 1988; 85:2538–2542. [PubMed: 2451828]
13. Briercheck DM, et al. The NMR structure of the RNA binding domain of *E. coli* rho factor suggests possible RNA-protein interactions. *Nat Struct Biol*. 1998; 5:393–399. [PubMed: 9587002]
14. Bogden CE, et al. The structural basis for terminator recognition by the Rho transcription termination factor. *Mol Cell*. 1999; 3:487–493. [PubMed: 10230401]
15. McSwiggen JA, et al. Interactions of *Escherichia coli* transcription termination factor rho with RNA. I. Binding stoichiometries and free energies. *J Mol Biol*. 1988; 199:609–622. [PubMed: 2451028]

16. Bear DG, et al. Interactions of Escherichia coli transcription termination factor rho with RNA. II. Electron microscopy and nuclease protection experiments. *J Mol Biol.* 1988; 199:623–635. [PubMed: 2451029]
17. Salstrom JS, et al. N-independent leftward transcription in coliphage lambda: deletions, insertions and new promoters bypassing termination functions. *Mol Gen Genet.* 1979; 168:211–230. [PubMed: 286866]
18. Morgan WD, et al. Rho-dependent termination of transcription. II. Kinetics of mRNA elongation during transcription from the bacteriophage lambda PR promoter. *J Biol Chem.* 1983; 258:9565–9574. [PubMed: 6223930]
19. Chen CY, et al. Transcription termination at lambda tR1 is mediated by interaction of rho with specific single-stranded domains near the 3' end of cro mRNA. *Cell.* 1986; 46:1023–1028. [PubMed: 2428503]
20. Schwartz A, et al. Noncanonical interactions in the management of RNA structural blocks by the transcription termination rho helicase. *Biochemistry.* 2007; 46:9366–9379. [PubMed: 17655325]
21. Skordalakes E, Berger JM. Structure of the Rho transcription terminator: mechanism of mRNA recognition and helicase loading. *Cell.* 2003; 114:135–146. [PubMed: 12859904]
22. Rabhi M, et al. RNA remodeling by hexameric RNA helicases. *RNA Biol.* 2010; 7:655–666. [PubMed: 21045542]
23. Richardson JP. Activation of rho protein ATPase requires simultaneous interaction at two kinds of nucleic acid-binding sites. *J Biol Chem.* 1982; 257:5760–5766. [PubMed: 6175630]
24. Wei RR, Richardson JP. Identification of an RNA-binding Site in the ATP binding domain of Escherichia coli Rho by H₂O₂/Fe-EDTA cleavage protection studies. *J Biol Chem.* 2001; 276:28380–28387. [PubMed: 11369775]
25. Skordalakes E, Berger JM. Structural insights into RNA-dependent ring closure and ATPase activation by the Rho termination factor. *Cell.* 2006; 127:553–564. [PubMed: 17081977]
26. Gogol EP, et al. Structure and assembly of the Escherichia coli transcription termination factor rho and its interaction with RNA. I. Cryoelectron microscopic studies. *J Mol Biol.* 1991; 221:1127–1138. [PubMed: 1719215]
27. Brennan CA, et al. Transcription termination factor rho is an RNA-DNA helicase. *Cell.* 1987; 48:945–952. [PubMed: 3030561]
28. Epshtein V, et al. An allosteric mechanism of Rho-dependent transcription termination. *Nature.* 2010; 463:245–249. [PubMed: 20075920]
29. Kalyani BS, et al. Interaction with the nascent RNA is a prerequisite for the recruitment of Rho to the transcription elongation complex in vitro. *J Mol Biol.* 2011; 413:548–560. [PubMed: 21920369]
30. Thomsen ND, Berger JM. Running in reverse: the structural basis for translocation polarity in hexameric helicases. *Cell.* 2009; 139:523–534. [PubMed: 19879839]
31. Lyubimov AY, et al. The nuts and bolts of ring-translocase structure and mechanism. *Curr Opin Struct Biol.* 2011; 21:240–248. [PubMed: 21282052]
32. Schwartz A, et al. A stepwise 2'-hydroxyl activation mechanism for the bacterial transcription termination factor Rho helicase. *Nat Struct Mol Biol.* 2009; 16:1309–1316. [PubMed: 19915588]
33. Faus I, Richardson JP. Structural and functional properties of the segments of lambda cro mRNA that interact with transcription termination factor Rho. *J Mol Biol.* 1990; 212:53–66. [PubMed: 2157021]
34. Steinmetz EJ, Platt T. Evidence supporting a tethered tracking model for helicase activity of Escherichia coli Rho factor. *Proc Natl Acad Sci U S A.* 1994; 91:1401–1405. [PubMed: 7509071]
35. Koslover DJ, et al. Binding and translocation of termination factor rho studied at the single-molecule level. *J Mol Biol.* 2012; 423:664–676. [PubMed: 22885804]
36. Gocheva V, et al. Direct observation of the translocation mechanism of transcription termination factor Rho. *Nucleic Acids Res.* 2015; 43:2367–2377. [PubMed: 25662222]
37. Darlix JL, Horaist M. Existence and possible roles of transcriptional barriers in T7 DNA early region as shown by electron microscopy. *Nature.* 1975; 256:288–292. [PubMed: 1143328]

38. Banerjee S, et al. Rho-dependent transcription termination: more questions than answers. *J Microbiol.* 2006; 44:11–22. [PubMed: 16554712]
39. Dutta D, et al. Transcription termination factor rho prefers catalytically active elongation complexes for releasing RNA. *J Biol Chem.* 2008; 283:20243–20251. [PubMed: 18482981]
40. Richardson JP. Rho-dependent termination and ATPases in transcript termination. *Biochim Biophys Acta.* 2002; 1577:251–260. [PubMed: 12213656]
41. Park JS, Roberts JW. Role of DNA bubble rewinding in enzymatic transcription termination. *Proc Natl Acad Sci U S A.* 2006; 103:4870–4875. [PubMed: 16551743]
42. Das A, et al. Isolation and characterization of conditional lethal mutants of *Escherichia coli* defective in transcription termination factor rho. *Proc Natl Acad Sci U S A.* 1976; 73:1959–1963. [PubMed: 132662]
43. Hollands K, et al. Riboswitch control of Rho-dependent transcription termination. *Proc Natl Acad Sci U S A.* 2012; 109:5376–5381. [PubMed: 22431636]
44. Boudvillain M, et al. Terminator still moving forward: expanding roles for Rho factor. *Curr Opin Microbiol.* 2013; 16:118–124. [PubMed: 23347833]
45. Grylak-Mielnicka A, et al. Transcription termination factor Rho: a hub linking diverse physiological processes in bacteria. *Microbiology.* 2016; 162:433–447. [PubMed: 26796109]
46. Jacob F, Monod J. Genetic regulatory mechanisms in the synthesis of proteins. *J Mol Biol.* 1961; 3:318–356. [PubMed: 13718526]
47. Franklin NC, Luria SE. Transduction by bacteriophage P-1 and the properties of the lac genetic region in *E. coli* and *S. dysenteriae*. *Virology.* 1961; 15:299–311. [PubMed: 13894683]
48. Imamoto F, Yanofsky C. Transcription of the tryptophan operon in polarity mutants of *Escherichia coli*. I. Characterization of the tryptophan messenger RNA of polar mutants. *J Mol Biol.* 1967; 28:1–23. [PubMed: 4860985]
49. Richardson JP, et al. Transcription termination factor rho activity is altered in *Escherichia coli* with *suA* gene mutations. *Proc Natl Acad Sci U S A.* 1975; 72:1725–1728. [PubMed: 1098042]
50. Imamoto F, Yanofsky C. Transcription of the tryptophan operon in polarity mutants of *Escherichia coli*. II. Evidence for normal production of tryp-mRNA molecules and for premature termination of transcription. *J Mol Biol.* 1967; 28:25–35. [PubMed: 4860986]
51. Proshkin S, et al. Cooperation between translating ribosomes and RNA polymerase in transcription elongation. *Science.* 2010; 328:504–508. [PubMed: 20413502]
52. Burmann BM, et al. A NusE:NusG complex links transcription and translation. *Science.* 2010; 328:501–504. [PubMed: 20413501]
53. Sullivan SL, Gottesman ME. Requirement for *E. coli* NusG protein in factor-dependent transcription termination. *Cell.* 1992; 68:989–994. [PubMed: 1547498]
54. Leela JK, et al. Rho-dependent transcription termination is essential to prevent excessive genome-wide R-loops in *Escherichia coli*. *Proc Natl Acad Sci U S A.* 2013; 110:258–263. [PubMed: 23251031]
55. Wu AM, et al. Tandem termination sites in the tryptophan operon of *Escherichia coli*. *Proc Natl Acad Sci U S A.* 1981; 78:2913–2917. [PubMed: 6265923]
56. Rossi J, et al. The *tyrT* locus: termination and processing of a complex transcript. *Cell.* 1981; 26:305–314. [PubMed: 7034960]
57. Liu B, et al. Expression of multiple *Bacillus subtilis* genes is controlled by decay of *slrA* mRNA from Rho-dependent 3' ends. *Nucleic Acids Res.* 2016; 44:3364–3372. [PubMed: 26857544]
58. Figueroa-Bossi N, et al. RNA remodeling by bacterial global regulator CsrA promotes Rho-dependent transcription termination. *Genes Dev.* 2014; 28:1239–1251. [PubMed: 24888591]
59. Kriner MA, Groisman EA. The Bacterial Transcription Termination Factor Rho Coordinates Mg(2+) Homeostasis with Translational Signals. *J Mol Biol.* 2015; 427:3834–3849. [PubMed: 26523680]
60. Doelling JH, Franklin NC. Effects of all single base substitutions in the loop of boxB on antitermination of transcription by bacteriophage lambda's N protein. *Nucleic Acids Res.* 1989; 17:5565–5577. [PubMed: 2527353]

61. Chattopadhyay S, et al. Bipartite function of a small RNA hairpin in transcription antitermination in bacteriophage lambda. *Proc Natl Acad Sci U S A*. 1995; 92:4061–4065. [PubMed: 7732031]
62. Roberts JW. RNA and protein elements of E. coli and lambda transcription antitermination complexes. *Cell*. 1993; 72:653–655. [PubMed: 8453661]
63. Ward DF, Gottesman ME. Suppression of transcription termination by phage lambda. *Science*. 1982; 216:946–951. [PubMed: 6281888]
64. Figueroa-Bossi N, et al. Caught at its own game: regulatory small RNA inactivated by an inducible transcript mimicking its target. *Genes Dev*. 2009; 23:2004–2015. [PubMed: 19638370]
65. Stewart V, et al. Rho-dependent transcription termination in the tryptophanase operon leader region of *Escherichia coli* K-12. *J Bacteriol*. 1986; 166:217–223. [PubMed: 2420781]
66. Martinez AK, et al. Interactions of the TnaC nascent peptide with rRNA in the exit tunnel enable the ribosome to respond to free tryptophan. *Nucleic Acids Res*. 2014; 42:1245–1256. [PubMed: 24137004]
67. Bischoff L, et al. Molecular basis for the ribosome functioning as an L-tryptophan sensor. *Cell Rep*. 2014; 9:469–475. [PubMed: 25310980]
68. Park SY, et al. A bacterial mRNA leader that employs different mechanisms to sense disparate intracellular signals. *Cell*. 2010; 142:737–748. [PubMed: 20813261]
69. Sevostyanova A, Groisman EA. An RNA motif advances transcription by preventing Rho-dependent termination. *Proc Natl Acad Sci U S A*. 2015; 112:E6835–6843. [PubMed: 26630006]
70. Hollands K, et al. Unusually long-lived pause required for regulation of a Rho-dependent transcription terminator. *Proc Natl Acad Sci U S A*. 2014; 111:E1999–2007. [PubMed: 24778260]
71. Morgan WD, et al. Rho-dependent termination of transcription. I. Identification and characterization of termination sites for transcription from the bacteriophage lambda PR promoter. *J Biol Chem*. 1983; 258:9553–9564. [PubMed: 6223929]
72. Zhu AQ, von Hippel PH. Rho-dependent termination within the trp t' terminator. II. Effects of kinetic competition and rho processivity. *Biochemistry*. 1998; 37:11215–11222. [PubMed: 9698367]
73. Shashni R, et al. Redundancy of primary RNA-binding functions of the bacterial transcription terminator Rho. *Nucleic Acids Res*. 2014; 42:9677–9690. [PubMed: 25081210]
74. Reynolds R, et al. Parameters affecting transcription termination by *Escherichia coli* RNA polymerase. I. Analysis of 13 rho-independent terminators. *J Mol Biol*. 1992; 224:31–51. [PubMed: 1372365]
75. Stitt BL. *Escherichia coli* transcription termination protein rho has three hydrolytic sites for ATP. *J Biol Chem*. 1988; 263:11130–11137. [PubMed: 3042765]
76. Brennan CA, et al. Specificity and efficiency of rho-factor helicase activity depends on magnesium concentration and energy coupling to NTP hydrolysis. *J Biol Chem*. 1990; 265:5440–5447. [PubMed: 1690711]
77. Romani A. Regulation of magnesium homeostasis and transport in mammalian cells. *Arch Biochem Biophys*. 2007; 458:90–102. [PubMed: 16949548]
78. Bennett BD, et al. Absolute metabolite concentrations and implied enzyme active site occupancy in *Escherichia coli*. *Nat Chem Biol*. 2009; 5:593–599. [PubMed: 19561621]
79. Lowery C, Richardson JP. Characterization of the nucleoside triphosphate phosphohydrolase (ATPase) activity of RNA synthesis termination factor p. I. Enzymatic properties and effects of inhibitors. *J Biol Chem*. 1977; 252:1375–1380. [PubMed: 138681]
80. Maguire ME, Cowan JA. Magnesium chemistry and biochemistry. *Biometals*. 2002; 15:203–210. [PubMed: 12206387]
81. Pontes MH, et al. When Too Much ATP Is Bad for Protein Synthesis. *J Mol Biol*. 2015; 427:2586–2594. [PubMed: 26150063]
82. McCarthy BJ. The effects of magnesium starvation on the ribosome content of *Escherichia coli*. *Biochim Biophys Acta*. 1962; 55:880–889.
83. Brandis G, et al. Autoregulation of the tufB operon in *Salmonella*. *Mol Microbiol*. 2016
84. Burgess BR, Richardson JP. RNA passes through the hole of the protein hexamer in the complex with the *Escherichia coli* Rho factor. *J Biol Chem*. 2001; 276:4182–4189. [PubMed: 11071888]

- What molecular determinants influence the specificity of target selection by the primary binding site of Rho?
- Is there any sequence specificity to the RNA-Rho SBS interaction and/or activation of Rho ATPase activity?
- What structural or sequence features of an RNA (such as RARE) allow it to promote or antagonize Rho activity?
- How does Rho cause release of RNA from the transcription elongation complex?
- What factors influence whether an initial Rho-RNA interaction leads to termination of transcription?
- What are the structural and/or mechanistic differences between constitutive and regulated Rho-dependent terminators?
- Why do particular transcription attenuation mechanisms employ Rho vs. an intrinsic terminator hairpin? Is regulation by Rho associated with certain physiological functions?

- The bacterial transcription termination factor Rho preserves integrity of the genome and gene expression
- Rho is associated with most transcription elongation complexes but only promotes transcription termination of a subset
- Rho specificity can be defined by sequences that mediate its recruitment, sequences that antagonize its activity, and determinants of RNA polymerase pausing
- A leader mRNA can exploit the multistep nature of Rho-dependent termination to integrate multiple physiological signals
- Rho controls transcription of all three magnesium transporters in *Salmonella*, revealing a physiological link between Rho and magnesium homeostasis
- By competing with translating ribosomes for nascent RNA, Rho acts as a sensor of translational status. This property facilitates regulation of particular genes in response to translational signals

Figure 1. The process of Rho-dependent termination and its regulatory checkpoints

The Rho-RNA complex undergoes several isomerization events before reaching a translocation-competent state. The initial complex consists of a transient interaction between an RNA substrate (black) and a positively charged surface of the Rho hexamer (blue) [21]. Sequence-specific contacts (yellow asterisks) between RNA and the Rho primary binding site lead to the ring opening and threading of the RNA through the central hole of the hexamer [21, 84]. The RNA then establishes contacts with the secondary binding site of Rho, leading to ring closure [25]. Using the energy derived from ATP hydrolysis, Rho translocates in a 5' to 3' direction until it catches up with a paused RNAP [23]. The steps of the pathway used as regulatory checkpoints in various gene leaders are shown with arrows and gene names in red.

Figure 2. Rho as a translational sensor

(A) Transcription-translation coupling. Normally, ribosomes translating nascent RNA emerging from RNA polymerase prevent Rho action by blocking Rho access to *rut* sites. This genome-wide effect leads to a positive correlation between transcription and translation [51].

(B) Transcriptional polarity. The uncoupling of transcription and translation allows Rho recruitment. Therefore, when translation is impaired (e.g., by a nonsense mutation (red star)), *rut* sites are no longer occluded by ribosomes and Rho can load to the RNA and promote transcription termination [50].

(C) Reverse polarity. When efficient ORF translation promotes Rho-dependent transcription termination, the outcome of transcriptional-translational coupling is reversed. In the case of the *Salmonella* Mg^{2+} transporter loci, efficient translation of a short ORF promotes Rho-dependent termination by favoring an RNA conformation that either brings together a composite *rut* site (*mgtA*, *corA*) [43, 59] or sequesters a Rho-antagonizing RNA element (RARE) in a stem-loop (*mgtCBR*) [69]. In the case of the *tufB* gene, slow translation elongation leads to accumulation of ribosomes that occlude a *rut* site [83]. By contrast, inefficient translation of the short ORF favors an RNA conformation that prevents Rho action, for instance by disrupting a *rut* site.

Figure 3. Mechanisms of regulated Rho-dependent termination

(A) RNA remodeling by a protein. The RNA-binding protein CsrA unfolds a stem-loop that sequesters the *rut* site in the leader of the *pgaABCD* operon in *E. coli* thereby making the *rut* site available to Rho and resulting in transcription termination (left) [58].

(B) Formation of alternative stem-loop structures. Rho recruitment in the *Salmonella mgtA* and *corA* gene leaders is controlled by selective formation of RNA structures that bring together or disrupt a multi-partite *rut* site [43, 59]. This allows Rho recruitment and transcription termination (left)

(C) Rho-antagonizing RNA element. The *Salmonella mgtCBR* leader RNA can adopt alternative conformations that either occlude a Rho-antagonizing RNA element (RARE) in a stem-loop or leave RARE in a single-stranded, active form. RARE induces a catalytically inactive conformation of Rho bound to the *mgtCBR* leader RNA without affecting the interaction between Rho and the *rut* site [69]. When RARE is sequestered in an alternative stem-loop, Rho forms a productive complex with the *rut* site resulting in transcription termination (left).

(D) RNA pausing. RNA structures in the *Salmonella mgtA* leader influence the longevity of RNAP pausing, which, in turn, affects the fraction of elongating transcription complexes that will be disrupted by Rho [70]. Extended RNAP pausing allows Rho to terminate transcription (left).