Rho-dependent transcription terminators participate in sophisticated genetic regulatory mechanisms, in both bacteria and phages; they occur in regulatory regions preceding the coding sequences of genes and within coding sequences, as well as at the end of transcriptional units, to prevent readthrough transcription. Most Rho-dependent terminators have been found in enteric bacteria, but they also occur in Gram-positive bacteria and may be widespread among bacteria. Rho-dependent termination requires both cis-acting elements, on the mRNA, and trans-acting factors. The only cis-acting element common to Rho-dependent terminators is richness in rC residues. Additional sequence elements have been observed at different Rho termination sites. These ‘auxiliary elements’ may assist in the termination process; they differ among terminators, their occurrence possibly depending on the function and sequence context of the terminator. Specific nucleotides required for termination have also been identified at Rho sites. Rho is the main factor required for termination; it is a ring-shaped hexameric protein with ATPase and helicase activities. NusG, NusA and NusB are additional factors participating in the termination process. Rho-dependent termination occurs by binding of Rho to ribosome-free mRNA, C-rich sites being good candidates for binding. Rho's ATPase is activated by Rho–mRNA binding, and provides the energy for Rho translocation along the mRNA; translocation requires sliding of the message into the central hole of the hexamer. When a polymerase pause site is encountered, the actual termination occurs, and the transcript is released by Rho's helicase activity. Many aspects of this process are still being studied. The isolation of mutants suppressing termination, site-directed mutagenesis of cis-acting elements in Rho-dependent termination, and biochemistry, are and will be contributing to unravelling the still undefined aspects of the Rho termination machinery. Analysis of the more sophisticated regulatory mechanisms relying on Rho-dependent termination may be crucial in identifying new essential elements for termination.

Introduction

Bacteria and their phages use two main modes of terminating transcription: Rho-independent or ‘intrinsic’ termination, mainly requiring elements located on the mRNA, and Rho-dependent termination, relying on both mRNA elements and trans-acting factors (reviewed by Richardson & Greenblatt, 1996). Rho-dependent termination has been intensively studied, and it has previously been extensively reviewed (Richardson, 2002, 2003). About half of the transcription terminators identified in *Escherichia coli* are Rho-dependent. These terminators lie at the natural end of genes or within cistrons and in control regions preceding the coding sequences of genes, where they play an important role in the regulation of gene expression.

Rho is a homohexameric protein with RNA-dependent ATPase and helicase activities (reviewed by Platt & Richardson, 1992), which binds to the mRNA. Essential sites on the mRNA are the Rho-binding site, known as the Rho utilization site (*rut*), and a distal region where the transcripts are terminated at multiple transcription stop points (*tsp*) and released, mediated by Rho interaction with RNA polymerase (reviewed by Richardson & Greenblatt, 1996). This process requires a ribosome-free transcript of at least 85–97 nt (Hart & Roberts, 1994). This transcript exhibits a high-C low-G content, which is consistent with the known requirements for rC residues and for an unstructured RNA by the Rho activities (reviewed by Richardson & Greenblatt, 1996; for a summary see Guérin et al., 1998).

Rho loads onto the mRNA at a binding site of 70–80 nt (Zhu & von Hippel, 1998a), smaller than the ribosome-free transcript required for the termination process. The Rho-binding site seems to be the primary and essential determinant for Rho termination activity (Guérin et al., 1998). Thus, the C richness of Rho site sequences could very well be the main requirement for Rho binding, consistent with the fact that this is the only feature common to Rho-dependent terminators.
In addition to C richness, other sequence elements, termed 'auxiliary elements' in this review, have been observed only at some Rho-dependent termination sites. For instance, rutA and rutB elements have been described in the λtR1 terminator (Chen & Richardson, 1987), and suggested to provide a strong attachment site for Rho (Graham & Richardson, 1998) on the mRNA. Similar rut elements have been found at the trp t' terminator of E. coli (Zalatan et al., 1993). Some Rho-dependent terminator regions show sequences with homology to boxA (Friedman & Olson, 1983), which has an antitermination function in the bacteriophage λ (Das, 1992) and in other systems (Heinrich et al., 1995; Vogel & Jensen, 1995); interestingly, boxA-like sequences in the tna operon of E. coli (Gong & Yanofsky, 2002) and in the his operon of Salmonella typhimurium (Ciampi et al., 1989; Carlomagno & Nappo, 2001) seem to be required for Rho-dependent termination. These and other potential 'auxiliary elements' (listed in Table 1), which are not common features of Rho-dependent terminators, could be part of a pool of elements, different combinations of which may contribute to Rho loading efficiency and to the efficiency of termination. Which 'auxiliary element(s)' occur at a specific Rho site may depend on the 'local' context of the terminator, and this would explain why not all terminators exhibit the same elements, other than C richness.

Rho binding to the message activates Rho’s 5’ to 3’ ATP dependent RNA–DNA helicase (Delagouette & von Hippel, 2003), allowing translocation of Rho along the message, toward polymerase pause sites. At these key sites interactions with RNA polymerase (Guarente, 1979) lead to termination and release of transcripts (Yager & von Hippel, 1987). Rho protein also interacts with other Rho-dependent termination factors, such as NusG (Sullivan & Gottesman, 1992; for a summary see Burns et al., 1999), NusA (Schmidt & Chamberlin, 1984; for a summary see Ingham et al., 1999) and NusB (Carlomagno & Nappo, 2001), which play an important role in the release of the terminated transcripts.

In this article I review the features of several Rho-dependent terminators, with the intent of drawing a picture of the various contexts in which terminators occur and of the different roles that they play, in relationship to the mechanism of Rho-dependent termination and to the regulation of gene expression. I also summarize older and more recent data on the various elements involved in factor-dependent termination, emphasizing important insights that affected this field and remaining problems. Models for Rho-dependent termination are also discussed.

I start with a survey of Rho-dependent terminators: for each terminator the facts most relevant to the role of the terminator and to the mechanism of termination are presented; similarities and differences among the terminators are highlighted. The terminators are classified into two groups: constitutive and regulated terminators (see Table 2). The regulated terminators are divided into two subgroups: conventionally regulated Rho-dependent terminators and intragenic terminators, most of the latter being those responsible for transcriptional polarity effects. The main focus of the discussion will not be on the regulatory mechanisms, rather on the features of the Rho-dependent terminators themselves. I then review cis-acting sequence elements and trans-acting factors required for Rho-dependent termination. The ‘auxiliary elements’ identified at some terminators are presented and discussed for the individual terminators. Finally, ideas and facts leading to the current models for Rho-dependent termination are summarized.

### Constitutive Rho-dependent terminators

Some Rho-dependent terminators occur at the end of transcriptional units, where transcription stops independently of any regulatory mechanism. As expected, these terminators are constitutive. Three such terminators are described in this section: the trp and tyrT operon terminators of E. coli, and the gene IV transcript terminator of the E. coli bacteriophage f1 (Table 2).

**trp operon of E. coli.** One of the most investigated Rho-dependent terminators is trp t’, located at the very end of the trp operon of E. coli, terminating the synthesis of the polycistronic trpA–E mRNA (Platt, 1981). In vitro studies of a DNA fragment carrying the terminator region revealed that the terminator is Rho-dependent and that

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**Table 1. ‘Auxiliary elements’ associated with Rho-dependent termination sites**

<table>
<thead>
<tr>
<th>Location of terminator (name)</th>
<th>Auxiliary elements</th>
</tr>
</thead>
<tbody>
<tr>
<td>trp operon of E. coli (trp t’)</td>
<td>rutA-like and rutB-like</td>
</tr>
<tr>
<td>tyrT operon of E. coli</td>
<td>R1 and R2</td>
</tr>
<tr>
<td>cro gene of λ (tR0)</td>
<td>CAAUCAA</td>
</tr>
<tr>
<td>Colicin E1 gene of E. coli</td>
<td>CAACAA</td>
</tr>
<tr>
<td>Rightward operon of λ (tR1)</td>
<td>rutA and rutB</td>
</tr>
<tr>
<td>hisG gene of S. typhimurium</td>
<td>CAACAA</td>
</tr>
<tr>
<td>tna operon of E. coli</td>
<td>boxA-like</td>
</tr>
<tr>
<td>ilv operon of E. coli</td>
<td>boxA-like</td>
</tr>
<tr>
<td>rutB boxA</td>
<td>CAUCA</td>
</tr>
<tr>
<td>CAUCGA</td>
<td></td>
</tr>
</tbody>
</table>
the actual sites of termination are spread over a region of about 100 nt (Wu et al., 1981).

Transcription of the DNA fragment from the gal promoter showed that the activity of the terminator is not promoter-specific, allowing deletion analysis of the first 105 bp sequence of the region and identification of a Rho ‘loading site’ and of a pair of elements within it (summarized by Zalatan et al., 1993). These elements are examples of what I have defined above as ‘auxiliary elements’; that is, they are not common to all terminators, but they are found in the specific context of one or a few terminators. Indeed, the pair of elements observed at the trp t’ terminator are similar to the rutA and rutB sequences identified in the λ tR1 terminator (see later) and proposed to be required for the ATPase activity of Rho (Chen & Richardson, 1987). In trp t’, the rutB-like element seems to be more important than the rutA-like element, which appears to be non-functional unless the rutB-like sequence is present. Two similar subelements, R1 and R2, within the Rho-loading site, have also been proposed to be important for Rho-dependent termination at trp t’ (Zalatan et al., 1993).

Efficient loading of Rho at trp t’ guarantees efficient and precise termination at the downstream termination zone; in fact, introduction of a secondary structure within the Rho loading site both reduces termination efficiency and shifts the initial termination site downstream (Zhu & von Hippel, 1998a). Zhu & von Hippel (1998a) also showed that approximately 97 nt of transcript are required by trp t’ to cause termination. They also found that termination efficiency depends on the sequence context at the 3’ end of the transcripts; the transcript terminus probably affects the interactions of components of the transcription complex with the transcription bubble and thereby affects pausing. Indeed, the efficiency of RNA release at the individual termination positions is controlled by a kinetic competition between RNA polymerase elongating the transcripts and Rho terminating them (Zhu & von Hippel, 1998b). These authors found no requirement for specific sequences, like the previously identified rut sites, under the conditions used in their experiments, which is consistent with the view that Rho loading occurs at sites that are simply devoid of secondary structures and that contain a number of rC-residues compatible with the activation of the RNA-dependent ATPase of Rho.

tyrT operon of E. coli. Transcription termination of the tyrT operon of E. coli, encoding tRNA<sup>Tyr</sup>, occurs at a Rho-dependent terminator, both in vivo (Rossi et al., 1981) and in vitro (Küpper et al., 1978), and is completely dependent on Rho-factor. The site of termination is A·T rich and the adjacent sequences on both sides are G·C rich (Küpper et al., 1978). An A·T rich region preceded by a small cluster of G·C pairs is common to some other Rho-dependent terminators, thought to cause a pause in transcription downstream. Small hairpin structures have been found at some termination sites, just before the end of the transcript, but they are absent at the tRNA<sup>Tyr</sup> terminator (Küpper et al., 1978). On the other hand, the potential ‘auxiliary sequence’ CAAUCAA, observed at some Rho sites (see later), is also found at the tyrT operon terminator (Küpper et al., 1978). Madden & Landy (1989) showed that progressive removal of the region preceding the tRNA<sup>Tyr</sup> terminator progressively reduces Rho-dependent

| Table 2. Classification and location of Rho-dependent terminators |
|-----------------|-----------------|-----------------|
| **Location of terminators (name)** | **Organism** | **Position of terminators** |
| **Constitutive terminators** | | |
| trp operon (trp t’) | E. coli | End of operon |
| tyrT operon | E. coli | End of operon |
| Gene IV | E. coli phage f1 | End of gene |
| **Conventionally regulated terminators** | | |
| Rightward operon (λtR1) | E. coli phage λ | Between cro and cII genes |
| tna operon | E. coli | Between leader region and structural genes |
| rho gene | E. coli | Leader and 5’ end regions |
| rdu gene | B. subtilis | Leader and 5’ end regions |
| **Intragenic regulated terminators** | | |
| his operon | S. typhimurium | Within hisG and hisC coding regions |
| trp operon | E. coli | Within trpE coding region |
| trp operon | B. subtilis | Within trpE coding region |
| ilv operon | E. coli | Within ilvGM region |
| lac operon | E. coli | Within lacZ coding region |
| gal operon | E. coli | Within galE coding region |
| malK-lamB operon | E. coli | Within lamB coding region |
| nifLA operon | K. pneumoniae | Within nifA coding region |
| Left operon (timm) | E. coli phage P4 | Within kil coding region |

http://mic.sgmjournals.org 2517
termination activity, consistent with the idea that upstream sequences mediate the efficiency of Rho-dependent termination.

Gene IV of bacteriophage \(\lambda\). A peculiar type of terminator lies at the end of gene IV of the \(E.\ coli\) bacteriophage \(\lambda\). Readthrough transcription at this terminator is observed in nonsense suppressor or \(\rho\) hosts (Moses & Model, 1984; La Farina \textit{et al.}, 1990), indicating that translating ribosomes are responsible for suppression of termination, through occupation of a region of RNA which is not normally translated. The observations further indicate that Rho factor is required for the activity of the terminator. The terminator is peculiar in the sense that it seems to be Rho-dependent, according to the above observations, but not according to other features of the terminator site. Indeed, the region of RNA between the nonsense codon for translation termination of gene IV and the 3' end of the Rho-terminated transcripts is 63 nt long and not sufficient to guarantee Rho binding, according to the minimum size of ribosome-free transcript required for Rho termination; this suggests that the common concept of a Rho-binding site needs to be refined. An additional, unusual feature of this terminator is that the termination site occurs within a predicted, fairly stable stem–loop structure (La Farina \textit{et al.}, 1990), in contrast with the generally accepted idea of the requirement of a mostly unstructured RNA by the Rho activities. Thus, the context in which Rho-dependent termination occurs at this site seems to be quite different from that of other characterized Rho sites. On the other hand, an analysis of the distal portion of the gene IV coding sequence revealed similarity with sequences observed in other Rho-terminated transcripts (La Farina \textit{et al.}, 1990).

The ‘auxiliary elements’ or potential ‘auxiliary elements’ found at the constitutive Rho sites described above are not common to the terminators of this class (Table 1); thus, these elements don’t seem to be specific to the role of constitutive terminators as natural terminators or to the location of these terminators at the end of transcriptional units. The next section describes Rho-dependent terminators whose activity is regulated.

Regulated Rho-dependent terminators

Most of the Rho-dependent terminators identified and studied are regulated by various and sophisticated mechanisms. Some show a conventional regulation, requiring \textit{trans}-acting factors, and others are regulated by \textit{cis}-acting elements, located within the coding regions, the latter being terminators responsible for natural polarity.

Conventionally regulated Rho-dependent terminators

Rightward operon of bacteriophage \(\lambda\). Rho plays a central role in the life cycle of the \(E.\ coli\) bacteriophage \(\lambda\), as a key function in the regulation of gene expression. The \(tR1\) terminator of \(\lambda\) is one of the best-studied Rho-dependent terminators. \(tR1\) lies within the major rightward operon of \(\lambda\), in the intercistronic region between the \(cro\) and \(cII\) genes, and it controls \(N\)-mediated expression of the downstream genes in the operon. Two regions were identified in \(\lambda \ tR1\), \(rut\) and \(tsp\), in the mRNA, binds Rho. It was dissected into two elements, \(rutA\) and \(rutB\), separated by the \(N\)-utilization element \(boxB\) (Chen & Richardson, 1987). The roles of these ‘auxiliary elements’ were tested \textit{in vivo}, assaying the effects of deletions of \(rut\) sequences both on the ability of the mutant transcripts to bind Rho and to activate Rho ATPase and on the overall activity of the terminator (Graham & Richardson, 1998). These studies suggested that the \(rut\) site guarantees correct positioning of the RNA with respect to the Rho protein, for activation of ATPase, in addition to providing a strong attachment site for Rho. Removal of \(rutA\) has a more dramatic effect on the function of \(tR1\) than does removal of \(rutB\) (Graham & Richardson, 1998). Indeed, \(rutA\) RNA is more C-rich than \(rutB\) RNA, consistent with the proposed role of C residues in the interaction of Rho with the mRNA (McSwiggen \textit{et al.}, 1988) and in the function of Rho-dependent terminators (Hart & Roberts, 1991; Zalatan & Platt, 1992). Deletion of the \(boxB\) sequence of the \(\lambda \ cro\) gene, which forms a small stem–loop structure in the RNA, has no effect on the affinity of the \(cro\) RNA for Rho, nor on the efficiency of termination at \(tR1\) (Graham & Richardson, 1998); this contrasts with the observation that small hairpin structures are generally found in transcripts to which Rho binds (Schneider \textit{et al.}, 1993). Since no differences were observed between the findings \textit{in vivo} and those in a purified \textit{in vitro} system with Rho factor only (Graham & Richardson, 1998), possible interaction of other factors does not, apparently, interfere with Rho action in the \(rut\) region. The \textit{in vitro} results seem to fully account for the \(rut\) functions \textit{in vivo} (Graham & Richardson, 1998), and suggest that no other \textit{cis}-acting sequences outside \(rut\) are necessary for \(tR1\) function.

The \(rutA\) element carries a sequence with only one mismatch with the consensus sequence of \(boxA\), a site of interaction of the host-encoded NusB, NusG and S10 proteins, for antitermination in bacteriophage \(\lambda\) (Mogridge \textit{et al.}, 1998). Similar ‘auxiliary elements’ have been identified in the \(his\) operon of \(Salmonella\) (Ciampi \textit{et al.}, 1989; Carломagno & Nappo, 2001) and in the \(tna\) operon of \(E.\ coli\) (Gong & Yanofsky, 2002) (Table 1), where they seem to be required for Rho-dependent termination.

The other region identified in \(\lambda \ tR1\), \(tsp\), carries three heterogeneous RNA stop points, which correspond to polymerase pause sites (Lau \textit{et al.}, 1983; Richardson & Richardson, 1996). This region can be substituted with an alien sequence, carrying polymerase pause sites, without affecting termination at the transcription stop points (Richardson & Richardson, 1996), which shows independence of the \(rut\) and \(tsp\) action in the termination. Termination at one of the RNA stop points of \(\lambda \ tR1\) occurs at the sequence CAAUCAA preceded by a potential weak
stem–loop structure in the RNA transcript (Rosenberg et al., 1978). Similar putative ‘auxiliary elements’ have also been found at the $\lambda$ TR0 Rho-dependent terminator (CAACAA) (Calva & Burgess, 1980), near Rho-dependent transcription stop points within the $\lambda$ ilv operon (CAUCAAG) (Wek et al., 1987) and at the Rho-dependent terminators following the tRNA$^\text{Tyr}$ (CAUAUCAA) (Küpper et al., 1978) and the colicin E1 (CAACAAA) (Ebina & Nakazawa, 1983) genes (Table 1); other terminators do not have CAUAUCAA-like sequences. Lau et al. (1984) tested the possibility that these features are sufficient to signal Rho-dependent termination at $\lambda$ tR1, and found that they are insufficient, suggesting that there are additional sequence requirements for Rho action.

Specific residues required for Rho–RNA interaction at $\lambda$ tR1 have recently been identified by Graham (2004): single nucleotide substitutions at eight positions in a 25 nt region, previously shown to be critical for termination, reduce termination. These substitutions occur at the 5' end of rutA and introduce, in most cases, guanosines, confirming Rho’s idiosyncrasy for Gs; not surprisingly, many substitutions result in the loss of cytidine residues, confirming Rho’s ‘sympathy’ for Cs.

The $\lambda$ tR1 terminator region shows several ‘auxiliary elements’ or potential ‘auxiliary elements’ (Table 1), as mentioned above. This suggests that the terminator may require the contribution of more elements for its activities than those needed at other terminator sites. These requirements may be dictated by the specific location of the terminator within the rightward operon of $\lambda$, in relation to its role of regulator of gene expression.

Some conventionally regulated Rho-dependent terminators occur in regulatory regions located at the beginning of transcriptional units. Such terminators have been found in the tna operon of E. coli and in the rho genes of E. coli and Bacillus subtilis (Table 2), and they are described below.

**tna operon of E. coli.** A Rho-dependent terminator, playing a key role in regulation of expression of the degradative tryptophanase (tna) operon of E. coli (Stewart et al., 1986), lies between the sequences of the tnaC leader and of the first structural gene of the operon, tnaA. It prevents expression of the operon when no tryptophan is available and termination is inhibited when the inducer tryptophan is provided. Studies of tna constitutive mutants disclosed elements that participate in Rho-dependent termination, mainly a Rho-utilization site (Gollnick & Yanofsky, 1990) and a sequence with similarity to boxA (Könan & Yanofsky, 2000). These studies suggest that, in the absence of inducer, Rho binds to the leader portion of the tna transcript, moves 5' to 3' along the transcript until it reaches a paused polymerase, and then drives the elongation complex to terminate transcription. Oponer induction requires the presence of a single, crucial, trp codon in the leader-peptide coding region and requires translation of tnaC. In the presence of tryptophan, the TnaC-peptidyl-tRNA remains bound to the translating ribosome, which is not released at the tnaC stop codon, preventing Rho factor access to the boxA and rut sites, and consequently inhibiting termination (Gong & Yanofsky, 2003). This ribosome release inhibition appears to be the crucial event that regulates Rho-dependent termination in the tna operon leader region.

**rho gene of E. coli and B. subtilis.** The expression of the E. coli rho gene itself is regulated through Rho-dependent termination (Matsumoto et al., 1986). In fact, Rho-dependent terminators have been identified in the mRNA leader and N-terminal regions of the rho gene, and they have been proposed to be responsible for reducing expression of rho in response to high levels of the coded protein.

Similarly, the rho gene of B. subtilis seems to use Rho-dependent terminators for autoregulation of the gene (Ingham et al., 1999). The ability to regulate Rho levels may allow the cell to modulate gene expression by using Rho-dependent terminators strategically located between genes and their promoters. Interestingly, Rho-dependent termination also plays a role in trp operon expression of B. subtilis (see later), these two cases being, to the best of my knowledge, the only known examples of gene regulation by Rho-dependent termination in Gram-positive bacteria.

**Intragenic regulated Rho-dependent terminators**

It has been known for a long time that Rho-dependent terminators are responsible for premature blocks of transcription within operons, in response to premature translation termination or inefficient translation initiation caused by stress conditions. Such a response is the result of the fact that transcription and translation are coupled. These intragenic terminators are cryptic; in fact, they don’t normally function, but are unmasked only by pathological conditions interfering with proper translation of polycistronic mRNAs and uncoupling transcription from translation as a consequence. Rho-dependent termination within operons is responsible for transcriptional polarity effects on expression of the genes downstream from the transcriptional block; such a mechanism may have evolved to conserve energy, by preventing the synthesis of unused transcripts (reviewed by Richardson, 1991). Rho-dependent terminators causing polarity effects have been identified and studied in a number of systems (Table 2). Several of these terminators are presented below.

**his operon of S. typhimurium.** In the first gene of the histidine (his) operon of S. typhimurium, hisG, a Rho-dependent terminator, is responsible for the polar effects caused by point mutations and by insertions of transposable elements Tn10 and Tn5 (Ciampi et al., 1982; Ciampi & Roth, 1988; Wang & Roth, 1988). This terminator was identified by the isolation and characterization of mutants suppressing the strongly polar hisG frameshift mutation hisG2148 (Ciampi et al., 1982; Ciampi & Roth, 1988).
In vivo analysis of the transcripts produced by the polar mutant unveiled the existence of five major transcription end points in hisG, all affected by Rho to a different extent (Ciampi et al., 1989). Three of these sites fall immediately downstream of sequences with the potential for forming secondary structures (Ciampi et al., 1989) and one of them, the first in the gene and the most affected in a rho background, occurs within a sequence (Ciampi et al., 1989) resembling the boxA consensus element. This Salmonella his sequence, cloned on a plasmid, was shown to enhance termination by interacting with the NusB factor of E. coli cells (Carlomagno & Nappo, 2001). Salmonella hisG sequences required for premature, Rho-dependent termination of transcription (Ciampi & Roth, 1988; Ciampi et al., 1989) were also cloned on a plasmid and used to show that NusA modulates this intragenic termination, in nusA E. coli hosts (Carlomagno & Nappo, 2003). Three of the termination sites in hisG are also weakly active in the normally translating wild-type strain, similarly to what was observed in the Salmonella hisC cistron (Alifano et al., 1988) and in the E. coli lacZ gene (Stanssens et al., 1986). These sites may coincide with polymerase pause sites, as described in other systems (Yager & von Hippel, 1987).

The histidine operon of S. typhimurium proved particularly useful for the isolation and study of polarity suppressor mutants. Several mutations affecting the hisG terminator have been isolated, all of which are deletions, suggesting that no sequence-specific element is required for Rho-dependent termination. Indeed, four point mutations scattered along a stretch of 18 bp removed by the smallest of the deletions do not damage the terminator (Ciampi & Roth, 1988). Two of the deletions do not remove any of the five transcription end-points within hisG (G. Lisanti & M. S. Ciampi, unpublished results) and they may identify a binding site for Rho factor (Ciampi & Roth, 1988). Other deletions show that progressive removal of the sequence including the transcription stop points has an increasing suppressing effect on polarity, leading to progressive increase of downstream gene expression (G. Lisanti & M. S. Ciampi, unpublished results). Mutations suppressing polarity by reinitation of transcription (Bianco et al., 1998) or of protein synthesis (M. R. Pasca, A. La Teana & M. S. Ciampi, unpublished results) within hisG were also isolated, consistent with transcription terminating in hisG in polar mutants and with the known interference of translation on Rho-dependent termination.

A cryptic Rho-dependent terminator is also responsible for premature termination of transcription in the hisG cistron of S. typhimurium (Alifano et al., 1988), where polarity suppressor mutations affecting initiation of translation or transcription have been isolated and studied (M. R. Pasca & M. S. Ciampi, unpublished results). Several 3′ transcription end points were mapped within the hisD gene, immediately following hisG in the operon, and polar hisA mutants also produce shorter mRNAs (Alifano et al., 1991), but possible action of Rho at these sites remains to be defined. Polar effects are also caused by mutations in the three remaining internal genes of the his operon, hisB, hisH and hisF (Ciampi et al., 1982); hence, cryptic Rho sites may be embedded in the coding sequences of these cistrons as well. In vivo studies with polarity suppressor mutants, including rho mutants, and in vitro transcription experiments with purified Rho on the hisD, hisB, hisH, hisA and hisF cistrons, would provide a complete picture of the distribution and features of Rho-dependent termination sites in the his operon. The scenery of the his operon could be common to most, if not all, bacterial operons; indeed, most operons may have evolved such a mechanism to cope with translation pathology.

The trp operon of E. coli and B. subtilis. The first gene of the trp operon of E. coli, trpE, also shows intragenic Rho-dependent termination, which is the cause of polarity effects (Korn & Yanofsky, 1976). Investigation of trpE revealed several termination sites and some pause sites within the coding sequence of the gene; however, the study of Rho-dependent termination in the trp operon did not focus on the features of the terminator, but rather on the ability of the polynucleotide-binding sites of the Rho protein to interact with the mRNA. This was investigated in rho mutants showing increased polarity in the trp and lac operons, by Tsurushita et al. (1989), who found that the primary Rho-binding site directs the efficiency of termination, while the secondary site selects the termination points.

Rho-dependent termination plays a sophisticated role in the expression of the trpE–A operon of B. subtilis, the mechanism involving transcription attenuation and translational control. Translational control relies on the formation of an RNA hairpin that sequesters the trpE Shine–Dalgarno (SD) sequence, blocking translation. Formation of the SD-blocking hairpin causes transcriptional polarity, by allowing Rho’s access to the nascent transcript, downstream from this RNA structure (Yakhnin et al., 2001).

The ilv operon of E. coli. Rho-dependent termination is responsible for translational polarity in the ilv operon of E. coli K-12. Three Rho-dependent termination sites were identified and partially characterized by in vitro transcription experiments with a restriction fragment containing the promoter-distal portion of ilvG and the proximal portion of ilvM, fused to the tac promoter (Wek et al., 1987). Several sequences with no dyad symmetry, required for Rho interaction with the mRNA, were identified: two of these sequences fall immediately upstream of two of the termination sites (Wek et al., 1987). Regions with regularly spaced cytidine residues, observed in other Rho-dependent terminators (Platt, 1986), lie upstream of the transcription stop points in the ilvGM′ region, and the sequence CAAUCA, found adjacent to some Rho-dependent termination sites (Lau et al., 1984) (Table 1), is also present, with one mismatch (CAAUCGA), near the three transcription stop points in the ilvGM′ region (Wek et al., 1987); however, a possible role for these potential
lac and gal operons of E. coli. Inefficient translation initiation causes premature transcription termination in the first gene of the lac operon of E. coli, lacZ (Stanisens et al., 1986). Four cryptic Rho-dependent termination sites, which are turned on by upstream blocks of protein synthesis, as well as by amino acid starvation, were identified in lacZ, by in vitro and in vivo studies; they correspond to polymerase pause sites (Ruteshouser & Richardson, 1989). A detailed analysis of the sequence showed a complex interplay of polymerase-pause and terminator elements, widely discussed by these authors. Two of these terminators in the early region of lacZ (tiZ1 and tiZ2) proved particularly useful in testing the requirement for the NusG and NusA factors in Rho-dependent termination: NusG has a stimulating effect on Rho-dependent termination in lacZ, while NusA inhibits termination (Burns et al., 1998) (see section on trans-acting factors).

Cryptic Rho-dependent terminators responsible for polarity effects also lie in the gal operon of E. coli (De Crombrugghe et al., 1973). The termination sites have not been identified and characterized at the molecular level, but this system has mostly been used to study the requirement for the E. coli NusG protein in Rho-dependent termination. Rho-induced polarity in the gal operon is suppressed in NusG-deficient cells and Rho-dependent termination in galE, the first gene of the operon, is entirely prevented upon NusG depletion (Sullivan & Gottesman, 1992) (see also section on trans-acting factors).

malK-lamB operon of E. coli. The presence of a Rho-dependent terminator has been inferred within the lamB gene of the malK-lamB operon of E. coli K-12 (Colonna & Hofnung, 1981). Expression of lamB, the structural gene for the λ receptor (Thirion & Hofnung, 1972), results in phage sensitivity. Expression of the operon requires an activator, the malT gene product. A malT mutant is resistant to λ, since lamB is not expressed; however, in the absence of the malT gene product, expression of lamB and sensitivity to λ infection are restored in a rho mutant, due to the activity of a cryptic promoter, located at the distal end of malK and unmasked in the mutant rho (Colonna & Hofnung, 1981). Transcription originating at this promoter is terminated by Rho at the inferred terminator within lamB, in a malT rho+ strain, and this prevents expression of lamB. The terminator’s structure and mechanism of function remain to be explored.

nifLA operon of Klebsiella pneumoniae. Intragenic Rho-dependent termination has been studied in the nifLA operon of K. pneumoniae, encoding the sensor–activator pair involved in the regulation of other nif genes. In this operon, altered translation of nifL activates Rho-dependent termination not in that gene, but in the downstream nifA (Govantes & Santero, 1996), which has been explained by the fact that L and A are translationally coupled (Govantes et al., 1998). These studies provide new insight into the putative role of regions of biased C and G composition in Rho-dependent termination. An analysis of transcripts terminated within the his operon, as well as in the lacZ cistron and at the Rho-dependent trp t' and λ rR1 terminators, led Alfano et al. (1991) to hypothesize that a region of high cytosine-over-guanosine content in the mRNA is an essential feature of Rho-dependent terminators. Such C>G regions were also found at the nifLA operon’s terminators; however, other C>G regions in the operon do not show termination activity (Govantes & Santero, 1996). Hence, a direct correlation between the occurrence of C>G regions and Rho-dependent termination could not be established by these authors, who suggest that the distribution of cytosines, yielding specific C/G profiles, may only be important in diagnosing the possible presence of a terminator.

Rho-dependent termination within coding sequences is not in all cases triggered by pathological conditions interfering with proper mRNA translation; in fact, intragenic termination can also be part of a physiological regulation of gene expression. One such case has been reported in the left operon of the E. coli bacteriophage P4.

Left operon of P4. A Rho-dependent terminator is involved in the establishment of P4 lysogeny, through a positive feedback mechanism on termination. The terminator, named timm, occurs within the coding sequence of the first translated gene, kil, of the left operon for replication of P4 and it seems to participate, with two upstream Rho-independent terminators, to attenuate expression of the left operon functions, early after infection (Briani et al., 2000). Translation of kil inhibits termination at timm (Forti et al., 1999), which may contribute to the production of a leader transcript, which is matured into a small, untranslated RNA, CI, a phage factor required for efficient termination. Once produced, CI acts as an antisense RNA, on the RNA leader of the left operon, where RNA–RNA interactions are essential for stringent termination at the downstream timm (Briani et al., 2000), which in turn prevents expression of the replication genes and guarantees immunity against superinfecting phages.

Summary of Rho-dependent terminators

Some of the regulated Rho-dependent terminators described above lie in regulatory regions separating genes from their promoters, which may provide the cell with a convenient mechanism for the control of gene expression. Most regulated Rho-dependent terminators occur within coding sequences, contributing to preventing wasteful expression of genes, but this location is also used to direct gene expression. Rho-dependent termination can also serve to end transcriptional units; however, this role seems to be minor among the cases identified so far.
Looking back over all Rho-dependent termination sites, their features don’t seem to depend on the common location or role of the terminators. ‘Auxiliary elements’ and potential ‘auxiliary elements’, observed at some of the Rho sites, do not appear to be specific to a specific class of terminators (Tables 1 and 2). These ‘auxiliary elements’ could be part of a pool of elements, one of which or combinations of which may contribute to Rho loading efficiency and to the overall efficiency of termination. The requirement for and effect on termination could be dictated by the ‘local’ context of the individual terminators, and this would explain why not all terminators exhibit the same ‘auxiliary elements’. How the individual sequence elements contribute to the structure of Rho-dependent terminators and to the termination mechanism is not fully understood yet. Analysis of the more sophisticated mechanisms relying on Rho-dependent termination may be crucial in identifying new essential elements of the Rho-dependent termination machinery. The isolation of mutants relieving transcriptional polarity, site-directed mutagenesis of the various sequence elements, and biochemistry, will contribute to uncovering the secrets of Rho-dependent termination.

**Cis-acting features of Rho-dependent terminators**

Various cis-acting elements have been observed at different Rho-dependent termination sites and they are discussed, for the individual Rho sites, in the section on Rho-dependent terminators. However, the only feature common to Rho-dependent terminators seems to be richness of rC residues in the mRNA. In fact, the strongest activators of NTP hydrolysis by Rho are poly(rC) and rC-rich oligonucleotides. The requirements for rC residues by the Rho activity have been extensively studied (reviewed by Richardson & von Hippel, 1993); besides, a 30 nt run of pure rUrC, introduced between a promoter and a non-terminator site, could be directly correlated with the efficiency of termination and with studies of Rho-dependent termination (Zalatan & Platt, 1992). Recently, however, Graham et al. (2004) showed that single nucleotide substitutions in the rut site of the trp t’ terminator did not identify a number or distribution of rC residues that could be directly correlated with the efficiency of termination (Zalatan & Platt, 1992). However, recent work (Alifano et al., 1991) suggests that the presence of a terminator (Govantes & Santero, 1996). Short rUrC oligomers stimulate Rho’s ATPase more than do rC homo-oligomers (Wang & von Hippel, 1993); besides, a 30 nt run of pure rUrC, introduced between a promoter and a non-terminator site, is sufficient to cause transcription termination (Guérin et al., 1998). This termination is dependent on the Salmonella Rho factor (identified by Housley & Whitfield, 1982), and is observed only in the absence of protein synthesis, consistent with the known incompatibility of Rho action with translation and with studies of Rho-dependent termination in the hisG gene of Salmonella (Ciampi et al., 1982; Ciampi & Roth, 1988). According to the results of Guérin et al. (1998), Rho-dependent termination seems to depend essentially on the Rho loading site.

**Trans-acting factors in Rho-dependent termination**

Rho factor. The hexameric protein Rho factor constitutes 0.1% of the total soluble protein of *E. coli* and *S. typhimurium* (Housley & Whitfield, 1982); it is an essential protein for the growth of *E. coli* and of its lambdoid phages (Das et al., 1976), for *Rhodobacter sphaeroides* (Gomelsky & Kaplan, 1996) and for *Micrococcus luteus* (Nowatzke et al., 1997); however, Rho is dispensable in *B. subtilis* (Quirk et al., 1993) and in *Staphylococcus aureus* (Washburn et al., 2001). Analysis of bacterial sequences suggests that *rho*-like homologues are ubiquitous in the bacteria and highly conserved (Opperman & Richardson, 1994; Washburn et al., 2001).

Rho acts as a 5’ to 3’ ATP dependent RNA–DNA helicase, triggered by the interaction with the mRNA at the Rho loading site (reviewed by Richardson, 2003, and by Delagoutte & von Hippel, 2003). ATP hydrolysis provides the free energy for Rho translocation 5’ to 3’ along the message, until the protein encounters the transcription elongation complex stalled at a pause site. At these sites transcription is terminated and the RNA is dissociated from the transcription complex, by Rho’s 5’ to 3’ helicase, which disrupts the transcript–template duplex, again powered by ATP hydrolysis.

Much of Rho’s structure and function were known (reviewed by Richardson, 2002), prior to the crystallographic studies of Skordalakes & Berger (2003). Rho functions as a homohexameric ring (Finger & Richardson, 1982; Geiselmann et al., 1992). Each Rho monomer bears two RNA-binding domains, the so-called primary and secondary sites (Richardson, 1982), located on the smaller N-terminal and larger C-terminal domains, respectively. Skordalakes and Berger’s work shows that the two RNA-binding sites, the primary one, responsible for mRNA recognition, and the secondary one, required for translocation and unwinding, point toward the centre of the ring, and that the complexed hexameric ring is split open (likened to a ‘lock washer’), a configuration likely required to allow Rho loading onto mRNA and translocation (reviewed by Richardson, 2003). Jeong et al. (2004), analysing the conformational changes of the Rho protein following nucleotide and nucleic acid binding, concluded that RNA binding changes the conformation of the Rho hexamer, resulting in opening of the subunit interfaces. ATP binding occurs and a new conformational change takes place, following RNA contact with the secondary sites lining the central channel of the Rho ring, which makes Rho capable of hydrolysing ATP and translocating.

NusG factor. Rho factor is sufficient to terminate transcription in *vitro* at most Rho-dependent terminators, whereas, in *vivo*, an additional factor, NusG, is required.
by several Rho-dependent terminators. NusG is an essential, abundant protein in *E. coli* (Li et al., 1992), whereas in *B. subtilis* NusG is not essential either for viability or for the autoregulation of Rho (Ingham et al., 1999). NusG was originally isolated as a factor aiding the N protein in antitermination of transcription in bacteriophage λ (Li et al., 1992); it is a transcription elongation factor, which stimulates the rate of transcription (Burova et al., 1995) and enhances suppression of class II polymerase pause sites (Artsimovitch & Landick, 2000). Orthologue *nusG* genes exist in all the bacteria whose genomes have been sequenced, including *Mycoplasma genitalium*, which lacks other important transcription factors (Fraser et al., 1995).

The requirement for NusG in Rho-dependent termination was first studied by Sullivan & Gottesman (1992), who showed that cells depleted of NusG have reduced efficiency of transcription termination at certain Rho-dependent terminators. However, some sites are fully dependent on NusG: Rho-dependent termination in *galE*, the first gene of the *gal* operon, is entirely prevented upon NusG depletion (Sullivan & Gottesman, 1992). *In vitro*, NusG is strongly required at the *tiZ1* terminator and its requirement is context dependent at *tiZ2* (Burns et al., 1999). NusG does not greatly affect *in vitro* termination at the *trp*′ (Nehrke et al., 1993) nor at the λ *tR1* (Burns et al., 1999) terminators; however, *in vivo*, the λ *tR1* terminator is strongly dependent on NusG (Sullivan & Gottesman, 1992). The discrepancy between *in vitro* and *in vivo* requirements at λ *tR1* may be due to the more-limiting context for Rho action *in vivo*, where the presence of ribosomes translating the message creates more pressure on Rho’s interaction with the mRNA. In fact, at *tiZ2* and λ *tR1* terminators NusG is required to overcome kinetic limitations that may be imposed on Rho function, depending on the properties of the specific terminator and on the position of the terminator in the transcriptional unit (Burns et al., 1999); this requirement may involve NusG-mediated modulation of the sensitivity of the transcription complex to Rho action. Alternatively, the NusG effect at λ *tR1* *in vivo* could be the consequence of its involvement in other events important for Rho activity, such as ribosome release. Indeed, *nusG* orthologues have been found in two organisms that lack Rho, *Mycoplasma genitalium* (Fraser et al., 1995) and *Methanococcus jannaschii* (Bult et al., 1996), which suggests a NusG role other than in Rho-dependent termination.

NusG has been suggested to connect Rho to the RNA polymerase–RNA complex, thereby facilitating recognition of the termination signals (summarized by Burns et al., 1999; reviewed by Richardson, 2002). Besides, the interaction of NusG with the RNA polymerase may cause this enzyme to become more susceptible to Rho actions that mediate release of the transcripts, as proposed by Burns et al. (1999), who have shown that NusG enhances the rate of release of the RNA from the transcription elongation complex. The NusG effect on release may also be the cause of the requirement for NusG at some terminators.

NusA factor. NusA has been reported to stimulate Rho-dependent termination (Kainz & Gourse, 1998); however, in most cases it inhibits Rho (Kainz & Gourse, 1998), consistent with the involvement of NusA with *mut* utilization in λ antitermination (Li et al., 1992) and with rRNA antitermination (Vogel & Jensen, 1997). NusA is an essential protein in *E. coli* (Kainz & Gourse, 1998), but it becomes dispensable in *rho* mutants with reduced termination efficiency (Zheng & Friedman, 1994), indicating a critical role in Rho-dependent termination. In *B. subtilis* NusA is essential; however, its requirement is independent of Rho (Ingham et al., 1999), which suggests the involvement of the protein in some process other than Rho-dependent termination. As for NusG, NusA orthologues exist in all the bacteria whose chromosomes have been sequenced.

NusA binds both to Rho factor and to *E. coli* RNA polymerase, which provides a way of coupling Rho to the elongation complex (Schmidt & Chamberlin, 1984). The Rho–NusA interaction may modulate Rho action at certain terminators. NusA is also a transcription elongation factor, which, unlike NusG, slows transcript elongation, by enhancing RNA polymerase pausing at some pause sites (Lau et al., 1983; Artsimovitch & Landick, 2000). Zheng & Friedman (1994) have proposed that the ability of NusA to enhance pausing, which results in tight coupling of transcription and translation, would interfere with the Rho–mRNA interactions, blocking termination of transcription as a consequence. NusA influences both pausing and Rho-dependent termination at λ *tR1*, by interaction with the C-terminal domain of the *α* subunit of *E. coli* RNA polymerase (Kainz & Gourse, 1998); however, a direct correlation has not been established, in this system, between the efficiency of Rho-dependent termination and the extent of pausing, two processes that appear to relate to each other in a complex manner.

Burns et al. (1998) tested the combinatorial effects of NusA and NusG on transcription elongation and on Rho-dependent termination at the *tiZ1* and *tiZ2* intragenic terminators, and it seems that the two factors act independently and at different sites: NusG enhances elongation and Rho-dependent termination, while NusA decreases both, and these opposing effects are balanced when both factors act. It would be interesting to know whether NusA and NusG affect the structural features of the transcription complex at a pause site.

Models for Rho-dependent termination

Models have been proposed to explain how Rho reaches the elongation complex stalled at a site downstream from the initial Rho loading site on the mRNA substrate, and how the nascent transcript is released. The possibility that Rho acts at a distance, simply by looping of the RNA, has been ruled out (Steinmetz et al., 1990), demanding that Rho translocates from the loading site to the termination site.
A physical model for the mechanism of translocation of Rho 5’ to 3’ along the mRNA was proposed by Geiselmann et al. (1993). This model, defined as the ‘pure’ tracking model, is a modification of the simple tracking model (Richardson, 1982), and it predicts that Rho detaches from the initial rut site of interaction to track 5’ to 3’ along the RNA chain to downstream sites, where the nascent RNA is released by the 5’ to 3’ RNA–DNA helicase activity of Rho (Geiselmann et al., 1993).

Later studies by Horiguchi et al. (1997) on the quaternary geometry of Rho factor highlighted structural resemblances between Rho and other helicases, such as RuvB and T7gp4. Thus, the finding that RuvB and T7gp4 interact with the RNA chain within the hole of the hexameric ring led Miwa et al. (1995) to speculate that the lining of the hole is a site of interaction with the RNA also for Rho, by analogy with the RuvB and T7gp4 helicases. This hypothesis was supported by the finding that rho mutations affecting the interaction of Rho with the RNA fall within the portion of the protein participating in the tertiary structure of the ATP-binding domain of Rho, and extending into the hole (Miwa et al., 1995). This finding, together with the possibility that Rho binding to the mRNA occurs by wrapping of the RNA chain around the N-terminal RNA-binding domains, located on one side of the Rho hexameric ring, led Horiguchi et al. (1997) to propose that Rho hooks the RNA through its N-terminal domain, and that interaction of the RNA with the central hole allows traction of the RNA.

A similar model had been proposed by Richardson & Richardson (1996), who suggested that, after the initial interaction of the 5’ end of the Rho utilization site of a nascent transcript with the RNA-binding domain surface of Rho, a dissociation and reassociation of subunits would drive the 5’ tail of the rut region of the transcript into the hole. This would be made possible by a break in the ring, observed in electron micrographs of unbound Rho (Gogol et al., 1991). After capturing of the RNA in the hole, Rho would move 5’ to 3’ along the RNA, with the aid of ATP hydrolysis, which provides the energy for dissociation of the transcript from the RNA polymerase.

The models proposed by Richardson & Richardson (1996) and by Horiguchi et al. (1997) assign a role to a part of Rho, the central hole, not previously implicated in translocation of the protein along the RNA, and they remind one of another major mode of translocation suggested by studies of Faus & Richardson (1990) and of Steinmetz & Platt (1994), and known as the ‘tethered tracking’ mechanism. In this model Rho is thought to remain bound to the rut site while translocating 5’ to 3’ along the mRNA, until it reaches the actual termination sites. The translocation would be mediated by interaction of the RNA with a secondary site – proposed by Richardson (1982) – separate from the primary rut-binding site.

The points of interaction of Rho with RNA were investigated by Burgess & Richardson (2001) and by Wei & Richardson (2001). Burgess & Richardson (2001) made Rho proteins with single cysteine residues, which where photo-cross-linked to various bound derivatives of E. coli RNA. Wei & Richardson (2001) explored the sites of Rho–RNA binding, through cleavage protection studies in the presence of bound ATP, RNA or DNA. These studies verified the existence of a secondary RNA-binding site on the Q-loop of Rho’s ATP-binding domain, inside the central hole of the Rho hexamer. The results of Burgess & Richardson (2001) and of Wei & Richardson (2001) are consistent with binding of the rut region of the RNA around the crown of the Rho hexamer and with interaction of the 3’ side of rut with the central hole, for activation of ATP hydrolysis and hence translocation. More recently Skordalakes & Berger (2003) determined the crystal structure of Rho bound to an ssDNA recognition site mimic or to an ssRNA and an ATP analogue. These structures show that Rho forms hexameric rings, with the primary RNA-binding sites facing the inside of the ring and leading the RNA 3’ side toward the hole of the hexamer. The structures also show that the Rho ring is stably open in the complexed state. These structural features clarify how Rho captures the 3’ side of the mRNA in the interior of the ring, before translocation starts.

Conclusions

Rho-dependent transcription termination may be widespread, but most Rho-dependent terminators characterized occur in enteric bacteria. Known examples of genes regulated by Rho in Gram-positive bacteria are the rho and trp genes of B. subtilis. Rho-dependent terminators lie in regulatory regions preceding genes, within coding sequences and at the end of transcriptional units. Some Rho-dependent terminators are constitutive, but most identified so far are regulated. This suggests that Rho-dependent termination, being a complex process involving transcription and translation elongation, is subject to control in many ways, allowing mechanisms to evolve to regulate termination by Rho, in response to a variety of conditions.

Rho is an RNA-binding protein with ATPase and helicase activities. It is an essential protein in E. coli and inessential in B. subtilis, but the two factors function similarly; therefore, the wider range of rho mutations one could isolate in B. subtilis might illuminate Rho’s function in E. coli and likely in S. typhimurium as well. Two additional factors, NusG and NusA, participate in Rho-dependent termination, by coupling Rho to the elongation complex. NusG enhances the rate of release of the RNA from the transcription elongation complex, and it may mediate Rho action on RNA polymerase, leading to termination of transcription. NusA enhances pausing of RNA polymerase, and it may participate in termination by lowering the rate of transcription elongation. NusA is essential in B. subtilis for reasons that seem to only partially apply to E. coli. The protein may be a key to understanding the differences in transcription elongation and Rho-dependent termination between these two bacteria, and ultimately to understanding the Rho-dependent termination mechanism. Indications of possible
effects of NusG and NusA on the structure of the transcription complex would illuminate how these factors mediate Rho action.

The RNA transcript plays a key role in Rho function, by mediating interaction between Rho and the transcription elongation complex. Rho binding to rut on the mRNA activates Rho’s RNA-dependent ATPase, which drives Rho 5’ to 3’ along the message, and the ATP-dependent RNA–DNA helicase, which ‘peels’ the transcript from its template. Rho-dependent terminators show a high content of C residues, required for activation of the Rho ATPase, and few Gs, minimizing RNA secondary structure and permitting Rho loading onto the transcript. These general features may be the only elements required for Rho–RNA binding on naked RNAs of appropriate length, even though specific nucleotides have been identified at some Rho-dependent terminators. Other terminator-specific cis-acting sequences, termed ‘auxiliary elements’ in this review, may aid in Rho loading, contributing to the efficiency of termination.

Models have been proposed to explain the mode of Rho–RNA interaction leading to termination of transcription. Rho-dependent termination is accomplished by the initial binding of Rho to rut, through the primary RNA-binding sites of the N-terminal domains of the Rho hexameric ring; subsequent sliding of the RNA into the central hole of the hexamer would then occur through a gap in the ring. Traction would translocate Rho 5’ to 3’ toward the RNA termination and release sites, while maintaining association with the initial binding site, as predicted by the ‘tethered tracking’ model, or following release of the initial binding site. Future structural studies will be needed to distinguish between these two models.

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