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Review

Transcription termination factor Rho: a hub linking diverse physiological processes in bacteria

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Factor-dependent termination of transcription in bacteria relies on the activity of a specific RNA helicase, the termination factor Rho. Rho is nearly ubiquitous in bacteria, but the extent to which its physiological functions are conserved throughout the different phyla remains unknown. Most of our current knowledge concerning the mechanism of Rho's activity and its physiological roles comes from the model micro-organism *Escherichia coli*, where Rho is essential and involved in the control of several important biological processes. However, the rather comprehensive knowledge about the general mechanisms of action and activities of Rho based on the *E. coli* paradigm cannot be directly extrapolated to other bacteria. Recent studies performed in different species favour the view that Rho-dependent termination plays a significant role even in bacteria where Rho is not essential. Here, we summarize the current state of the ever-increasing knowledge about the various aspects of the physiological functions of Rho, such as limitation of deleterious foreign DNA expression, control of gene expression, suppression of pervasive transcription, prevention of R-loops and maintenance of chromosome integrity, focusing on similarities and differences of the activities of Rho in various bacterial species.

Introduction

Transcription termination is a critical step in gene regulation in all living organisms. In bacteria, transcription termination is well known to be essential for the generation of different types of functional RNAs, the definition of the boundaries of the transcriptional units, the release of RNA polymerase (RNAP) and the regulation of gene expression via the mechanism known as transcription attenuation (Peters *et al.*, 2011; Santangelo & Artsimovitch, 2011).

However, recent studies have revealed new roles of transcriptional termination, e.g. those linked to the maintenance of genome integrity or degradation of untranslated mRNAs. Particular attention is now paid to transcription termination due to its crucial role in the control of pervasive transcription. This type of genome-wide transcription, not associated with annotated genome features such as protein-coding genes, is a universal phenomenon for all the three domains of life and viruses (Georg & Hess, 2011; Wade & Grainger, 2014). In eukaryotes, pervasive transcription arises mainly from bidirectional promoters that synthesize both mRNA and diverse non-coding RNAs, but this phenomenon is also controlled by selective transcriptional termination (Kapranov *et al.*, 2007; Schulz *et al.*, 2013). Recently, an essential role of transcription termination in the control of pervasive transcription was demonstrated for both Gram-positive and Gram-negative model micro-organisms *Bacillus subtilis* and *Escherichia coli* (Nicolas *et al.*, 2012; Peters *et al.*, 2012).

In bacteria, transcription termination is achieved by two mechanisms: factor-independent (intrinsic) and factor-dependent termination. Intrinsic termination is strongly associated with sequence-specific signals characterized by a GC-rich symmetrical element followed by a 'T stretch' sequence encoding a RNA terminator hairpin with a 'U-tract' essential for pausing and disruption of the transcription elongation complex (TEC) (Gusarov & Nudler, 1999; Epshtein *et al.*, 2007). Recognition of this structure by TEC with the consequent release of RNAP does not require any additional factors (reviewed by Peters *et al.*, 2011; Santangelo & Artsimovitch, 2011). Intrinsic terminators and terminator-like sequences were identified in >2000 sequenced bacterial chromosomes (Mitra *et al.*, 2011). However, numerous bacteria are devoid of canonical intrinsic terminators downstream from putative transcriptional units, suggesting the existence of other termination mechanisms (Washio *et al.*, 1998; de Hoon *et al.*, 2005; Mitra *et al.*, 2009; Peters *et al.*, 2011).

Several factors were reported to act on RNAP during the elongation stage, causing TEC dissociation and release of

Abbreviations: asRNA, antisense RNA; BCM, bicyclomycine; ChIP, chromatin immunoprecipitation; DSB, double-strand break; RNAP, RNA polymerase; sRNA, small regulatory RNA; TEC, transcription elongation complex.

a transcript (reviewed by Merrikh *et al.*, 2012; Washburn & Gottesman, 2015). Some of them assure transcription termination under certain conditions. For example, *E. coli* transcription-repair coupling factor (Mfd) was shown to remove RNAP stalled by DNA template lesions and DNA-bound proteins (reviewed by Borukhov *et al.*, 2005). However, the principal factor-dependent termination pathway relies on the activity of a specific protein, transcription factor Rho (Roberts, 1969; Richardson, 2002). In contrast to intrinsic terminators, sequences required for the function of Rho (called Rho utilization or *rut* sites) are complex and do not show any conserved features (reviewed by Ciampi, 2006; Peters *et al.*, 2011). Most of our current knowledge about the mechanisms of Rho activity and its physiological roles comes from *E. coli* where Rho is an essential and abundant protein (Bubunenko *et al.*, 2007; Li *et al.*, 2014). In this model Gram-negative bacterium, Rho mediates $>20\,\%$ of all transcription termination events and assures the formation of the 3′ end of different types of RNA (Peters *et al.*, 2009).

E. coli Rho is known to be involved in the control of a variety of important biological processes, including (i) enforcement of transcription–translation coupling and termination of transcription of untranslated mRNAs (well known also as a phenomenon of Rho-dependent transcriptional polarity) (reviewed by Ciampi, 2006; Peters *et al.*, 2011); (ii) suppression of pervasive antisense transcription (Peters *et al.*, 2012); (iii) assistance in preventing deleterious R-loops (Harinarayanan & Gowrishankar, 2003; Leela *et al.*, 2013) and maintenance of genome integrity by prevention of conflicts between transcription and replication machineries (Dutta *et al.*, 2011; Washburn & Gottesman, 2011); (iv) silencing of horizontally transferred DNA (Cardinale *et al.*, 2008; Menouni *et al.*, 2013); and (v) regulation of gene expression mediated by small regulatory RNAs (sRNA) and riboswitches (Bossi *et al.*, 2012; Hollands *et al.*, 2012; Proshkin *et al.*, 2014). Thus, Rho-dependent transcription termination plays an important role in linking transcription to other vital cellular processes.

Several of these processes have been shown to rely on Rho cooperating with various endogenous factors. Amongst them, the most important role was attributed to the transcription elongation factors NusG and NusA, which bind to Rho and RNAP, and modulate Rho-dependent termination at certain terminators (Burns *et al.*, 1998; Ciampi, 2006; Peters *et al.*, 2012; Boudvillain *et al.*, 2013). Both *nusG* and *nusA* are conserved, but not always essential in different bacteria. In *E. coli*, other putative protein partners of Rho were identified as components of the interaction network containing conserved and essential proteins (Butland *et al.*, 2005).

At present, the extent to which molecular mechanisms and physiological functions of Rho are conserved throughout the bacterial phyla remains elusive despite the fact that phylogenetic analysis has shown that *rho* is nearly ubiquitous in bacteria (D'Heygère *et al.*, 2013). The current state of knowledge concerning Rho from bacterial species other than *E. coli* is restricted to several representatives. In each case, only discrete aspects of Rho functionality were examined. However, although limited, these analyses indicate that other bacteria deviate from the *E. coli* paradigm as the activity and function of Rho may be influenced by species-specific features, such as its cellular abundance, conservation of Rho protein partners or the occurrence of sequence determinants associated with regions of Rho-dependent termination (de Hoon *et al.*, 2005).

In this review, we present general information about Rho and Rho-dependent termination in bacteria and summarize the ever-increasing knowledge about the various aspects of the physiological activity of Rho, such as limitation of deleterious foreign DNA expression, control of gene expression, suppression of pervasive transcription, prevention of R-loops and maintenance of chromosome integrity. Special attention is paid to the role of Rho in *Bacillus subtilis* where its inactivation has been shown to significantly modify the transcriptome (Nicolas *et al.*, 2012). Based on updated data, we discuss the similarities and differences of Rho activity in different bacterial species.

Features and phylogeny of Rho

Transcription termination factor Rho was initially characterized in 1969 by J. W. Roberts as a factor that boosts the 'accuracy' of *in vitro* transcription on the bacteriophage λ DNA template by terminating RNAP at specific sites (Roberts, 1969). The progress in understanding the structure and mechanisms of action of Rho, based on *E. coli* Rho as an experimental model, provides a rather comprehensive view of Rho that is convenient to export to other bacterial species.

Rho is a homo-hexameric protein with ATP-dependent RNA helicase-translocase activity that causes the TEC to dissociate (reviewed by Richardson 2002; Peters *et al.*, 2011; Boudvillain *et al.*, 2013). Complex multistep binding of Rho to the nascent transcript involves different structural regions within the Rho hexamer known as primary and secondary binding sites (Richardson, 1982; Skordalakes & Berger, 2003; Skordalakes *et al.*, 2005). The primary binding site is jointly formed by the N-terminal subdomains of Rho monomers. It is responsible for the initial, ATP-independent binding of Rho to *rut* sites – complex RNA sequences with a high cytidine/low guanosine content and relatively little secondary structure. Each monomeric subdomain comprises a characteristic OB-fold (oligonucleotide/oligosaccharide binding fold) able to bind two pyrimidine bases, preferentially cytosines (Bogden *et al.*, 1999; reviewed by Ciampi, 2006; Peters *et al.*, 2011). This explains Rho's utilization of the *rut* sites for RNA binding. Upon RNA binding, the Rho hexamer adopts an asymmetrical ring conformation, with RNA enclosed within its central channel containing the secondary RNA-binding site (reviewed by Peters *et al.*, 2011; Boudvillain *et al.*, 2013). Contacts between RNA and the secondary site lead to activation of ATP binding by the C-proximal ATPase domains of Rho. ATP hydrolysis stimulates 5′→3′ translocation of Rho along the RNA and, finally, results in

dissociation of TEC (reviewed by Richardson, 2002; Peters *et al.*, 2011) (Fig. 1). Important details of the mechanism of Rho translocation and RNAP dissociation on Rho-dependent terminators are still debated and different models have been proposed (Park & Roberts, 2006; Epshtein *et al.*, 2010; Koslover *et al.*, 2012; Gocheva *et al.*, 2015). Discussion of these models is beyond the scope of this review. As mentioned earlier, molecular mechanisms used by Rho were studied mainly for *E. coli* Rho protein, with several exceptions of *Micrococcus luteus* (Nowatzke & Richardson, 1996; Nowatzke *et al.*, 1996, 1997a, b), *Rhodobacter sphaeroides* (Ingham, 1999) and *Mycobacterium tuberculosis* (Kalarickal *et al.*, 2010; Mitra *et al.*, 2014; D'Heygère *et al.*, 2015) Rho proteins. Despite some controversial data on helicase activity of *Mycobacterium tuberculosis* Rho (Kalarickal *et al.*, 2010; D'Heygère *et al.*, 2015) and the existence of structural differences between Rho proteins (see below), the basic principles of the action of Rho are conserved across species (D'Heygère *et al.*, 2015). Intimate knowledge of the structure of Rho was used for rational design of potential inhibitors of Rho activity in the Gram-negative coccobacillus *Brucella melitensis* – the infectious agent of brucellosis disease (Pradeepkiran *et al.*, 2015).

The most complete phylogenetic analysis of Rho so far performed revealed that Rho is a well-conserved protein across different bacterial phyla, with the corresponding gene found in >90 % of sequenced bacterial genomes (D'Heygère *et al.*, 2013). Bacteria devoid of Rho, such as all *Cyanobacteria* and *Mollicutes*, but also some members of the *Clostridia*, *Bacilli* and *Negativicutes*, frequently contain small AT-rich genomes. Although it was proposed that Rho conservation is linked to some form of genome complexity, the evolutionary loss of *rho* by some bacteria defies explanation.

In this context it should be noted that the relative importance of Rho-dependent termination of transcription differs between bacterial species. Whilst most bacteria contain genes homologous to *rho*, the homologue is not necessarily an essential gene. Alongside *E. coli*, Rho is essential for the viability of *Salmonella enterica*, *Klebsiella aerogenes*, *Shigella flexneri* (Miloso *et al.*, 1993), *R. sphaeroides* (Gomelsky & Kaplan, 1996), *Mycobacterium tuberculosis* (Sassetti *et al.*, 2003; Griffin *et al.*, 2011), *Bacteroides fragilis* (Veeranagouda *et al.*, 2014), *Pseudomonas aeruginosa* (Morita *et al.*, 2010) and *Micrococcus luteus* (Nowatzke *et al.*, 1997a, b). However, it was found to be dispensable under conditions of growth in rich media in *Bacillus subtilis* (Quirk *et al.*, 1993; Nicolas *et al.*, 2012), *Streptomyces lividans* (Ingham *et al.*, 1996) and *Staphylococcus aureus* (Washburn *et al.*, 2001). Alternatively, a requirement for Rho activity can increase under specific conditions, e.g. in *Caulobacter crescentus*; otherwise dispensable Rho becomes essential for survival under oxidative stress (Italiani & Marques, 2005).

Comparative analysis of Rho homologues has demonstrated that the key residues involved in Rho's oligomerization, RNA binding, ATP hydrolysis and RNA translocation are conserved through different species, consequently

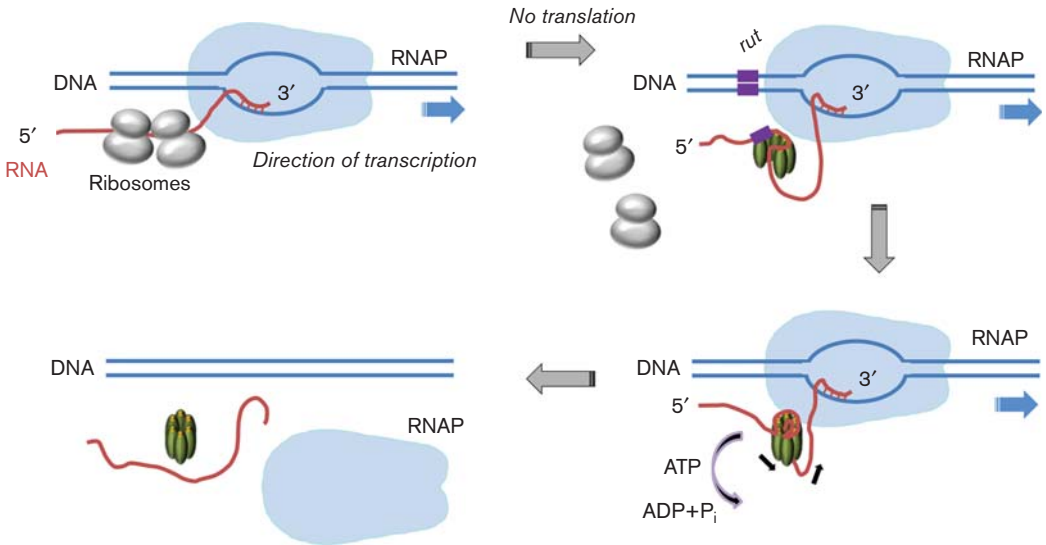

Fig. 1. Schematic representation of Rho-dependent termination (elements not to scale). During coupled transcription–translation of protein-coding genes, RNAP (in blue) is closely followed by ribosomes; the Rho utilization site (*rut*, in dark violet) is not accessible. In the absence of translating ribosomes, the open form of the Rho hexamer (in green) loads on nascent RNA (in brown) at the *rut* site through its primary binding site. Adaptation of the ring conformation and contacts between RNA and the Rho secondary binding site stimulate activation of ATP hydrolysis and 5′→3′ translocation of Rho along the RNA until Rho catches up with RNAP and dissociates the TEC.

suggesting conserved mechanisms of action (D'Heygère *et al.*, 2013). However, the RNA-binding domain of Rho shows more variability than its ATP-binding domain (Opperman & Richardson, 1994; Italiani & Marques, 2005). The most significant difference between Rho proteins corresponds to a large and variable insertion within the N-terminal domain found in ~30 % of bacterial genomes. At present, the functional significance of these structural modifications of Rho is not well understood. Such an insertion was proposed to facilitate Rho–RNA binding in *Actinobacteria* (Nowatzke *et al.*, 1997a, b; D'Heygère *et al.*, 2013). A recent study in the *Mycobacterium tuberculosis* system has shown that deletion of the N-terminal insertion subdomain of Rho provokes RNA-binding defects and modifies ATPase activity (D'Heygère *et al.*, 2015).

In the majority of bacteria, the size of Rho is ~420 aa (D'Heygère *et al.*, 2013), but it varies amongst different species. For example, it is longer in *Actinobacteria* and *Bacteroidetes* due to extensions and/or insertions of the N-terminal domain. The longest Rho sequence was found in *Thermaerobacter marianensis* (865 aa) – a marine extremophile belonging to the *Firmicutes*. The shortest Rho sequences, lacking some RNA-binding motifs, were detected in *Colwellia psychrerythraea* (314 aa) and *Marinomonas* sp. MWYL1 (318 aa), both belonging to the *Gammaproteobacteria*. However, both genomes contain an additional full-sized *rho* ORF, carrying supplementary motifs (D'Heygère *et al.*, 2013).

With regard to the involvement of Rho in the control of diverse processes in bacteria (see below), a better understanding of its evolutionary loss and/or conservation across bacterial phyla appears to be important.

Regulation of gene expression

Rho is known to enforce transcription–translation coupling by interrupting transcription of messages that are not translated (reviewed by Richardson, 1991). According to the *E. coli* model, under optimal translational conditions, the ribosome immediately following RNAP occludes the nascent RNA and consequently physically blocks the access of Rho to the *rut* sites which are presumed to occur frequently in mRNAs (Boudvillain *et al.*, 2013). In the absence of translating ribosomes, Rho binds to the available *rut* sites of the nascent transcript and proceeds to terminate transcription. This underlies the well-known phenomenon of transcriptional polarity, when a nonsense mutation within a gene represses expression of the downstream genes in the same operon (Proshkin *et al.*, 2010).

The above mechanism of Rho function was deduced for *E. coli* where Rho is an abundant protein, and the amount of Rho, in the form of a hexamer, corresponds to ~38–64 % of the RNAP level (Li *et al.*, 2014; Wang *et al.*, 2015a). However, this model of Rho activity cannot be directly extrapolated to Gram-positive bacteria such as *Bacillus subtilis* and *Staphylococcus aureus*, given the low cellular abundance of Rho and low Rho/RNAP ratio in these bacteria (Ingham *et al.*, 1999; Maass *et al.*, 2011; Nicolas *et al.*, 2012; Muntel *et al.*, 2014). In *Bacillus subtilis*, the cellular level of Rho estimated using immunoblotting analysis does not exceed 50 hexamers per cell (Ingham *et al.*, 1999). Similarly, the copy number of Rho determined by the absolute quantification of the *Bacillus subtilis* cytosolic proteome does not exceed 80 Rho hexamers per cell, which corresponds to ~0.8 % of RNAP (Muntel *et al.*, 2014). Measurement of *Bacillus subtilis* Rho cellular abundance using a GFP-Rho fusion estimated the Rho hexamers at ~5 % of the level of RNAP (Nicolas *et al.*, 2012). Thus, *Bacillus subtilis* Rho cannot be present at the majority of TECs, contrary to other general transcription factors such as NusG and NusA, whose cellular levels are near-equimolar to RNAP (Doherty *et al.*, 2006; reviewed by Lewis *et al.*, 2008).

Transcriptional polarity in *Bacillus subtilis* was described for the tryptophan biosynthesis *trpEDCFBA* operon (Babitzke & Gollnick, 2001; Yakhnin *et al.*, 2001). Another example was reported for the *rplJL* operon encoding the ribosomal L10$(L12)_4$ complex. This operon is regulated by an increased translation of the leader region that leads to reduced Rho-dependent termination and relief of transcriptional attenuation (Yakhnin *et al.*, 2015). Despite the limited number of examples of Rho-dependent regulation, the potential role of Rho in transcription–translation coupling in *Bacillus subtilis* and other Gram-positive bacteria should not be underestimated. In *Bacillus subtilis*, Rho prevents synthesis of the untranslated antisense RNAs (asRNAs) initiated from 31 promoter sequences (Nicolas *et al.*, 2012). This argues that despite its low availability, Rho acts to control transcription–translation coupling in *Bacillus subtilis* at the genome scale. One can suggest that in *Bacillus subtilis* some endogenous cellular factors may increase Rho affinity to individual untranslated mRNAs via protein-mediated recruitment mechanism.

New mechanisms of gene regulation which use Rho-dependent termination were revealed recently. In *Salmonella enterica*, translational inhibition leading to Rho-dependent transcription termination is due to the action of sRNA, as was shown by analysis of ChiX sRNA which negatively regulates the *chiPQ* operon involved in oligosaccharide uptake (Bossi *et al.*, 2012). ChiX binds to the ribosome-binding site of the first gene of the operon, *chiP*. As a result, ribosome binding and translation of *chiP* are abolished, inducing premature Rho-dependent transcription termination within the early portion of the *chiPQ* operon. Consequently, the expression of the downstream *chiQ* gene is also downregulated (Bossi *et al.*, 2012). In *E. coli*, the 109 nt long Spot 42 sRNA encoded by the *spf* gene is known to downregulate expression of *galK*, the third gene of the galactose operon *galETKM*, thereby inhibiting GalK production (Møller *et al.*, 2002). Recent studies suggested that Spot 42 regulates *galK* expression at two levels: RNA degradation and enhancement of Rho-dependent transcription termination at the

galT–galK junction (Wang *et al.*, 2015b). To explain how Rho assures termination at the end of the *galT* gene, Wang *et al.* (2015b) proposed that the binding site of Spot 42 on the *galT–galK* junction RNA overlaps with a putative *rut* site. Spot 42 binding enhances the disassembly of the ribosome at the stop codon of *galT* and exposes the *rut* site. Rho would finally catch up with the RNAP transcribing downstream DNA and terminate transcription (Wang *et al.*, 2015b).

A new Rho-dependent mechanism was described for expression of *Salmonella* genes involved in Mg^{2+} transport, suggesting that Rho links Mg^{2+} uptake to translational signals (Kriner & Groisman, 2015). Expression of the Mg^{2+} channel gene *corA* was shown to be regulated by a Rho-dependent terminator located within its 5′ leader region. Accessibility of the *rut* site depending on RNA conformation was shown to be modulated by translation of *corL*, a short *orf* located within the *corA* gene (Kriner & Groisman, 2015).

Another example of gene regulation implicating Rho is the regulation of the *E. coli pgaABCD* operon by the CsrA protein (Figueroa-Bossi *et al.*, 2014). The 5′ UTR of the *pgaABCD* operon was shown to contain a *rut* site which is sequestered by stable RNA secondary structure. Binding of CsrA to the RNA prevents formation of this secondary structure, thus making the *rut* site accessible for Rho binding, and consequently promotes Rho-dependent transcriptional attenuation (Figueroa-Bossi *et al.*, 2014). The authors suggested that in terms of regulatory responses, transcription termination and anti-termination can be equated to repression and activation of transcription initiation (Figueroa-Bossi *et al.*, 2014). This hypothesis is supported by the involvement of Rho in the widespread regulatory system using riboswitches.

In bacteria, riboswitches are RNA-based regulatory elements that control expression of biosynthetic and transport proteins as a result of binding to particular ligands (ions or metabolites) (reviewed by Mellin & Cossart, 2015). The significance of riboswitch function lies in the transduction of ligand binding into changes in expression of the downstream gene. Recently, it was shown that some riboswitches use Rho to attenuate transcription, thus linking Rho to the process of sensing and regulating gene expression in response to environmental cues (Proshkin *et al.*, 2014).

Regulation of Rho-dependent termination by riboswitches was described for the Mg^{2+}-sensing *mgtA* riboswitch from *Salmonella enterica* (Hollands *et al.*, 2012, 2014) for the flavin mononucleotide-sensing *ribB* riboswitch from *E. coli* (Hollands *et al.*, 2012) and flavin mononucleotide riboswitch from the Gram-positive *Corynebacterium glutamicum* (Takemoto *et al.*, 2015). In general, depending on the ligand concentration (e.g. Mg^{2+} or flavin), the leader region of the riboswitch can exist in two alternative conformations. When a ligand is highly available, the riboswitch binds to it and exposes the *rut* sequence. Consequently, Rho interacts with the nascent RNA and induces transcription termination. Otherwise, at low ligand concentrations, the *rut* site is inaccessible and thus regular gene transcription can occur.

Furthermore, it was speculated that Rho-dependent transcription termination is a common, integral part of riboswitches that is actually underestimated. This point of view is based on the fact that a number of riboswitches found in different mRNA leader sequences in *E. coli* are deprived of obvious intrinsic, Rho-independent terminators (Proshkin *et al.*, 2014).

It should be noted that for several proven cases of Rho-dependent gene regulation, the corresponding molecular mechanisms remain unknown currently. Amongst them are the Rho-dependent mechanisms of oxidative stress survival in *Caulobacter crescentus* and *E. coli* (Italiani & Marques, 2005; Kawamura *et al.*, 2005) or repression of the osmotically regulated *proU* operon in *E. coli* and *Salmonella enterica* (Rajkumari & Gowrishankar, 2001). Rho-dependent regulation of the pyrimidine *de novo* biosynthesis *pyr* operon was demonstrated recently in *Corynebacterium glutamicum*. Rho inactivation in this bacterium leads to a two- to fourfold increase of mRNA levels of the pyrimidine biosynthesis genes (Tanaka *et al.*, 2015). Interestingly, an opposite situation is observed with the *pyr* operon of *Bacillus subtilis* cells (Turner *et al.*, 1994): transcriptome analysis of the *Bacillus subtilis rho* mutant revealed a net decrease of *pyr* operon transcription (Nicolas *et al.*, 2012). This strongly suggests that in both *Corynebacterium glutamicum* and *Bacillus subtilis*, Rho regulates *pyr* operon expression, but by diverse unravelled regulatory mechanisms with opposite physiological effects.

Gram-positive bacteria also use a variety of regulatory mechanisms based on the association of proteins, sRNAs or metabolites with mRNA for control of gene expression (Mandal *et al.*, 2003). In *Bacillus subtilis*, ~70 genes are known to be controlled by riboswitches (Mandal *et al.*, 2003; Irnov *et al.*, 2010). Recently, a wide variety of ssRNAs was identified in *Bacillus subtilis*, *Bacillus anthracis*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Streptomyces coelicolor* (Irnov *et al.*, 2010 and references therein; Oliva *et al.*, 2015). However, involvement of Rho-dependent termination in these regulatory mechanisms in Gram-positive bacteria remains to be demonstrated.

Limitation of deleterious foreign DNA expression

Among different physiological functions of Rho, silencing of horizontally transferred foreign DNA was revealed in *E. coli* within the past 10 years. Inactivation of Rho by the Rho-specific antibiotic bicyclomycine (BCM) in *E. coli* resulted in a global increase in the expression of prophages genes (Cardinale *et al.*, 2008). Additionally, RNAP chromatin immunoprecipitation and microarray ('ChIP-chip') experiments in BCM-treated cells revealed a significant association of Rho-dependent terminators

with foreign DNA, suggesting that horizontally transferred gene islands are 'hotspots' for Rho-dependent termination (Peters *et al.*, 2009). Alternative *in silico* analysis confirmed this hypothesis and suggested that Rho can act as a part of the 'cellular immune mechanism' protecting against phage-related or xenogenic DNA not only in *E. coli*, but also in other proteobacteria species (Mitra & Nagaraja, 2012).

So far several hypotheses explaining this function of Rho have been proposed (reviewed by Peters *et al.*, 2011). First, foreign (mostly phage-related) DNA may be rich in Rho-dependent terminators involved in the regulation of gene expression. For example, in *E. coli*, the Rho-specific terminator t_{imm} blocks induction of toxin genes from the *rac* prophage (Cardinale *et al.*, 2008). Rho has been also shown to control the lysogenic state of *E. coli* prophage KplE1 by inhibiting the expression of the *torI* gene that mediates excisive recombination (Menouni *et al.*, 2013). A second hypothesis implies that codon usage in foreign DNA could inhibit translation and thus expose *rut* sequences, which are Rho targets. It was also hypothesized that the insertion of foreign DNA into active transcriptional units alters the activity of natural terminators, disrupting translation–transcription coupling and consequently increasing recruitment of Rho (Peters *et al.*, 2009). Finally, it was proposed that there could be progressive selection against hairpin-encoding sequences (like intrinsic terminators) to facilitate Rho action within horizontally acquired islands (Mitra & Nagaraja, 2012). Regardless of which hypothesis is accurate, Rho activity appears to be important for controlling expression of foreign DNA in *E. coli*.

An involvement of Rho in the control of horizontally transferred genes in other bacteria has not been addressed. It should be noted that *Rhodobacter sphaeroides* 2.4.1, *Bacteroides fragilis* 638R and *Mycobacterium tuberculosis* H37Rv strains in which *rho* was identified as an essential gene are derived from lysogenic strains (Gomelsky & Kaplan, 1996; Cole *et al.*, 1998; Patrick *et al.*, 2010; Griffin *et al.*, 2011; Kontur *et al.*, 2012). In contrast, a *Staphylococcus aureus rho* knockout mutant was obtained from prophage-free RN4220 strain (Washburn *et al.*, 2001; Nair *et al.*, 2011).

However, this activity of Rho appears not to be universal in bacteria as a lack of Rho does not stimulate expression of prophage-related genes in *Bacillus subtilis* (Nicolas *et al.*, 2012). The largest asRNAs detected in *Bacillus subtilis rho* mutant strain were specific to ICE *Bs1* element, SPβ prophage and SKIN element. These asRNAs could negatively interfere with expression of the corresponding genes, thus preventing prophage expression (see below). No asRNAs were detected for PBXS prophage, but transcription of the operon encoding putative phage structural proteins, terminase subunits and lysis functions was strongly down-regulated (Nicolas *et al.*, 2012). These observations suggest that Rho could take part in regulation of foreign DNA expression in *Bacillus subtilis*, but differently than in *E. coli*. Expression of toxin genes *txpA* and *yonT* from SKIN and SPβ prophages was not significantly modified in the absence of Rho.

Importantly, although *E. coli* strains cured from prophages are relatively resistant to BCM, *rho* remains an essential gene in these strains (Cardinale *et al.*, 2008; Tran *et al.*, 2011; Washburn & Gottesman, 2011). This pinpoints other activities of Rho which are vital for viability.

Suppression of pervasive transcription

Genome-wide overlapping transcription has been described for different bacterial transcriptomes (Dornenburg *et al.*, 2010; Sharma *et al.*, 2010; Georg & Hess, 2011; Nicolas *et al.*, 2012; Peters *et al.*, 2012; Voigt *et al.*, 2014). This widespread phenomenon was designated 'pervasive' transcription, where non-canonical transcription is not delineated by any defined ends, which means that it can occur at nearly any place in the genome. Resulting transcripts are usually non-coding RNA, not demarcated by gene boundaries, and are frequently antisense (Wade & Grainger, 2014). Although the number of asRNAs with identified function is limited (reviewed by Thomason & Storz, 2010; Georg & Hess, 2011; Schultze *et al.*, 2014), it is generally assumed that asRNAs could play a role in the regulation of gene expression via a variety of mechanisms, such as transcriptional interference, transcription attenuation, and modulation of degradation by nucleases and of ribosome binding (Thomason & Storz, 2010). Recently, a novel role of pervasive transcription in the surveillance of genome damage and efficient nucleotide excision repair was proposed (Kamarthapu & Nudler, 2015). However, it has also been suggested that pervasive transcription may have no functional role and be a form of transcriptional noise (Peters *et al.*, 2012; Raghavan *et al.*, 2012).

In any case, a high level of pervasive transcription could have deleterious effects, interfering with sense transcription, chromosome replication and genome stability (see below); it could also compromise cellular energy levels. This implies the existence of molecular mechanisms to control such type of transcription.

The role of Rho in the suppression of pervasive, primary antisense, transcription was demonstrated for both Gram-negative and Gram-positive model micro-organisms *E. coli* and *Bacillus subtilis* (Peters *et al.*, 2009, 2012; Nicolas *et al.*, 2012). In *E. coli*, most antisense transcription suppressed by Rho has been shown to arise either from a large uncharacterized set of antisense promoters within genes or from continuation of sense transcription past the ends of genes (read-through) into divergently oriented downstream genes. The mean size of these asRNAs is ~700 nt (Peters *et al.*, 2012). Peters *et al.* (2012) observed that an increase in antisense transcription caused by sub-lethal inhibition of Rho did inhibit sense transcription, which is consistent with the idea that most antisense transcription is transcriptional noise. ChIP-chip experiments also showed that, for preventing antisense transcription,

Rho-specific termination is more important than intrinsic terminators as coding requirements for factor-independent terminators are not always consistent with a protein-coding gene on the opposite strand (Peters *et al.*, 2009).

The major role of Rho in the suppression of pervasive transcription was also demonstrated in *Bacillus subtilis* (Nicolas *et al.*, 2012). The study, aimed at examination of *Bacillus subtilis* WT and *rho* mutant transcriptomes, revealed that 13 % of the protein-coding genes of the WT strain were targeted by asRNAs. A majority of antisense transcripts arise from incomplete termination of transcription. Subsequently, transcriptome analysis of the *rho*-null strain revealed that in the absence of Rho, antisense transcription in *Bacillus subtilis* is largely increased. In total, Rho was shown to prevent antisense transcription in >93 chromosomal regions, comprising 367 genes. In 62 regions, asRNAs corresponded to extended mRNA up to 16 000 nt long and were associated with Rho-specific or partially efficient intrinsic terminators at the 3′ ends of transcriptional units. The rest of the Rho-controlled asRNAs were associated with a large increase in the activity of the promoters and could attain 12 000 nt with a mean size of ∼5400 nt (Nicolas *et al.*, 2012). This last observation led to the suggestion that *Bacillus subtilis* Rho might act very shortly after initiation and promote premature termination of transcription initiated at spurious promoter-like sequences across the genome due to the lack of coupling with translation. Interestingly, in some chromosomal regions of the *rho* mutant an increase of asRNA correlated with a decrease of sense RNA transcription (Nicolas *et al.*, 2012; our unpublished results). This negative correlation between sense and antisense transcription suggests that, unlike *E. coli*, Rho-controlled asRNAs may influence gene expression in *Bacillus subtilis* cells. The biological significance of this observation needs further investigations.

Pervasive transcription can be also suppressed by several other mechanisms during transcription initiation and elongation, and by RNA degradation involving the activity of different bacterial proteins (reviewed by Wade & Grainger, 2014).

In *E. coli*, both the histone-like nucleoid-structuring protein H-NS and transcription factor NusG were shown to contribute to Rho-mediated suppression of antisense transcription (Saxena & Gowrishankar, 2011; Peters *et al.*, 2012). H-NS binds DNA at high-affinity sites, and forms nucleoprotein filaments that spread on AT-rich DNA and bridge distant DNA sites (reviewed by Seshasayee, 2014; Landick *et al.*, 2015). H-NS is known to play an important role in the silencing of horizontally acquired genes and in the suppression of non-coding transcription by inhibition of both transcription initiation and elongation (Saxena & Gowrishankar, 2011; Peters *et al.*, 2012; Singh *et al.*, 2014). H-NS filaments cause RNAP trapping at the promoter region by binding to AT-rich DNA, which is abundant in spurious promoters (Singh *et al.*, 2014) and characteristic for many horizontally acquired genes in *E. coli* (Chandraprakash & Seshasayee, 2014). ChIP-chip analysis demonstrated a strong association between sites of H-NS filament formation and Rho-dependent termination (Peters *et al.*, 2012). It was also shown that bridged H-NS filaments directly inhibit elongating RNAP and promote Rho-dependent termination by expanding the kinetic window for Rho action (Kotlajich *et al.*, 2015).

Premature termination governed by Rho can be increased through the binding of NusG. NusG is a universally conserved transcription factor in prokaryotes. In *E. coli*, it was shown to be indispensable for termination of transcription governed by Rho (reviewed by Tomar & Artsimovitch, 2013). *E. coli* NusG physically couples transcription and translation as it binds to RNAP, and concurrently, either Rho or NusE – a ribosomal S10 protein (Burmann *et al.*, 2010). Rho–NusG interaction was proven to increase efficiency of termination at weak *rut* sites, characterized by lower C/G ratio sequence (Peters *et al.*, 2012; Shashni *et al.*, 2014). It was also shown that NusG influences termination efficiency at ∼20 % of antisense and sense factor-dependent terminators. However, some NusG homologous proteins regulate transcription processivity differently. In *Mycobacterium* species, NusG does not act as a transcription elongation factor and is unable to bind Rho, although it weakly stimulates intrinsic termination (Czyz *et al.*, 2014; Kalyani *et al.*, 2015). Similarly, *Bacillus subtilis* and *Thermus thermophilus* NusG proteins were shown to stimulate RNAP pausing rather than facilitate transcription elongation (Yakhnin *et al.*, 2008; Sevostyanova & Artsimovitch, 2010).

Moreover, H-NS is absent from the *Bacillus subtilis* genome, while NusG is dispensable in this bacteria (Ingham *et al.*, 1999). Therefore, the above mechanisms assisting Rho activity in *E. coli* are not conserved in other bacterial systems.

Finally, pervasive antisense transcription can be controlled at the level of RNA degradation (Lasa *et al.*, 2011; Laalami *et al.*, 2014). It is possible that, under certain circumstances, Rho can participate in this process, although the experimental data supporting this hypothesis are limited at present. For example, in *E. coli*, Rho was identified as a component of the RNase E-based ribonucleoprotein complex purified under specific oxygen-dependent conditions (Tuckerman *et al.*, 2011). Cells expressing a mutant form of Rho showed a decrease in the half-life of bulk mRNA which was attributed to the altered RNA-binding activity of the mutated Rho protein (Sozhamannan & Stitt, 1997). Deletion of *pcnB*, encoding poly(A) polymerase I which polyadenylates and consequently destabilizes RNAs, or *rppH*, encoding a pyrophoshohydrolase which triggers 5′-end-dependent mRNA degradation, renders *E. coli* cell more sensitive to Rho inactivation (Tran *et al.*, 2011). However, Tran *et al.* (2011) do not exclude that these effects are indirect and can be due to activation of the alternative pathways for RNA degradation.

The most direct evidence for the involvement of Rho in RNA degradation comes from *Rhodobacter capsulatus*, where Rho was found to be a major component of the

RNase E-based RNA degradosome (Jäger *et al.*, 2001). The level of Rho considerably increased under anaerobic growth, suggesting a role of Rho in the regulatory response to changing environment (Jäger *et al.*, 2004). At present, no systematic analysis, either by classical genetic approaches or by transcriptomic studies, has been performed to verify and understand the intriguing link that appears to exist between Rho-dependent transcription termination and mRNA degradation.

Taken together, these data provide strong evidence that Rho is a major factor responsible for suppression of pervasive antisense transcription in bacteria. Future detailed analysis of Rho is certainly needed to understand the still elusive role of pervasive transcription in prokaryotes.

Rho as a factor of genome stability

Maintenance of genome stability is a recently discovered biological function of *E. coli* Rho. This activity of this Rho is tightly linked to the control of RNAP backtracking (i.e. spontaneous reversed translocation of RNAP on the template during transcription elongation) and prevention of R-loop formation. R-loops are the three-stranded RNA/DNA hybrids in which RNA is base-paired with its template DNA, leading to extrusion of the non-template single strand from the DNA duplex (Fig. 2). Although the mechanisms of R-loop formation are still debated, it is well documented that formation of RNA/DNA hybrids is a co-transcriptional process favoured by negative supercoiling of DNA, its high G+C content, the absence of RNA secondary structures, and the uncoupling of transcription and translation (reviewed by Drolet *et al.*, 2003; Gowrishankar & Harinarayanan, 2004; Li & Manley, 2006; Dutta *et al.*, 2011; Gowrishankar *et al.*, 2013). Formation of R-loops is also stimulated by RNAP backtracking (Nudler *et al.*, 1997; reviewed by Nudler, 2012). Normally, RNAP backtracking is minimized by translating ribosomes which closely follow the elongation complex and push it forward (Proshkin *et al.*, 2010 and references therein). By premature termination of the untranslated transcripts, Rho plays an important role in the control of spontaneous RNAP backtracking and consequently limits R-loop formation (Gowrishankar & Harinarayanan, 2004).

R-loops have proved to be harmful for genome stability in all organisms, as they can provoke hyper-recombination, mutagenesis and formation of chromosomal double-

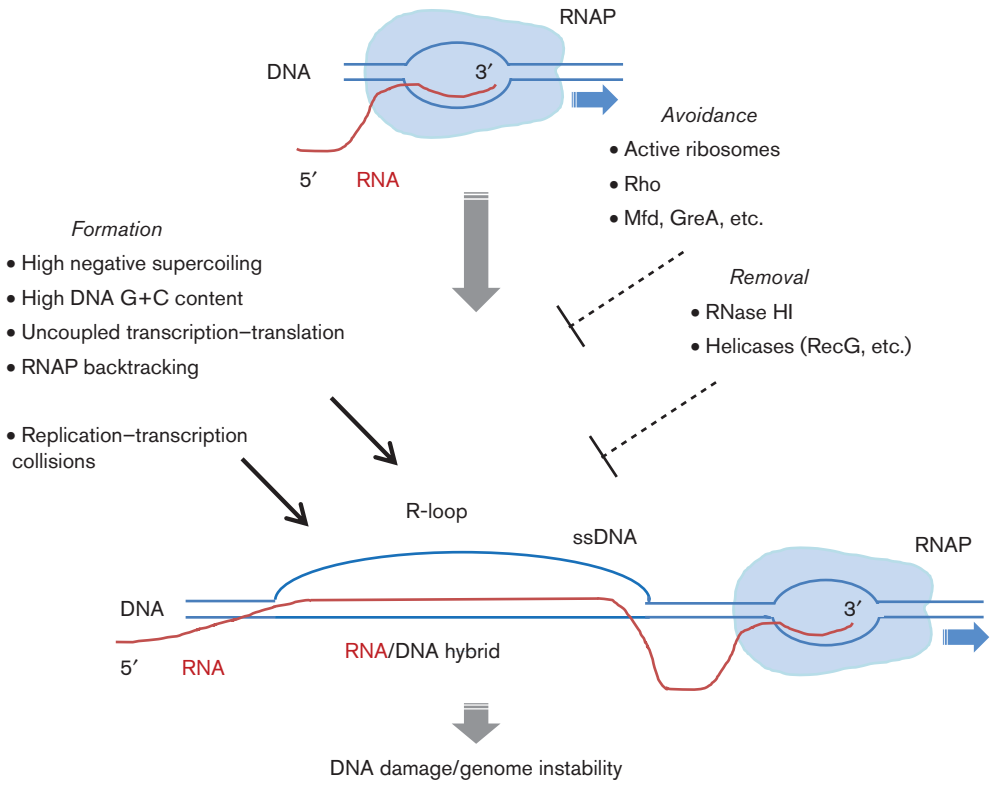

Fig. 2. Schematic representation of R-loop formation. Factors that favour R-loop formation (DNA high negative supercoiling, high DNA G+C content, uncoupled transcription–translation, RNAP backtracking and transcription–replication conflicts) are listed on the left. Anti-backtracking factors preventing R-loop formation (translating ribosomes, Rho, Mfd, GreA) and factors negatively affecting R-loops (RNase HI, helicase RecG active on DNA/DNA and RNA/DNA hybrids) are listed on the right.

strand breaks (DSBs), which are lethal to cells if not repaired (reviewed by Li & Manley, 2006; Aguilera & García-Muse, 2012; Nudler, 2012; Gowrishankar *et al.*, 2013; Wimberly *et al.*, 2013). One of the main sources of DSBs in all genera is the recurrent collisions between the replication and transcription machineries. In bacteria, these collisions are frequent due to the absence of temporal separation of transcription and replication, and to a higher rate of replication fork progression, compared with the elongating RNAP (Washburn & Gottesman, 2011 and references therein). Replication–transcription collisions lead to the replication forks stalling and collapsing, and provoke the formation of DSBs by different mechanisms which, on their own, depend on the nature of the arrest (Washburn & Gottesman, 2011; De Septenville *et al.*, 2012; Dutta *et al.*, 2011).

It has been shown that detrimental effects of replication–transcription collisions can be suppressed by overproduction of RNase HI, an enzyme which specifically hydrolyses the RNA moiety of RNA/DNA hybrids (Boubakri *et al.*, 2010; Dutta *et al.*, 2011; Gan *et al.*, 2011). These observations clearly indicate that R-loops contribute to DSB formation during replication–transcription collisions.

There is also a body of evidence implicating Rho in the avoidance of such deleterious events in *E. coli*. (i) Viability of BCM-treated cells depends on elongation factor GreA (Dutta *et al.*, 2011) and DNA translocase Mfd (Washburn & Gottesman, 2011), both acting as anti-backtracking factors for RNAP in addition to Rho (reviewed by Borukhov *et al.*, 2005). Cells become less sensitive to BCM (i.e. less dependent on Rho) by introduction of the *rpoB**35 mutation which destabilizes TECs and makes them less prone to backtracking (Trautinger & Lloyd, 2002; Washburn & Gottesman, 2011). (ii) *E. coli rho* and *nusG* missense mutants with reduced termination activity show an increased requirement for the anti-R-loop functions of RNase HI and RecG – an enzyme which unwinds the RNA moiety of the RNA/DNA hybrid (Harinarayanan & Gowrishankar, 2003). Consistently, *rho* missense and *recG* knockout mutations are synthetically lethal (Harinarayanan & Gowrishankar, 2003). Most strikingly, the lethal phenotype of *E. coli* cells deleted for *rho* or *nusG* genes can be suppressed by the phage T4-borne helicase UvsW acting on the RNA/DNA hybrids (Leela *et al.*, 2013). Leela *et al.* (2013) also provide evidence for an increased incidence of the R-loops in the chromosome of the *nusG* mutant defective for Rho-dependent termination. (iii) Inactivation of functions needed for the restoration of the collapsed replication forks and replication restart renders *E. coli* cells more sensitive to BCM (Washburn & Gottesman, 2011). This lethality is linked to the considerable increase of chromosomal DSBs upon BCM treatment (Washburn & Gottesman, 2011). Finally, (iv) DSB formation induced by BCM can be suppressed by overexpression of RNase HI or by addition of the replication inhibitor hydroxyurea, which indicates that the DSBs originate from collisions between transcription and replication machineries (Dutta *et al.*, 2011; Washburn & Gottesman, 2011).

Taken together, these data prove that, by limiting RNAP backtracking and R-loop formation, Rho acts to avoid replication–transcription collisions, and consequently to diminish replication stress and DNA damage by breakage. Removal of the backtracked RNAP in front of replication forks and/or prevention of excessive genome-wide formation of R-loops are considered as the reasons of Rho essentiality in *E. coli* cells (Washburn & Gottesman, 2011; Leela *et al.*, 2013; Gowrishankar *et al.*, 2013). In the absence of Rho, cells fail to withstand massive DNA damage.

The extent to which Rho is implicated in the maintenance of genome stability in bacteria where it is non-essential has not yet been addressed experimentally. In the case of *Bacillus subtilis*, it seems plausible that the pervasive transcripts accumulated in the *rho* mutant, most of which are kilobases in size, could engage in the formation of R-loops. It is also remarkable that 28 out of the 31 promoters activated in the absence of Rho are oriented oppositely to chromosome replication (head-on orientation) (Nicolas *et al.*, 2012). However, the *Bacillus subtilis rho* mutant grows normally in rich medium (Quirk *et al.*, 1993; Nicolas *et al.*, 2012), which is in sharp contrast to the low viability of cells experiencing head-on replication–transcription collisions in either the *E. coli* (Boubakri *et al.*, 2010) or *Bacillus subtilis* (Srivatsan *et al.*, 2010) model systems. The mechanisms underlying the robustness of *Bacillus subtilis rho* mutant cells are unknown and need to be established. It was proposed that RNase HI and RecG enzymes responsible for R-loop removal might be more active in bacteria where Rho is non-essential compared with *E. coli* (Gowrishankar *et al.*, 2013). This interesting hypothesis awaits experimental validation, alongside the analysis of other functions potentially able to prevent and to repair deleterious consequences of the loss of Rho-dependent transcription termination in these bacteria.

Intrinsic inhibitors of Rho

The importance of Rho-dependent transcription termination for the fine control of different cellular processes is further supported by the occurrence of intrinsic negative regulators of Rho. Initially, inhibitors of Rho activity were discovered in bacteriophages, which develop several original strategies to suppress Rho-dependent terminators present in their genomes. One of the best-studied examples is N protein encoded by lambdoid phages. N protein, assisted by bacterial elongation factors, modifies RNAP and suppresses intrinsic and Rho-dependent terminators present in phage DNA, thereby assuring transcription of the middle and late phage genes (Mason *et al.*, 1992). N protein overcomes Rho action in multiple ways: it forms an inactive complex with Rho–NusA, prevents Rho–RNAP interaction, removes NusA from Rho-dependent termination pathway and perturbs the Rho–NusG interaction (Muteeb *et al.*, 2012). *E. coli*

cells encode two more Rho-specific inhibitor proteins, Psu and YaeO. The polarity suppression protein Psu is encoded by the *E. coli* defective prophage P4. Psu interacts with Rho specifically, thus affecting ATP binding and RNA-dependent ATP hydrolysis which may reduce Rho translocation along the RNA and thereby the termination efficiency (Pani *et al.*, 2006). YaeO protein binds to the Rho hexamer in a 1 : 1 monomer/monomer ratio in the vicinity to the primary binding site and inhibits the early stages of Rho binding to RNA (Gutiérrez *et al.*, 2007).

YaeO protein exhibits some topological similarities with the pleiotropic regulator of gene expression, RNA-binding protein Hfq (Gutiérrez *et al.*, 2007). Recently, it was shown that *E. coli* Hfq also plays a specific role in Rho-dependent transcription regulation by direct association with Rho and trapping the Rho–RNA complex into an inactive configuration (Rabhi *et al.*, 2011). Rabhi *et al.* (2011) suggested that functional Rho–Hfq interactions are frequent in *E. coli*, although the specifically targeted transcription units remain currently unknown. Interestingly, despite high conservation of the *hfq* gene in a wide range of bacterial genomes, it does not play an important role in regulation in Gram-positive bacteria. For example, in *Bacillus subtilis*, the absence of Hfq had no global effects on the transcriptome (Hämmerle *et al.*, 2014; Rochat *et al.*, 2015). As mentioned earlier, Hfq is a highly conserved protein, and the *yaeO* and *psu* homologous genes are present in some genomes of *E. coli* and related enterobacteria and several prophages. However, one cannot rule out the possibility that other proteins with Rho-specific inhibition activity exist in other bacterial species.

Conclusions

The importance of Rho-dependent transcription termination in bacteria is now commonly recognized. During the last two decades considerable progress has been made in our understanding of the structure and the molecular mechanism of Rho action, thus providing a solid basis for the study of its physiological roles in bacterial cell (Fig. 3). However, whilst the molecular mechanism of Rho activity, based on the *E. coli* Rho model, seems to be mostly conserved, some Rho features may vary considerably amongst different species. Recent data also suggest that apart from the universal functions, e.g. in the control of transcription–translation coupling and pervasive transcription, Rho might manage other functions more or less characteristic for different bacteria. Additional

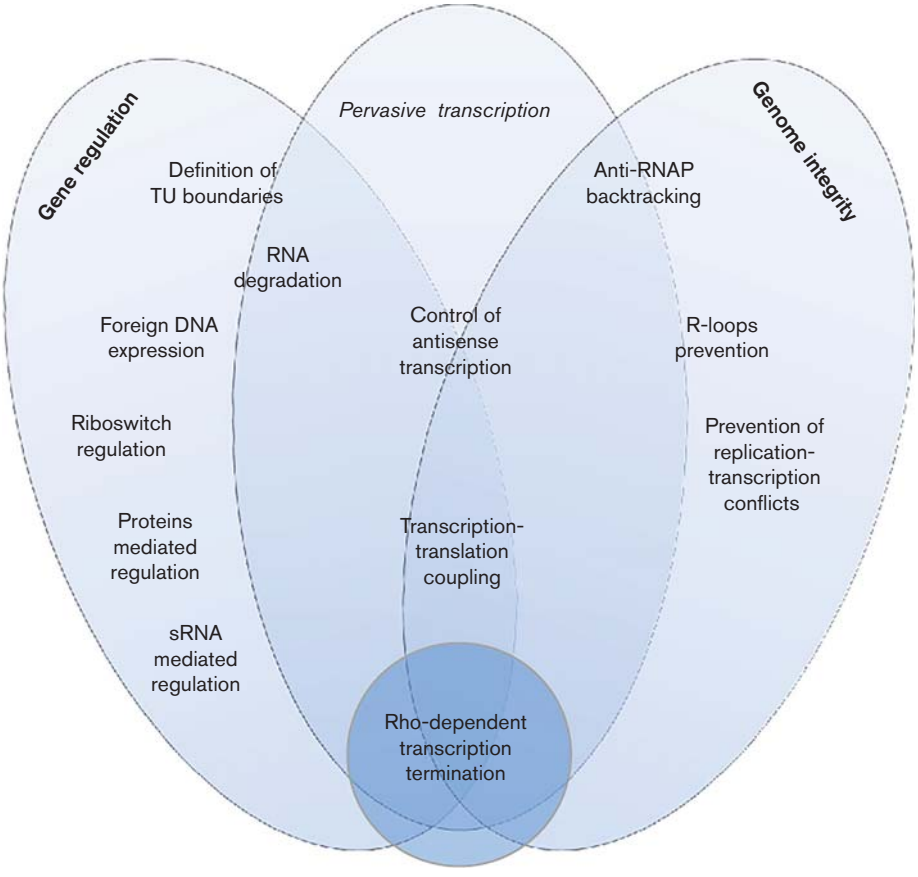

Fig. 3. Involvement of Rho-dependent termination in different inter-related cellular processes. TU, Transcriptional unit.

questions must be addressed by future experimental studies. How does Rho assure transcription termination of untranslated mRNAs in bacteria where it is present in low amounts? What is the extent of Rho employment in the control of horizontally transferred genes across bacterial phyla? What are the compensatory functions and back-ups for Rho activity in the control of genome stability in species where Rho is dispensable? Does Rho-controlled pervasive transcription have a regulatory role for genes expression? This list of the questions is certainly not exhaustive. Elucidation of the species-specific activities of Rho, and its structural and functional interactions with other proteins, promises to be gratifying for fundamental and applied research, especially relating to the discovery of new antimicrobial agents.

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References

Aguilera, A. & García-Muse, T. (2012). R loops: from transcription by products to threats to genome stability. *Mol Cell* **46**, 115–124.

Babitzke, P. & Gollnick, P. (2001). Posttranscription initiation control of tryptophan metabolism in *Bacillus subtilis* by the *trp* RNA-binding attenuation protein (TRAP), anti-TRAP, and RNA structure. *J Bacteriol* **183**, 5795–5802.

Bogden, C. E., Fass, D., Bergman, N., Nichols, M. D. & Berger, J. M. (1999). The structural basis for terminator recognition by the Rho transcription termination factor. *Mol Cell* **3**, 487–493.

Borukhov, S., Lee, J. & Laptenko, O. (2005). Bacterial transcription elongation factors: new insights into molecular mechanism of action. *Mol Microbiol* **55**, 1315–1324.

Bossi, L., Schwartz, A., Guillemardet, B., Boudvillain, M. & Figueroa-Bossi, N. (2012). A role for Rho-dependent polarity in gene regulation by a noncoding small RNA. *Genes Dev* **26**, 1864–1873.

Boubakri, H., de Septenville, A. L., Viguera, E. & Michel, B. (2010). The helicases DinG, Rep and UvrD cooperate to promote replication across transcription units *in vivo*. *EMBO J* **29**, 145–157.

Boudvillain, M., Figueroa-Bossi, N. & Bossi, L. (2013). Terminator still moving forward: expanding roles for Rho factor. *Curr Opin Microbiol* **16**, 118–124.

Bubunenko, M., Baker, T. & Court, D. L. (2007). Essentiality of ribosomal and transcription antitermination proteins analyzed by systematic gene replacement in *Escherichia coli*. *J Bacteriol* **189**, 2844–2853.

Burmann, B. M., Schweimer, K., Luo, X., Wahl, M. C., Stitt, B. L., Gottesman, M. E. & Rösch, P. (2010). A NusE : NusG complex links transcription and translation. *Science* **328**, 501–504.

Burns, C. M., Richardson, L. V. & Richardson, J. P. (1998). Combinatorial effects of NusA and NusG on transcription elongation and Rho-dependent termination in *Escherichia coli*. *J Mol Biol* **278**, 307–316.

Butland, G., Peregrín-Alvarez, J. M., Li, J., Yang, W., Yang, X., Canadien, V., Starostine, A., Richards, D., Beattie, B. & other authors (2005). Interaction network containing conserved and essential protein complexes in *Escherichia coli*. *Nature* **433**, 531–537.

Cardinale, C. J., Washburn, R. S., Tadigotla, V. R., Brown, L. M., Gottesman, M. E. & Nudler, E. (2008). Termination factor Rho and its cofactors NusA and NusG silence foreign DNA in *E. coli*. *Science* **320**, 935–938.

Chandraprakash, D. & Seshasayee, A. S. (2014). Inhibition of factor-dependent transcription termination in *Escherichia coli* might relieve xenogene silencing by abrogating H-NS-DNA interactions *in vivo*. *J Biosci* **39**, 53–61.

Ciampi, M. S. (2006). Rho-dependent terminators and transcription termination. *Microbiology* **152**, 2515–2528.

Cole, S. T., Brosch, R., Parkhill, J., Garnier, T., Churcher, C., Harris, D., Gordon, S. V., Eiglmeier, K., Gas, S. & other authors (1998). Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nature* **393**, 537–544.

Czyz, A., Mooney, R. A., Iaconi, A. & Landick, R. (2014). Mycobacterial RNA polymerase requires a U-tract at intrinsic terminators and is aided by NusG at suboptimal terminators. *MBio* **5**, e00931–e00e14.

D'Heygère, F., Rabhi, M. & Boudvillain, M. (2013). Phyletic distribution and conservation of the bacterial transcription termination factor Rho. *Microbiology* **159**, 1423–1436.

D'Heygère, F., Schwartz, A., Coste, F., Castaing, B. & Boudvillain, M. (2015). ATP-dependent motor activity of the transcription termination factor Rho from *Mycobacterium tuberculosis*. *Nucleic Acids Res* **43**, 6099–6111.

de Hoon, M. J., Makita, Y., Nakai, K. & Miyano, S. (2005). Prediction of transcriptional terminators in *Bacillus subtilis* and related species. *PLoS Comput Biol* **1**, e25.

De Septenville, A. L., Duigou, S., Boubakri, H. & Michel, B. (2012). Replication fork reversal after replication-transcription collision. *PLoS Genet* **8**, e1002622.

Doherty, G. P., Meredith, D. H. & Lewis, P. J. (2006). Subcellular partitioning of transcription factors in *Bacillus subtilis*. *J Bacteriol* **188**, 4101–4110.

Dornenburg, J. E., Devita, A. M., Palumbo, M. J. & Wade, J. T. (2010). Widespread antisense transcription in *Escherichia coli*. *MBio* **1**, e00024–e00e10.

Drolet, M., Broccoli, S., Rallu, F., Hraiky, C., Fortin, C., Massé, E. & Baaklini, I. (2003). The problem of hypernegative supercoiling and R-loop formation in transcription. *Front Biosci* **8**, d210–d221.

Dutta, D., Shatalin, K., Epshtein, V., Gottesman, M. E. & Nudler, E. (2011). Linking RNA polymerase backtracking to genome instability in *E. coli*. *Cell* **146**, 533–543.

Epshtein, V., Cardinale, C. J., Ruckenstein, A. E., Borukhov, S. & Nudler, E. (2007). An allosteric path to transcription termination. *Mol Cell* **28**, 991–1001.

Epshtein, V., Dutta, D., Wade, J. & Nudler, E. (2010). An allosteric mechanism of Rho-dependent transcription termination. *Nature* **463**, 245–249.

Figueroa-Bossi, N., Schwartz, A., Guillemardet, B., D'Heygère, F., Bossi, L. & Boudvillain, M. (2014). RNA remodeling by bacterial global regulator CsrA promotes Rho-dependent transcription termination. *Genes Dev* **28**, 1239–1251.

Gan, W., Guan, Z., Liu, J., Gui, T., Shen, K., Manley, J. L. & Li, X. (2011). R-loop-mediated genomic instability is caused by impairment of replication fork progression. *Genes Dev* **25**, 2041–2056.

Georg, J. & Hess, W. R. (2011). *cis*-Antisense RNA, another level of gene regulation in bacteria. *Microbiol Mol Biol Rev* **75**, 286–300.

Gocheva, V., Le Gall, A., Boudvillain, M., Margeat, E. & Nollmann, M. (2015). Direct observation of the translocation mechanism of transcription termination factor Rho. *Nucleic Acids Res* **43**, 2367–2377.

Gomelsky, M. & Kaplan, S. (1996). The *Rhodobacter sphaeroides* 2.4.1 *rho* gene: expression and genetic analysis of structure and function. *J Bacteriol* **178**, 1946–1954.

Gowrishankar, J. & Harinarayanan, R. (2004). Why is transcription coupled to translation in bacteria? *Mol Microbiol* **54**, 598–603.

Gowrishankar, J., Leela, J. K. & Anupama, K. (2013). R-loops in bacterial transcription: their causes and consequences. *Transcription* **4**, 153–157.

Griffin, J. E., Gawronski, J. D., Dejesus, M. A., Ioerger, T. R., Akerley, B. J. & Sassetti, C. M. (2011). High-resolution phenotypic profiling defines genes essential for mycobacterial growth and cholesterol catabolism. *PLoS Pathog* **7**, e1002251.

Gusarov, I. & Nudler, E. (1999). The mechanism of intrinsic transcription termination. *Mol Cell* **3**, 495–504.

Gutiérrez, P., Kozlov, G., Gabrielli, L., Elias, D., Osborne, M. J., Gallouzi, I. E. & Gehring, K. (2007). Solution structure of YaeO, a Rho-specific inhibitor of transcription termination. *J Biol Chem* **282**, 23348–23353.

Hämmerle, H., Amman, F., Večerek, B., Stülke, J., Hofacker, I. & Bläsi, U. (2014). Impact of Hfq on the *Bacillus subtilis* transcriptome. *PLoS One* **9**, e98661.

Harinarayanan, R. & Gowrishankar, J. (2003). Host factor titration by chromosomal R-loops as a mechanism for runaway plasmid replication in transcription termination-defective mutants of *Escherichia coli*. *J Mol Biol* **332**, 31–46.

Hollands, K., Proshkin, S., Sklyarova, S., Epshtein, V., Mironov, A., Nudler, E. & Groisman, E. A. (2012). Riboswitch control of Rho-dependent transcription termination. *Proc Natl Acad Sci U S A* **109**, 5376–5381.

Hollands, K., Sevostiyanova, A. & Groisman, E. A. (2014). Unusually long-lived pause required for regulation of a Rho-dependent transcription terminator. *Proc Natl Acad Sci U S A* **111**, E1999–E2007.

Ingham, C. J. (1999). Characterisation of the enzymatic and RNA-binding properties of the *Rhodobacter sphaeroides* 2.4.1.Rho homologue. *Biochim Biophys Acta* **1446**, 115–125.

Ingham, C. J., Hunter, I. S. & Smith, M. C. (1996). Isolation and sequencing of the *rho* gene from *Streptomyces lividans* ZX7 and characterization of the RNA-dependent NTPase activity of the overexpressed protein. *J Biol Chem* **271**, 21803–21807.

Ingham, C. J., Dennis, J. & Furneaux, P. A. (1999). Autogenous regulation of transcription termination factor Rho and the requirement for Nus factors in *Bacillus subtilis*. *Mol Microbiol* **31**, 651–663.

Irnov, I., Sharma, C. M., Vogel, J. & Winkler, W. C. (2010). Identification of regulatory RNAs in *Bacillus subtilis*. *Nucleic Acids Res* **38**, 6637–6651.

Italiani, V. C. & Marques, M. V. (2005). The transcription termination factor Rho is essential and autoregulated in *Caulobacter crescentus*. *J Bacteriol* **187**, 4290–4294.

Jäger, S., Fuhrmann, O., Heck, C., Hebermehl, M., Schiltz, E., Rauhut, R. & Klug, G. (2001). An mRNA degrading complex in *Rhodobacter capsulatus*. *Nucleic Acids Res* **29**, 4581–4588.

Jäger, S., Hebermehl, M., Schiltz, E. & Klug, G. (2004). Composition and activity of the *Rhodobacter capsulatus* degradosome vary under different oxygen concentrations. *J Mol Microbiol Biotechnol* **7**, 148–154.

Kalarickal, N. C., Ranjan, A., Kalyani, B. S., Wal, M. & Sen, R. (2010). A bacterial transcription terminator with inefficient molecular motor action but with a robust transcription termination function. *J Mol Biol* **395**, 966–982.

Kalyani, B. S., Kunamneni, R., Wal, M., Ranjan, A. & Sen, R. (2015). A NusG paralogue from *Mycobacterium tuberculosis*, Rv0639, has evolved to interact with ribosomal protein S10 (Rv0700) but not to function as a transcription elongation-termination factor. *Microbiology* **161**, 67–83.

Kamarthapu, V. & Nudler, E. (2015). Rethinking transcription coupled DNA repair. *Curr Opin Microbiol* **24**, 15–20.

Kapranov, P., Cheng, J., Dike, S., Nix, D. A., Duttagupta, R., Willingham, A. T., Stadler, P. F., Hertel, J., Hackermüller, J. & other authors (2007). RNA maps reveal new RNA classes and a possible function for pervasive transcription. *Science* **316**, 1484–1488.

Kawamura, N., Kurokawa, K., Ito, T., Hamamoto, H., Koyama, H., Kaito, C. & Sekimizu, K. (2005). Participation of Rho-dependent transcription termination in oxidative stress sensitivity caused by an *rpoB* mutation. *Genes Cells* **10**, 477–487.

Kontur, W. S., Schackwitz, W. S., Ivanova, N., Martin, J., Labutti, K., Deshpande, S., Tice, H. N., Pennacchio, C., Sodergren, E. & other authors (2012). Revised sequence and annotation of the *Rhodobacter sphaeroides* 2.4.1 genome. *J Bacteriol* **194**, 7016–7017.

Koslover, D. J., Fazal, F. M., Mooney, R. A., Landick, R. & Block, S. M. (2012). Binding and translocation of termination factor rho studied at the single-molecule level. *J Mol Biol* **423**, 664–676.

Kotlajich, M. V., Hron, D. R., Boudreau, B. A., Sun, Z., Lyubchenko, Y. L. & Landick, R. (2015). Bridged filaments of histone-like nucleoid structuring protein pause RNA polymerase and aid termination in bacteria. *eLife* **4**, e04970.

Kriner, M. A. & Groisman, E. A. (2015). The bacterial transcription termination factor Rho coordinates Mg^{2+} homeostasis with translational signals. *J Mol Biol* **427**, 3834–3849.

Laalami, S., Zig, L. & Putzer, H. (2014). Initiation of mRNA decay in bacteria. *Cell Mol Life Sci* **71**, 1799–1828.

Landick, R., Wade, J. T. & Grainger, D. C. (2015). H-NS and RNA polymerase: a love-hate relationship? *Curr Opin Microbiol* **24**, 53–59.

Lasa, I., Toledo-Arana, A., Dobin, A., Villanueva, M., de los Mozos, I. R., Vergara-Irigaray, M., Segura, V., Fagegaltier, D., Penadés, J. R. & other authors (2011). Genome-wide antisense transcription drives mRNA processing in bacteria. *Proc Natl Acad Sci U S A* **108**, 20172–20177.

Leela, J. K., Syeda, A. H., Anupama, K. & Gowrishankar, J. (2013). Rho-dependent transcription termination is essential to prevent excessive genome-wide R-loops in *Escherichia coli*. *Proc Natl Acad Sci U S A* **110**, 258–263.

Lewis, P. J., Doherty, G. P. & Clarke, J. (2008). Transcription factor dynamics. *Microbiology* **154**, 1837–1844.

Li, X. & Manley, J. L. (2006). Cotranscriptional processes and their influence on genome stability. *Genes Dev* **20**, 1838–1847.

Li, G. W., Burkhardt, D., Gross, C. & Weissman, J. S. (2014). Quantifying absolute protein synthesis rates reveals principles underlying allocation of cellular resources. *Cell* **157**, 624–635.

Maass, S., Sievers, S., Zühlke, D., Kuzinski, J., Sappa, P. K., Muntel, J., Hessling, B., Bernhardt, J., Sietmann, R. & other authors (2011). Efficient, global-scale quantification of absolute protein amounts by integration of targeted mass spectrometry and two-dimensional gel-based proteomics. *Anal Chem* **83**, 2677–2684.

Mandal, M., Boese, B., Barrick, J. E., Winkler, W. C. & Breaker, R. R. (2003). Riboswitches control fundamental biochemical pathways in *Bacillus subtilis* and other bacteria. *Cell* **113**, 577–586.

Mason, S. W., Li, J. & Greenblatt, J. (1992). Host factor requirements for processive antitermination of transcription and suppression of pausing by the N protein of bacteriophage lambda. *J Biol Chem* **267**, 19418–19426.

Mellin, J. R. & Cossart, P. (2015). Unexpected versatility in bacterial riboswitches. *Trends Genet* **31**, 150–156.

Menouni, R., Champ, S., Espinosa, L., Boudvillain, M. & Ansaldi, M. (2013). Transcription termination controls prophage maintenance in *Escherichia coli* genomes. *Proc Natl Acad Sci U S A* **110**, 14414–14419.

Merrikh, H., Zhang, Y., Grossman, A. D. & Wang, J. D. (2012). Replication-transcription conflicts in bacteria. *Nat Rev Microbiol* **10**, 449–458.

Miloso, M., Limauro, D., Alifano, P., Rivellini, F., Lavitola, A., Gulletta, E. & Bruni, C. B. (1993). Characterization of the rho genes of *Neisseria gonorrhoeae* and *Salmonella typhimurium*. *J Bacteriol* **175**, 8030–8037.

Mitra, A. & Nagaraja, V. (2012). Under-representation of intrinsic terminators across bacterial genomic islands: Rho as a principal regulator of xenogenic DNA expression. *Gene* **508**, 221–228.

Mitra, A., Angamuthu, K., Jayashree, H. V. & Nagaraja, V. (2009). Occurrence, divergence and evolution of intrinsic terminators across eubacteria. *Genomics* **94**, 110–116.

Mitra, A., Kesarwani, A. K., Pal, D. & Nagaraja, V. (2011). WebGeSTer DB – a transcription terminator database. *Nucleic Acids Res* **39** (Database), D129–D135.

Mitra, A., Misquitta, R. & Nagaraja, V. (2014). *Mycobacterium tuberculosis* Rho is an NTPase with distinct kinetic properties and a novel RNA-binding subdomain. *PLoS One* **9**, e107474.

Møller, T., Franch, T., Udesen, C., Gerdes, K. & Valentin-Hansen, P. (2002). Spot 42 RNA mediates discoordinate expression of the *E. coli* galactose operon. *Genes Dev* **16**, 1696–1706.

Morita, Y., Narita, S., Tomida, J., Tokuda, H. & Kawamura, Y. (2010). Application of an inducible system to engineer unmarked conditional mutants of essential genes of *Pseudomonas aeruginosa*. *J Microbiol Methods* **82**, 205–213.

Muntel, J., Fromion, V., Goelzer, A., Maaβ, S., Mäder, U., Büttner, K., Hecker, M. & Becher, D. (2014). Comprehensive absolute quantification of the cytosolic proteome of *Bacillus subtilis* by data independent, parallel fragmentation in liquid chromatography/mass spectrometry (LC/MS(E)). *Mol Cell Proteomics* **13**, 1008–1019.

Muteeb, G., Dey, D., Mishra, S. & Sen, R. (2012). A multipronged strategy of an anti-terminator protein to overcome Rho-dependent transcription termination. *Nucleic Acids Res* **40**, 11213–11228.

Nair, D., Memmi, G., Hernandez, D., Bard, J., Beaume, M., Gill, S., Francois, P. & Cheung, A. L. (2011). Whole-genome sequencing of *Staphylococcus aureus* strain RN4220, a key laboratory strain used in virulence research, identifies mutations that affect not only virulence factors but also the fitness of the strain. *J Bacteriol* **193**, 2332–2335.

Nicolas, P., Mäder, U., Dervyn, E., Rochat, T., Leduc, A., Pigeonneau, N., Bidnenko, E., Marchadier, E., Hoebeke, M. & other authors (2012). Condition-dependent transcriptome reveals high-level regulatory architecture in *Bacillus subtilis*. *Science* **335**, 1103–1106.

Nowatzke, W. L. & Richardson, J. P. (1996). Characterization of an unusual Rho factor from the high G+C Gram-positive bacterium *Micrococcus luteus*. *J Biol Chem* **271**, 742–747.

Nowatzke, W., Richardson, L. & Richardson, J. P. (1996). Purification of transcription termination factor Rho from *Escherichia coli* and *Micrococcus luteus*. *Methods Enzymol* **274**, 353–363.

Nowatzke, W. L., Burns, C. M. & Richardson, J. P. (1997a). Function of the novel subdomain in the RNA binding domain of transcription termination factor Rho from *Micrococcus luteus*. *J Biol Chem* **272**, 2207–2211.

Nowatzke, W. L., Keller, E., Koch, G. & Richardson, J. P. (1997b). Transcription termination factor Rho is essential for *Micrococcus luteus*. *J Bacteriol* **179**, 5238–5240.

Nudler, E. (2012). RNA polymerase backtracking in gene regulation and genome instability. *Cell* **149**, 1438–1445.

Nudler, E., Mustaev, A., Lukhtanov, E. & Goldfarb, A. (1997). The RNA-DNA hybrid maintains the register of transcription by preventing backtracking of RNA polymerase. *Cell* **89**, 33–41.

Oliva, G., Sahr, T. & Buchrieser, C. (2015). Small RNAs, 5′ UTR elements and RNA-binding proteins in intracellular bacteria: impact on metabolism and virulence. *FEMS Microbiol Rev* **39**, 331–349.

Opperman, T. & Richardson, J. P. (1994). Phylogenetic analysis of sequences from diverse bacteria with homology to the *Escherichia coli rho* gene. *J Bacteriol* **176**, 5033–5043.

Pani, B., Banerjee, S., Chalissery, J., Muralimohan, A., Loganathan, R. M., Suganthan, R. B. & Sen, R. (2006). Mechanism of inhibition of Rho-dependent transcription termination by bacteriophage P4 protein Psu. *J Biol Chem* **281**, 26491–26500.

Park, J. S. & Roberts, J. W. (2006). Role of DNA bubble rewinding in enzymatic transcription termination. *Proc Natl Acad Sci U S A* **103**, 4870–4875.

Patrick, S., Blakely, G. W., Houston, S., Moore, J., Abratt, V. R., Bertalan, M., Cerdeño-Tárraga, A. M., Quail, M. A., Corton, N. & other authors (2010). Twenty-eight divergent polysaccharide loci specifying within- and amongst-strain capsule diversity in three strains of *Bacteroides fragilis*. *Microbiology* **156**, 3255–3269.

Peters, J. M., Mooney, R. A., Kuan, P. F., Rowland, J. L., Keles, S. & Landick, R. (2009). Rho directs widespread termination of intragenic and stable RNA transcription. *Proc Natl Acad Sci U S A* **106**, 15406–15411.

Peters, J. M., Vangeloff, A. D. & Landick, R. (2011). Bacterial transcription terminators: the RNA 3′-end chronicles. *J Mol Biol* **412**, 793–813.

Peters, J. M., Mooney, R. A., Grass, J. A., Jessen, E. D., Tran, F. & Landick, R. (2012). Rho and NusG suppress pervasive antisense transcription in *Escherichia coli*. *Genes Dev* **26**, 2621–2633.

Pradeepkiran, J. A., Kumar, K. K., Kumar, Y. N. & Bhaskar, M. (2015). Modeling, molecular dynamics, and docking assessment of transcription factor rho: a potential drug target in *Brucella melitensis* 16M. *Drug Des Devel Ther* **9**, 1897–1912.

Proshkin, S., Rahmouni, A. R., Mironov, A. & Nudler, E. (2010). Cooperation between translating ribosomes and RNA polymerase in transcription elongation. *Science* **328**, 504–508.

Proshkin, S., Mironov, A. & Nudler, E. (2014). Riboswitches in regulation of Rho-dependent transcription termination. *Biochim Biophys Acta* **1839**, 974–977.

Quirk, P. G., Dunkley, E. A. Jr., Lee, P. Jr. & Krulwich, T. A. (1993). Identification of a putative *Bacillus subtilis rho* gene. *J Bacteriol* **175**, 647–654.

Rabhi, M., Espéli, O., Schwartz, A., Cayrol, B., Rahmouni, A. R., Arluison, V. & Boudvillain, M. (2011). The Sm-like RNA chaperone Hfq mediates transcription antitermination at Rho-dependent terminators. *EMBO J* **30**, 2805–2816.

Raghavan, R., Sloan, D. B. & Ochman, H. (2012). Antisense transcription is pervasive but rarely conserved in enteric bacteria. *MBio* **3**, e00156–e00e12.

Rajkumari, K. & Gowrishankar, J. (2001). *In vivo* expression from the RpoS-dependent P1 promoter of the osmotically regulated *proU* operon in *Escherichia coli* and *Salmonella enterica* serovar

Typhimurium: activation by *rho* and *hns* mutations and by cold stress. *J Bacteriol* **183**, 6543–6550.

Richardson, J. P. (1982). Activation of rho protein ATPase requires simultaneous interaction at two kinds of nucleic acid-binding sites. *J Biol Chem* **257**, 5760–5766.

Richardson, J. P. (1991). Preventing the synthesis of unused transcripts by Rho factor. *Cell* **64**, 1047–1049.

Richardson, J. P. (2002). Rho-dependent termination and ATPases in transcript termination. *Biochim Biophys Acta* **1577**, 251–260.

Roberts, J. W. (1969). Termination factor for RNA synthesis. *Nature* **224**, 1168–1174.

Rochat, T., Delumeau, O., Figueroa-Bossi, N., Noirot, P., Bossi, L., Dervyn, E. & Bouloc, P. (2015). Tracking the elusive function of *Bacillus subtilis* Hfq. *PLoS One* **10**, e0124977.

Santangelo, T. J. & Artsimovitch, I. (2011). Termination and antitermination: RNA polymerase runs a stop sign. *Nat Rev Microbiol* **9**, 319–329.

Sassetti, C. M., Boyd, D. H. & Rubin, E. J. (2003). Genes required for mycobacterial growth defined by high density mutagenesis. *Mol Microbiol* **48**, 77–84.

Saxena, S. & Gowrishankar, J. (2011). Compromised factor-dependent transcription termination in a *nusA* mutant of *Escherichia coli*: spectrum of termination efficiencies generated by perturbations of Rho, NusG, NusA, and H-NS family proteins. *J Bacteriol* **193**, 3842–3850.

Schultze, T., Izar, B., Qing, X., Mannala, G. K. & Hain, T. (2014). Current status of antisense RNA-mediated gene regulation in *Listeria monocytogenes*. *Front Cell Infect Microbiol* **4**, 135.

Schulz, D., Schwalb, B., Kiesel, A., Baejen, C., Torkler, P., Gagneur, J., Soeding, J. & Cramer, P. (2013). Transcriptome surveillance by selective termination of noncoding RNA synthesis. *Cell* **155**, 1075–1087.

Seshasayee, A. S. N. (2014). Gene expression homeostasis and chromosome architecture. *BioArchitecture* **4**, 221–225.

Sevostyanova, A. & Artsimovitch, I. (2010). Functional analysis of *Thermus thermophilus* transcription factor NusG. *Nucleic Acids Res* **38**, 7432–7445.

Sharma, C. M., Hoffmann, S., Darfeuille, F., Reignier, J., Findeiß, S., Sittka, A., Chabas, S., Reiche, K., Hackermüller, J. & other authors (2010). The primary transcriptome of the major human pathogen *Helicobacter pylori*. *Nature* **464**, 250–255.

Shashni, R., Qayyum, M. Z., Vishalini, V., Dey, D. & Sen, R. (2014). Redundancy of primary RNA-binding functions of the bacterial transcription terminator Rho. *Nucleic Acids Res* **42**, 9677–9690.

Singh, S. S., Singh, N., Bonocora, R. P., Fitzgerald, D. M., Wade, J. T. & Grainger, D. C. (2014). Widespread suppression of intragenic transcription initiation by H-NS. *Genes Dev* **28**, 214–219.

Skordalakes, E. & Berger, J. M. (2003). Structure of the Rho transcription terminator: mechanism of mRNA recognition and helicase loading. *Cell* **114**, 135–146.

Skordalakes, E., Brogan, A. P., Park, B. S., Kohn, H. & Berger, J. M. (2005). Structural mechanism of inhibition of the Rho transcription termination factor by the antibiotic bicyclomycin. *Structure* **13**, 99–109.

Sozhamannan, S. & Stitt, B. L. (1997). Effects on mRNA degradation by *Escherichia coli* transcription termination factor Rho and pBR322 copy number control protein Rop. *J Mol Biol* **268**, 689–703.

Srivatsan, A., Tehranchi, A., MacAlpine, D. M. & Wang, J. D. (2010). Co-orientation of replication and transcription preserves genome integrity. *PLoS Genet* **6**, e1000810.

Takemoto, N., Tanaka, Y. & Inui, M. (2015). Rho and RNase play a central role in FMN riboswitch regulation in *Corynebacterium glutamicum*. *Nucleic Acids Res* **43**, 520–529.

Tanaka, Y., Teramoto, H. & Inui, M. (2015). Regulation of the expression of *de novo* pyrimidine biosynthesis genes in *Corynebacterium glutamicum*. *J Bacteriol* **197**, 3307–3316.

Thomason, M. K. & Storz, G. (2010). Bacterial antisense RNAs: how many are there, and what are they doing? *Annu Rev Genet* **44**, 167–188.

Tomar, S. K. & Artsimovitch, I. (2013). NusG-Spt5 proteins - universal tools for transcription modification and communication. *Chem Rev* **113**, 8604–8619.

Tran, L., van Baarsel, J. A., Washburn, R. S., Gottesman, M. E. & Miller, J. H. (2011). Single-gene deletion mutants of *Escherichia coli* with altered sensitivity to bicyclomycin, an inhibitor of transcription termination factor Rho. *J Bacteriol* **193**, 2229–2235.

Trautinger, B. W. & Lloyd, R. G. (2002). Modulation of DNA repair by mutations flanking the DNA channel through RNA polymerase. *EMBO J* **21**, 6944–6953.

Tuckerman, J. R., Gonzalez, G. & Gilles-Gonzalez, M. A. (2011). Cyclic di-GMP activation of polynucleotide phosphorylase signal-dependent RNA processing. *J Mol Biol* **407**, 633–639.

Turner, R. J., Lu, Y. & Switzer, R. L. (1994). Regulation of the *Bacillus subtilis* pyrimidine biosynthetic (*pyr*) gene cluster by an autogenous transcriptional attenuation mechanism. *J Bacteriol* **176**, 3708–3722.

Veeranagouda, Y., Husain, F., Tenorio, E. L. & Wexler, H. M. (2014). Identification of genes required for the survival of *Bacteroides fragilis* using massive parallel sequencing of a saturated transposon mutant library. *BMC Genomics* **15**, 429.

Voigt, K., Sharma, C. M., Mitschke, J., Lambrecht, S. J., Voß, B., Hess, W. R. & Steglich, C. (2014). Comparative transcriptomics of two environmentally relevant cyanobacteria reveals unexpected transcriptome diversity. *ISME J* **8**, 2056–2068.

Wade, J. T. & Grainger, D. C. (2014). Pervasive transcription: illuminating the dark matter of bacterial transcriptomes. *Nat Rev Microbiol* **12**, 647–653.

Wang, M., Herrmann, C. J., Simonovic, M., Szklarczyk, D. & von Mering, C. (2015a). Version 4.0 of PaxDb: protein abundance data, integrated across model organisms, tissues, and cell-lines. *Proteomics* **15**, 3163–3168.

Wang, X., Ji, S. C., Jeon, H. J., Lee, Y. & Lim, H. M. (2015b). Two-level inhibition of *galK* expression by Spot 42: degradation of mRNA mK2 and enhanced transcription termination before the *galK* gene. *Proc Natl Acad Sci U S A* **112**, 7581–7586.

Washburn, R. S. & Gottesman, M. E. (2011). Transcription termination maintains chromosome integrity. *Proc Natl Acad Sci U S A* **108**, 792–797.

Washburn, R. S. & Gottesman, M. E. (2015). Regulation of transcription elongation and termination. *Biomolecules* **5**, 1063–1078.

Washburn, R. S., Marra, A., Bryant, A. P., Rosenberg, M. & Gentry, D. R. (2001). *rho* is not essential for viability or virulence in *Staphylococcus aureus*. *Antimicrob Agents Chemother* **45**, 1099–1103.

Washio, T., Sasayama, J. & Tomita, M. (1998). Analysis of complete genomes suggests that many prokaryotes do not rely on hairpin formation in transcription termination. *Nucleic Acids Res* **26**, 5456–5463.

Wimberly, H., Shee, C., Thornton, P. C., Sivaramakrishnan, P., Rosenberg, S. M. & Hastings, P. J. (2013). R-loops and nicks initiate DNA breakage and genome instability in non-growing *Escherichia coli*. *Nat Commun* **4**, 2115.

Yakhnin, H., Babiarz, J. E., Yakhnin, A. V. & Babitzke, P. (2001). Expression of the *Bacillus subtilis trpEDCFBA* operon is influenced by translational coupling and Rho termination factor. *J Bacteriol* **183**, 5918–5926.

Yakhnin, A. V., Yakhnin, H. & Babitzke, P. (2008). Function of the *Bacillus subtilis* transcription elongation factor NusG in hairpin-dependent RNA polymerase pausing in the *trp* leader. *Proc Natl Acad Sci U S A* **105**, 16131–16136.

Yakhnin, H., Yakhnin, A. V. & Babitzke, P. (2015). Ribosomal protein $L10(L12)_4$ autoregulates expression of the *Bacillus subtilis rplJL* operon by a transcription attenuation mechanism. *Nucleic Acids Res* **43**, 7032–7043.