Bacterial gene expression

How to take down the terminator

NELLY SAID[1], IRINA ARTSIMOVITCH[2], MARKUS C. WAHL[1,3]
[1] FU BERLIN, INSTITUTE OF CHEMISTRY AND BIOCHEMISTRY, LABORATORY OF STRUCTURAL BIOCHEMISTRY
[2] DEPARTMENT OF MICROBIOLOGY AND CENTER FOR RNA BIOLOGY, THE OHIO STATE UNIVERSITY, COLUMBUS, OH, USA
[3] HELMHOLTZ-ZENTRUM BERLIN FÜR MATERIALIEN UND ENERGIE, MACROMOLECULAR CRYSTALLOGRAPHY, BERLIN

In bacteria, production of aberrant RNAs and transcription of foreign genes, including those on phages, are readily terminated by a hexameric ATPase, Rho. However, to make necessary transcripts, particularly during stress, bacteria depend on mechanisms to temper Rho activity. Similarly, phages have evolved diverse Rho-inhibitory mechanisms to enable the expression of their own genomes. In recent years, the structural bases of many such anti-termination mechanisms have been elucidated.

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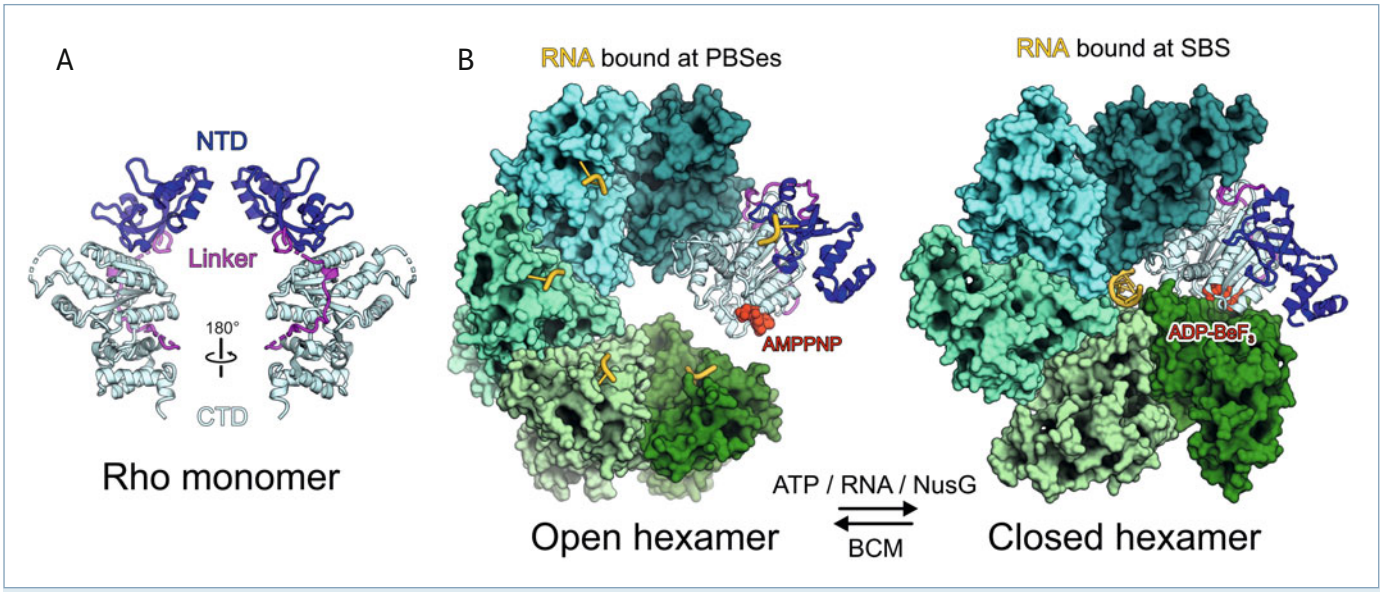
The molecular motor protein, Rho, is long known as a transcription terminator that defines the boundaries of many transcription units in bacteria. More recent studies have portrayed Rho as a global gene regulator and a sentinel of the bacterial genome. Rho is ubiquitous in bacteria and essential in most species, predominantly as it prevents production of "useless" or harmful transcripts.

Rho is a hexameric, ring-shaped, ATPase-driven RNA translocase and helicase. A Rho monomer contains an N-terminal (NTD) and a C-terminal domain (CTD) connected by a regulatory linker (**Fig. 1A**). The NTD contains a primary RNA-binding site (PBS) that preferentially binds pyrimidine-rich, single-stranded RNAs called *rho-utilization* (*rut*) sites. The CTDs jointly form a secondary RNA-binding site (SBS) at the center of the Rho ring and ATP-binding/ATPase sites at the CTD interfaces. Rho can adopt an ATPase-inactive open-ring conformation and an ATPase-competent closed-ring conformation (**Fig. 1B**). Activation of Rho is initiated by RNA binding at the PBSes. ATP binding and trapping of RNA at the SBS induce ring closure and stimulate ATPase-driven RNA translocation. *In vitro*, termination can proceed *via* two pathways. In an RNA-dependent path-



▲ **Fig. 1:** Structure of termination factor Rho. **A**, Diametric cartoon plots of a Rho subunit (PDB ID 1PVO, chain A). Blue: Rho N-terminal domain (NTD) containing a primary RNA binding site (PBS); pale cyan: Rho C-terminal domain (CTD) containing determinants of the secondary RNA binding site (SBS) and of the ATPase/translocase activities; magenta: regulatory linker. **B**, Left, cartoon and surface plot of hexameric Rho in an open conformation in complex with AMPPNP (red spheres) and RNA at the PBSes (gold cartoon; PDB ID 1PVO). Right, cartoon and surface plot of hexameric Rho in a closed conformation in complex with ADP-BeF_3 (red spheres) and RNA at the SBS (gold cartoon; PDB ID 3ICE). One Rho subunit is shown as cartoon, colored as in (A), the other Rho subunits are shown in surface view, colored in different shades of green and cyan. Binding of ATP, binding of RNA at the PBSes and at the SBS, and binding of transcription factor NusG support Rho ring closure, while the Rho-targeting antibiotic, bicyclomycin (BCM), supports ring opening.

way, Rho engages *rut* sites in the nascent transcript *via* its PBSes, captures a neighboring RNA region at the SBS, converts into a closed ring, and translocates on the RNA in 5'-to-3' direction powered by ATP hydrolysis (**Fig. 2**, center right) [1, 2]. When catching up with a transcription elongation complex (EC), Rho extracts the RNA or pushes RNA polymerase (RNAP) forward, leading to dissociation of RNAP from the DNA. In an EC-dependent pathway, Rho associates with an actively transcribing EC without immediately initiating termination, held in check by RNAP-associated factors (**Fig. 2**, center, left) [3, 4]. EC pausing can trigger a cascade of conformational changes in Rho and the EC that lead to RNAP inactivation. The moribund RNAP can then either release the RNA directly or with assistance from Rho, as in the RNA-dependent pathway.

To prevent Rho from terminating the desired transcripts, bacteria resort to several mechanisms. In a first strategy, they restrict Rho access to nascent RNAs or the EC. A second strategy relies on proteins that directly bind and inhibit Rho. A third strategy of Rho control *via* reversible aggregation or condensation is also emerging.

Indirect EC-based anti-termination strategies

Protein-coding RNAs are protected from Rho by the translating ribosome. In bacteria, transcription and translation occur in the same membrane-bound compartment, and a lead ribosome can rapidly engage the nascent RNA, catch up to the transcribing RNAP, and closely trail the EC thereafter. In such transcription/translation-coupled expressomes [5], Rho-binding sites on the EC are shielded by the lead ribosome and the nascent RNA exposed between the coupled machineries is too short for Rho to load (**Fig. 2**, top). In case of untranslated RNAs, such as ribosomal (r)RNAs, bacteria employ dedicated anti-termination complexes in which physical barriers are erected on RNAP that likewise block Rho attack. For example, we recently revealed how during rRNA synthesis in *Escherichia coli*, transcription factors NusA, NusB, NusE (identical to r-protein S10), NusG, inositol monophosphatase SuhB and r-protein S4 bind signal sequences in the leader and spacer regions of the nascent rRNA and assemble a multifactorial RNA-protein complex (RNP) around the RNA exit channel of RNAP (**Fig. 2**, top) [6]. Strikingly, phages employ a phage-encoded protein to nucleate a similar RNP on RNAP to fend off Rho (**Fig. 2**, bottom) [7].

Direct inhibition of Rho

Three bacterial and one phage protein are known to directly modulate Rho activity. The RNA chaperone Hfq can bind Rho, reinforced by RNA, and inhibit the Rho ATPase. A structural homolog of Hfq, the Sm-like protein Rof, also binds Rho and prevents Rho from engaging RNAP or RNA. YihE, a protein kinase, also inhibits Rho by direct interactions. Finally, Psu, a phage P4 coat protein, can inhibit Rho to allow P4 gene expression. Several of these proteins have been known as Rho antagonists for decades, but their modes of action and physiological roles have remained largely unresolved. We and others recently elucidated 3D structures of Rho-Rof and Rho-Psu complexes using cryogenic electron microscopy (cryoEM) in combination with single particle analysis.

CryoEM structures of Rho-Rof complexes show five Rof proteins bound to an open Rho hexamer (**Fig. 2**, bottom, [8, 9]). Each Rof binds between two neighboring Rho NTDs near the PBSes but does not block a "core" PBS; instead, Rof prevents RNA engagement at an "extended" PBS. Functional studies confirmed that Rho residues important for Rof binding are also required for RNA-PBS interactions, and that Rof blocks RNA binding to all sites on Rho. Furthermore, comparison to structures of Rho-modified ECs [3, 4] showed that Rof also sterically blocks Rho from engaging an EC. Thus, Rof can impede both the RNA-dependent and the EC-dependent termination pathways.

P4 is an enterobacterial satellite phage that can only replicate when the host bacterium is co-infected by its helper phage, P2. In the absence of P2, P4 integrates its genome into the bacterial chromosome and propagates as a prophage. Almost half of the sequenced *E. coli* genomes harbor P4-like elements that encode Psu, which has been shown to prevent termination by directly binding Rho, but the exact mechanism of Psu action has long remained elusive. Our interaction studies and cryoEM analysis revealed a dynamic ensemble of Rho-Psu complexes. Psu dimers laterally

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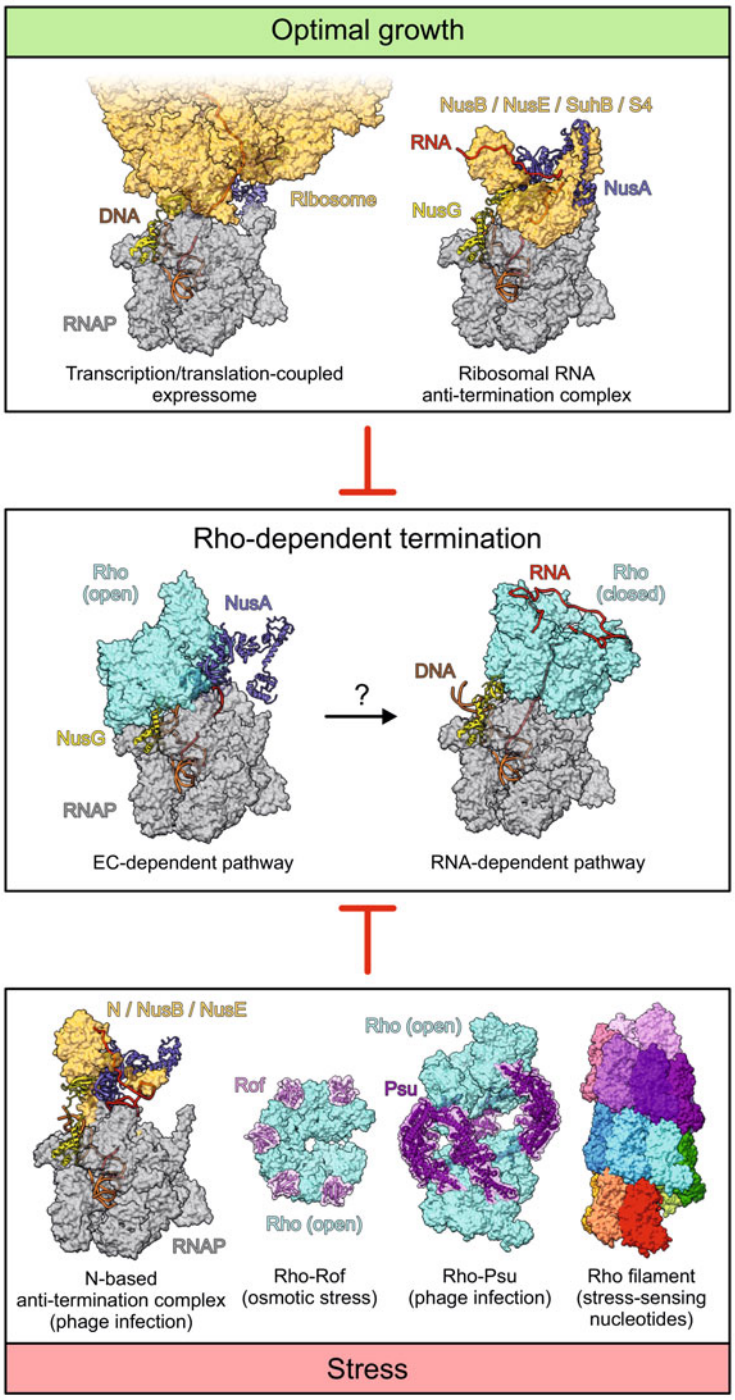

◀ **Fig. 2:** Regulation of Rho activity during optimal growth and stress. **Center**, Rho-mediated transcription termination can proceed *via* two pathways. In an RNA-dependent pathway (right; PDB ID 8E6W/8E6X), Rho in an open conformation employs its primary RNA-binding sites to engage pyrimidine-rich, unstructured regions in the nascent transcript, followed by capture of neighboring RNA regions at the secondary RNA-binding site and ring closure. Powered by ATP hydrolysis, Rho then translocates 5'-to-3' on the transcript. When it catches up with an elongation complex (EC), Rho can extract the nascent RNA or push the EC forward without accompanying nucleotide addition, leading to termination. In an EC-dependent pathway (left; PDB ID 6Z9P), Rho passively traffics on an EC without immediately terminating transcription. Upon EC pausing, Rho, aided by general transcription factors NusA and NusG, can induce inhibitory conformational changes in RNA polymerase (RNAP), resulting in a displacement of the DNA:RNA hybrid, RNAP opening and transcription stalling. Rho and nucleic acids may be released from this moribund state followed by Rho unwinding the DNA:RNA hybrid; alternatively, Rho might engage and translocate the transcript as in the RNA-dependent pathway to ultimately achieve termination ("?"). **Top**, under optimal growth conditions, transcription and translation are tightly coupled, such that a lead ribosome prevents EC attack by Rho (PDB ID 6X7K). A Nus-factor/SuhB/S4-RNA complex on RNAP likewise safeguards production of non-coding RNAs (PDB ID 6TQO). **Bottom**, phages can achieve anti-termination by assembling a protein N-based anti-termination complex (PDB ID 6GOV) or by producing Rho-inhibitory proteins, such as Psu (PDB ID 8PEU). Under other stress conditions, when translation is inefficient, ρ activity can be regulated by cellular proteins such as Rof (PDB ID 8PTM), or *via* an aggregation strategy (PDB ID 8Q3Q).

bridge two open Rho rings, counteracting ring closure and RNA binding to the SBS (**Fig. 2**, bottom). Additionally, each Psu molecule bridges the CTDs of neighboring Rho subunits across the ATP-binding sites, thereby downregulating Rho nucleotide transactions. Surprisingly, Psu also permits stretching of the Rho spiral, allowing additional Rho subunits to join the rings. Thus, apart from inhibiting RNA-SBS interactions and ATPase activity, Psu induces Rho hyper-oligomerization by cross-strutting two open rings and facilitating their expansion to at least the nonamer level, thereby reducing the number of Rho molecules available to assemble into active hexamers.

Reversible aggregation as an emerging principle of Rho regulation

The Psu mechanism of action suggests that modulation of the oligomerization state may be an efficient means to regulate Rho activity. Indeed, *Clostridium botulinum* Rho has been found to assemble into inactive amyloid-like structures [10], while *Bacteroides thetaiotaomicron* Rho can form phase-separated condensates that enhance Rho termination activity [11]. Reversible aggregation or condensation of these Rho orthologs depend on large, intrinsically disordered insertions in their NTDs. We recently found that *E. coli* Rho can also form inactive, higher-order polymers and filaments in response to stress-sensing nucleotides bound in place of ATP (**Fig. 2**, bottom). Thus, protein-dependent or -independent reversible aggregation appears to be another widespread principle to tune Rho activity to enable cellular adaptation.

Fine-tuning Rho activity during stress

Bacteria frequently experience stress and starvation and mount diverse adaptive responses, when Rho activity must be tightly regulated to balance necessary and unwanted transcription. For example, when protein biosynthesis is reduced in response to stress, uncoupled ECs become vulnerable to Rho. Rho must be downregulated to allow expression of genes whose products are still required under these conditions, and upregulated when conditions improve. Proteins produced in response to stress that directly inhibit Rho would provide means to quickly tune its cellular activity. Indeed, YihE is pro-

duced during envelope stress and Hfq modulates responses to diverse stresses. We found that Rof overexpression induces growth defects in *E. coli* lacking RpoS, a master regulator of the general stress response [8], and others showed that Rof inhibits *E. coli* growth under osmotic stress and promotes *Salmonella* pathogenicity [9]. Apparently, bacteria have diverse mechanisms at their disposal to adjust Rho activity to tune termination during both optimal and adverse conditions.

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Corresponding authors:
Prof. Markus C. Wahl, PhD
Institute of Chemistry and Biochemistry
Freie Universität Berlin
Takustraße 6
D-14195 Berlin
markus.wahl@fu-berlin.de

Prof. Irina Artsimovitch, PhD
The Ohio State University
Department of Microbiology
318 West 12th Ave.
Columbus, OH 43210
USA
artsimovitch.1@osu.edu

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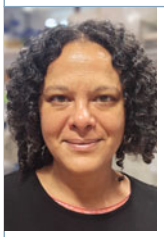
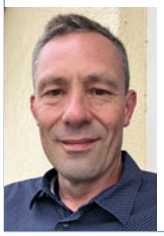


Nelly Said
2002–2008 studies of Biochemistry at FU Berlin. **2011–2016** Doctorate at Freie Universität Berlin under supervision of Prof. Dr. M. Wahl, PhD. Since **2016** Postdoc with Prof. Dr. M. Wahl, PhD at FU Berlin.

Irina Artsimovitch
1984–1989 studies of Biochemistry at Moscow State University, Moscow, Russia. **1992–1996** PhD in Microbiology at The University of Tennessee, USA, mentored by Prof. M. Howe, PhD. **1996–2001** Postdoc with Prof. R. Landick, PhD at the University of Wisconsin, USA,. Since **2001**, Professor of Microbiology at The Ohio State University.



Markus Wahl
1987–1993 studies of Biochemistry. **1993–1996** PhD at The Ohio State University mentored by Prof. M. Sundaralingam, PhD. **1997–2002** Postdoc with Prof. Dr. R. Huber, FRS, Max-Planck-Institute for Biochemistry, Martinsried. **2002–2008** Group Leader, Max-Planck-Institute for Biophysical Chemistry, Göttingen. **2008** Professor for Macromolecular Crystallography, University Medical Center Göttingen. Since **2009**, Professor for Structural Biochemistry, FU Berlin.

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