vastly outnumbered by cells with defective viral genomes, which are mostly irrelevant from the perspective of a cure[14].

Compared with uninfected cells, the rare cells likely to contain an intact HIV genome expressed higher levels of 'immune-checkpoint' molecules, which are negative regulators of T-cell function. This finding confirms and extends results from previous studies that reported high expression of immune-checkpoint molecules in reservoir cells[15–17]; such molecules are known to promote HIV latency[18,19]. Notably, HIV-infected cells also expressed several binding partners (ligands) of the immune-checkpoint molecules, suggesting a mechanism by which HIV-infected cells might dampen the killing capacity of immune cells, thereby providing the reservoir cells with a selective advantage in their ability to evade destruction by the immune system.

In contrast to the case with infected cells in the blood, reservoir cells from lymphoid tissue had only a limited number of the proteins that confer protection against immune-cell-mediated killing. Instead, lymphoid reservoir cells showed features associated with resistance to a type of cell death called apoptosis, and signs of expression of molecules that promote survival. This finding showed that the mechanisms responsible for the evolutionary selection of HIV-infected cells differ between the blood and other tissues.

Using distinct but complementary approaches, these two studies reveal features of HIV-infected cells that allow such cells to persist. Because they measure different aspects, the transcriptomic and phenotypic analyses identified different molecules that contribute to the survival of HIV. Nevertheless, the ways in which these molecules promote HIV persistence largely overlapped, suggesting functional redundancy in the underlying mechanisms (Fig. 1).

In both studies, molecules that contribute to HIV transcriptional silencing were found to be more highly expressed in HIV-infected cells than in their uninfected counterparts. Whether the interaction identified by Sun *et al.* between immune-checkpoint molecules and their ligands leads to the upregulation of factors that silence the expression of HIV sequences, as reported by Clark *et al.*, is not known. Also unknown is whether the increased activity of pro-survival and proliferation signalling pathways in HIV-infected cells, as revealed by the gene-expression analysis, is a result of the engagement of the survival-promoting cell-surface proteins indicated by the phenotypic results. Studies combining RNA and protein analyses will be needed to investigate this possibility.

The characteristics of infected cells that have survived years of ART reveal the pressures to which the reservoir is exposed. Given that reservoir cells often express molecules that protect them from being destroyed by immune cells, this points to a mechanism that might be exploited to accelerate the eradication of HIV. For example, blocking immune-checkpoint ligands would make reservoir cells more sensitive to killing by immune cells.

Both studies reveal that the persistence of HIV-infected cells goes beyond the mechanisms that underpin the exquisite capacity of memory $CD4^+$ T cells to endure and to maintain lifelong immunity. Silencing of HIV genes and escape from immune-system pressure are other skills that the long-lived reservoir must use to survive. Whether all three mechanisms must act in each infected cell to ensure its long-term existence remains unclear.

These findings might suggest that we redirect our efforts to eradicate the reservoir, but at what cost? Although it is too early to tell whether all three mechanisms must be targeted simultaneously to eliminate reservoir cells, any approach that would slightly reduce the reservoir but affect the health of people on stable ART would be unacceptable. Unlike the cellular factors that contribute to the longevity of T-cell immunity, which might be difficult to counteract without compromising immunological memory, the cellular and viral molecules that drive HIV latency and escape from destruction by immune cells might represent more-realistic targets for curative strategies.

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Structural biology

# Views of a debated transcription complex

**Fahad Rashid & James Berger**

High-resolution structures of the bacterial Rho protein in complex with an RNA polymerase enzyme and partner proteins provide support for the long-held model of how Rho helps to terminate gene transcription. **See p.367**

Over time, scientific concepts in textbooks can get enshrined into dogma. Occasionally, however, these assumptions are shaken up when new data arise that challenge those cherished models. In 2020, two cryo-electron microscopy studies[1,2] called into question long-standing assumptions about a key facet of bacterial gene regulation known as transcription termination. Now, Molodtsov *et al.*[3] (page 367) describe structures that restore the classical framework. Their results are an elegant demonstration that science sometimes needs to lurch sideways before it can move forwards.

The story begins with a bacterial protein known as Rho, which was discovered[4] in 1969. Not to be confused with an enzyme family of the same name found in nucleus-bearing (eukaryotic) cells, Rho was found to promote the termination of gene transcription — a crucial step in gene regulation that ensures that messenger RNA transcripts are the appropriate length. Over time, several properties of Rho that support this presumed role have been defined. For instance, Rho acts on a large subset of bacterial genes[5]. It forms six-subunit rings that bind to long stretches (more than 50 nucleotides) of RNA[6]. Its sequence is also remarkably similar to



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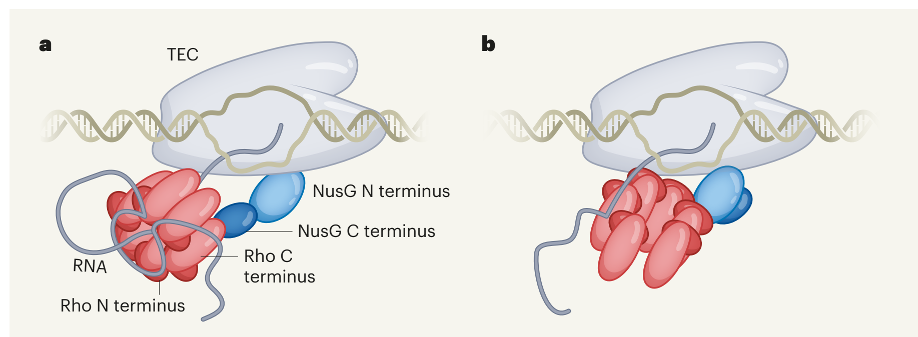


**Figure 1 | New support for an old model. a**, The textbook model for termination of transcription in bacteria indicates that six Rho proteins form a ring-shaped hexamer, which binds to newly transcribed RNA through the protein's amino-terminal (N) region and in the central channel, made up of carboxy-terminal (C) regions. Interaction with the protein NusG helps anchor Rho to an RNA polymerase enzyme, which is part of the transcription elongation complex (TEC) that mediates transcription. Rho then moves along the RNA to separate this nucleic acid from the TEC. **b**, Two studies[1,2] published in 2020 resolved high-resolution structures of the Rho complex and found that it adopted an unexpected configuration that did not fit with the textbook model. The Rho ring was open, RNA did not pass through the central channel, and NusG interacted with unexpected Rho domains. Now, Molodtsov *et al.*[3] describe new structures that support the original model in part **a**.

that of $F_1$ ATP synthase[7,8], a rotary motor protein that produces the energy-carrying molecule ATP. Like that enzyme, Rho seems to use the sequential hydrolysis of ATP molecules around its ring[9] to power movement — in the case of Rho, actively translocating an RNA substrate through its interior to promote the substrate's removal from DNA[10–13].

Observations from dozens of laboratories gradually led to a 'textbook' picture of how Rho functions at the molecular level (Fig. 1a). First, an open-ring form of the protein would bind to an exposed RNA region adjacent to a stalled RNA polymerase (the enzyme responsible for synthesizing RNA chains during transcription). Next, the ring would snap shut around the RNA and move along it to either push RNA polymerase off its DNA template or pull the RNA from the polymerase's active site, terminating RNA synthesis[14]. Although Rho could act alone in this capacity, it could also be assisted by another protein called NusG, which (among its many roles) binds to RNA polymerase and helps recruit Rho to act on non-ideal RNA sequences[15].

The textbook view enjoyed a reasonably quiet acceptance until 2020, when a pair of studies[1,2] emerged suggesting that it might be wrong. The studies reported cryo-electron microscopy structures of Rho in the presence of RNA polymerase, NusG and a small portion of RNA partially bound to a 'melted' DNA structure known as a bubble (which forms during transcription), mimicking a termination state. These structures displayed almost none of the interactions that would be predicted under the conventional model (Fig. 1b). For instance, RNA did not pass through the central pore of the Rho hexamer, nor did it bind to a set of the protein's known RNA-binding domains. Instead, the ring was cracked open, rather than closed. There also did not seem to be a clear role for ATP hydrolysis in powering RNA translocation and NusG did not associate with Rho's carboxy-terminal motor domains, as had been predicted[16]. It seemed the textbooks would need to be rewritten.

But science benefits from debate, and these Rho structures spurred Molodtsov and colleagues to re-examine our understanding of the protein. They first developed long nucleic-acid substrates that (unlike the DNA bubble from the 2020 work) enable full recapitulation of Rho-dependent and NusG-coupled transcription termination in biochemical assays. Next, they used those substrates, along with a non-hydrolysable analogue of ATP, to freeze Rho as it attempts to engage and terminate a stalled RNA polymerase. They then imaged the complex in these frozen states using cryo-electron microscopy.

**"Collectively, the structures convincingly reinstate the textbook view of Rho protein function."**

The outcome was a suite of structures that reprise all the features predicted by the textbook model[17,18]. The structures show interactions between Rho and RNA, and between Rho and NusG, that had been seen in previous structures[16] captured in the absence of RNA polymerase. They also confirm that the Rho ring is closed and in a nucleotide-bound state that is consistent with a mechanism involving sequential ATP hydrolysis[11,13,16].

Molodtsov and colleagues' structures also yield fresh and unexpected mechanistic insights into Rho-mediated transcription termination. For instance, they show that NusG not only functions as a recruitment factor for Rho but also, together with two regions on RNA polymerase, acts as a stator (the stationary part of a rotary motor). This role for NusG echoes that of the $b_2\delta$ 'stator stalk'[19], which holds the $F_1$ ATPase ring in place while the motor cycles to create ATP.

Collectively, the structures convincingly reinstate the textbook view of Rho function. But what should researchers make of the structures from the 2020 studies? Do those images represent off-pathway intermediates, early stages of the complex that form before termination, or states that have yet to have a cellular function ascribed to them? The answer is unclear, but a paper published on the preprint server bioRxiv indicates that both Molodtsov and colleagues' configuration and those reported in 2020 can simultaneously exist during complex formation[20]. This observation, along with the extensive protein–protein interactions seen in the 2020 structures, suggest that there is still much to learn about how transcription termination is regulated.

Fortunately, future efforts will now be able to take advantage of the molecular insights provided by Molodtsov and co-workers' study to devise targeted experiments that resolve the debate. Although it's too soon to know for certain, it would not be a bad bet to expect that nature has more surprises in store.

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