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RNA polymerase's relationship with the ribosome: Not so physical, most of the time

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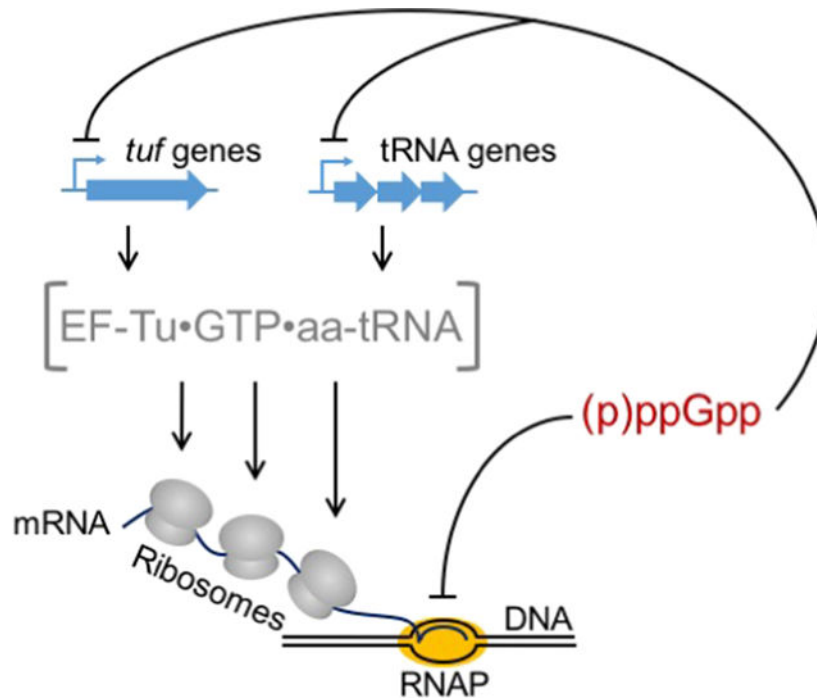
Abstract

In bacteria, the rates of transcription elongation and translation elongation are coordinated, changing together in response to growth conditions. It has been proposed that this is due to physical coupling of RNA polymerase and the lead ribosome on nascent mRNA, an interaction important for preventing premature transcription termination by Rho factor. Recent studies challenge this view and provide evidence that coordination is indirect, mediated in *E. coli* by the alarmone (p)ppGpp. Here, we discuss these new findings and how they shape our understanding of the functional relationship between RNA polymerase and the ribosome as well as the basis of transcriptional polarity.

Graphical abstract

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Keywords

transcription; translation; ppGpp; Rho; premature transcription termination

Transcription and translation take place together in the cytoplasm in Bacteria and Archaea [1]. It has been known for decades that translating ribosomes can functionally impact RNA polymerase (RNAP), exemplified by the phenomena of transcriptional polarity and transcription attenuation. In the 1960s, investigators noticed that nonsense or frameshift mutations in the *lacZ* gene abolished synthesis of LacY and LacA, encoded downstream in the same operon [2–4]. This resulted from an absence of downstream mRNA, due to premature transcription termination (PTT) by Rho [5]. This phenomenon, known as transcriptional polarity, was later reported for the *trp* operon [6], the *ilv* operon [7], and the *his* operon [8], and hence seemed to be general. In the 1970s, it was found that ribosomes can influence RNAP in another way, a phenomenon termed transcription attenuation. In the *trp* operon, the leader region contains a small open reading frame with tandem tryptophan codons upstream of a transcription terminator. Stalling of the ribosome at these codons due to low levels of Trp-tRNA^{Trp} promotes formation of an anti-terminator structure and continuation of transcription of the entire operon [9–13]. Attenuation is a well-known mechanism to regulate the biosynthesis of amino acids and other metabolites in bacteria [14–17].

Vogel and Jensen (1994) first showed that rates of polypeptide and mRNA chain elongation are normally correlated [18]. Using the *lacZ* gene, they measured the appearance of full-length mRNA and protein products upon isopropyl-β-d-thiogalactopyranoside (IPTG) induction, allowing calculation of chain elongation rates for both transcription and

translation. Cells growing rapidly exhibited the highest rates of chain elongation, while cells growing at progressively slower rates showed reductions in transcription and translation elongation rates, always of equivalent magnitude. Such coordination of chain elongation rates implies an important functional purpose.

A model of RNAP-ribosome coupling

In 2010, Nudler and coworkers confirmed that transcription and translation of *lacZ* are tightly coordinated under different growth conditions. They also provided compelling evidence that slowing the ribosome, by using a mutation in *rpsL* (encoding ribosomal protein S12) or the antibiotic chloramphenicol (Cm, which inhibits peptidyl transfer), also slows RNAP to the same degree. The authors proposed that by “pushing” the RNAP forward and preventing it from backtracking, the lead ribosome can control the rate of transcription [19]. In other words, the two macromolecules work like coupled train locomotives, each being able to influence the other [20, 21]. Concurrent work by Gottesman and coworkers showed that the transcription factor NusG can interact with both RNAP and ribosomal protein S10 (also known as NusE) [22]. It was envisaged that NusG acts as the molecular coupler, physically linking RNAP to the lead ribosome [23–25].

A number of groups have since explored the idea of physical coupling between RNAP and the ribosome, using cryo-electron microscopy (cryo-EM) and biochemical approaches. In 2017, the structure of an RNAP-ribosome complex formed in an *in vitro* transcription-translation system, termed the “expressome”, was solved by cryo-EM [26]. In this structure, the mRNA exit tunnel of RNAP docks right onto the mRNA entry tunnel of the 30S subunit of the ribosome, resulting in seamless protection of the mRNA. In independent work, the structure of RNAP bound to the small subunit (30S) of the ribosome was solved by cryo-EM [27]. In this structure, the mRNA exit region of RNAP is near the mRNA exit tunnel of the 30S subunit, a conformation at odds with the “expressome” structure and difficult to rationalize functionally. Recent biochemical studies showed that RNAP core enzyme is capable of interacting with the 30S subunit, the 50S subunit, and the 70S ribosome, all with a similar affinity [28]. These structural and biochemical data are puzzling and lend little congruent support for the coupling model.

There are other caveats to the physical coupling model. First, it has been shown that the elongation rate of ribosomal RNA (rRNA) transcription also varies as a function of growth rate, in a similar manner as mRNA transcription [18]. By necessity, rRNA synthesis rate itself cannot depend on translating ribosomes. Second, S10 is known to play moonlighting roles off the ribosome [29, 30], for example as part of the λ N and *tmn* antitermination complexes, which also include NusA, NusB and NusG [31–33]. Whether the role of S10 in transcription processivity involves the ribosome or another S10-containing complex is difficult to address and remains unclear.

Evidence that coordination of transcription and translation is indirect

Chen and Fredrick (2018) tested the idea that RNAP is normally coupled to the lead ribosome on the nascent mRNA chain [34]. They reasoned that if this is true, the ratio of

corresponding decreases in transcription elongation speed [36]. Because (p)ppGpp levels also control production of EFTu•GTP•aa-tRNA ternary complexes and translation elongation speed depends on ternary complex concentration [37, 45, 46], it was suggested that (p)ppGpp functions as the global coordinator, modulating transcription and translation speeds simultaneously [36].

Transcriptional polarity results from artificial disruption of transcription-translation coordination

Hwa and coworkers also described transcriptional polarity in quantitative terms [36]. As mentioned above, introduction of a nonsense mutation in *lacZ* reduced its transcript levels markedly. A graded reduction of mRNA as a function of gene position was observed, consistent with premature transcription termination (PTT). This was largely eliminated by bicyclomycin (a Rho inhibitor), demonstrating that the PTT observed was mediated by Rho. Similarly, translation inhibitors FA and Cm both caused obvious PTT, even though Cm did not affect measured translation elongation rates. In these cases, PTT was less pronounced than in the case of the nonsense mutation. The authors suggest that in contrast to FA, which slows translocation of all ribosomes, Cm gains access to only a subset of ribosomes but fully stalls their progress. Such distinct modes of inhibition can explain the *in vivo* effects of the two antibiotics [36]. Importantly, PTT was only observed under situations when translation is artificially inhibited, for example by nonsense mutation or antibiotic treatment. No PTT was seen when nutrient limitation is used to slow translation [36].

Ribosome traffic and its role in transcription processivity

It has been suggested that physical coupling between RNAP and the lead ribosome normally prevents PTT [47–49]. But, as detailed above, a growing body of evidence argues against any stable linkage between RNAP and the lead ribosome. And yet, ribosome traffic can clearly influence RNAP processivity. How can these observations be explained? Fig. 2 depicts the fate of a paused transcription elongation complex under three different conditions. When ribosome traffic on a natural mRNA is high (Fig. 2A), due to high translation initiation rate, there is a large probability that RNAP will escape the pause, either spontaneously or in a ribosome-assisted manner. Ribosome traffic will occlude Rho, even when Rho-utilization (*rut*) sites are present, and the lead ribosome may push RNAP forward (or inhibit backtracking). When translation of the same mRNA is artificially inhibited, for example due to introduction of a nonsense mutation (Fig. 2B), Rho will have open access to the *rut*-containing nascent chain and PTT will likely occur. Importantly, this differs from the case of a natural mRNA which normally exhibits light ribosome traffic, due to low translation initiation rate (Fig. 2C). Even though light ribosome traffic may be sufficient to largely inhibit Rho [50, 51], other determinants are likely at play. These mRNAs may have idiosyncratically evolved to become Rho-resistant, for example via increased structure and the lack of *rut* sites. Consistent with this possibility, coding regions of polycistronic mRNAs fold independently, and the degree of structure in these ORF-centric domains is inversely correlated with translation efficiency [52].

Why are transcription and translation elongation rates coordinated?

The fact that transcription elongation is at least as fast as translation elongation makes sense. This prevents excessive queuing of ribosomes, which would effectively limit translational control. But, why does transcription elongation speed match rather than exceed translation elongation speed? We suspect that, fundamentally, this is driven by the economics of cell growth. In coordinating chain elongation rates, the cell makes a given mRNA no faster than it can be translated—in other words, with optimal efficiency. Synthesizing even part of an mRNA before it can be used provides no obvious benefit, only potential costs. Regulatory mechanisms, such as Rho-dependent PTT and transcriptional attenuation, certainly rely on the coordination between RNAP and the ribosome. But such regulatory mechanisms may have evolved later to sense transcription-translation coordination, which was already in place. Notably, almost all studies that have looked at transcription-translation coordination have used the model organism *E. coli*. Comparative studies with other bacteria may provide new mechanistic insight and evolutionary perspective on this important aspect of gene expression.

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Highlights

- Rates of transcription elongation and translation elongation normally match one another, and the basis of this coordination has remained unclear.
- Recent evidence suggests that RNA polymerase and the lead ribosome move independently, and coordination is mediated by the alarmone (p)ppGpp.
- Transcription is normally processive; premature transcription termination by Rho occurs when ribosome traffic is eliminated via nonsense mutation or antibiotic treatment.

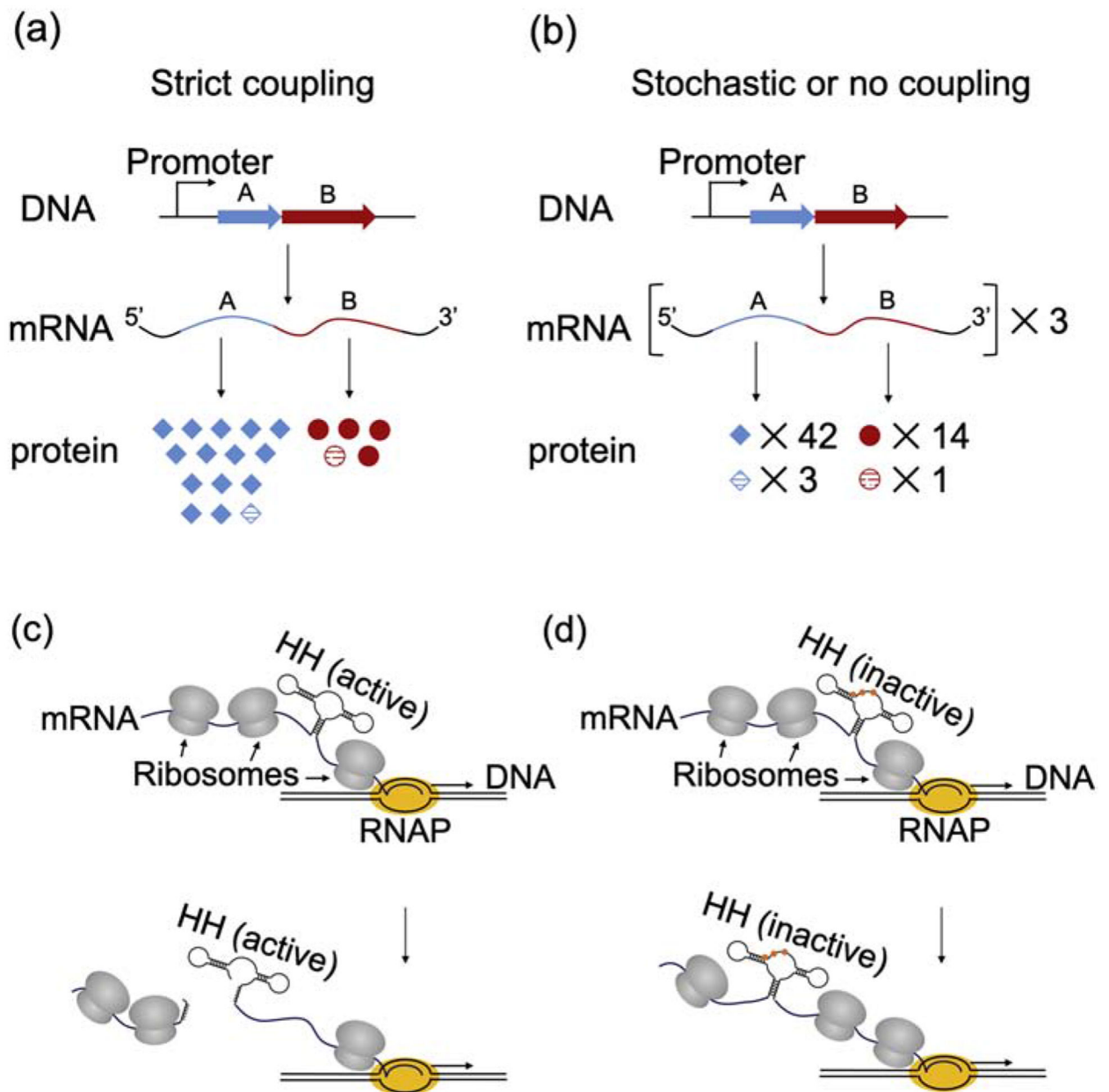


Fig. 1. Rationale and approach of Chen and Fredrick (2018). (A-B) Hypothetical protein products of strict versus stochastic coupling. (A) A scenario in which RNAP and the lead ribosome are strictly coupled. Genes A and B are co-transcribed, resulting in stoichiometric levels of A and B mRNA (1:1). The first round of translation by the RNAP-coupled ribosome generates equivalent amounts of proteins A and B (1:1, striped symbols), whereas multiple-round translation yields different amounts of total protein (3:1, all symbols). (B) A scenario in which transcription and translation are uncoupled or stochastically coupled. In this case, RNAP has effectively no impact on the lead ribosome, and hence the ratio of protein products made during transcription (3:1, striped symbols) matches that of multiple-round translation (3:1, all symbols). (C-D) A hammerhead ribozyme enables direct comparison of

'single' - (co-transcriptional) versus multiple-round translation. (C) The active hammerhead (HH) quickly self-cleaves after being made, so only a ribosome tailgating RNAP will produce full-length LacZ. (D) Three point mutations (colored dots) inactivate the hammerhead without altering the encoded polypeptide, enabling multiple-round translation to be measured.

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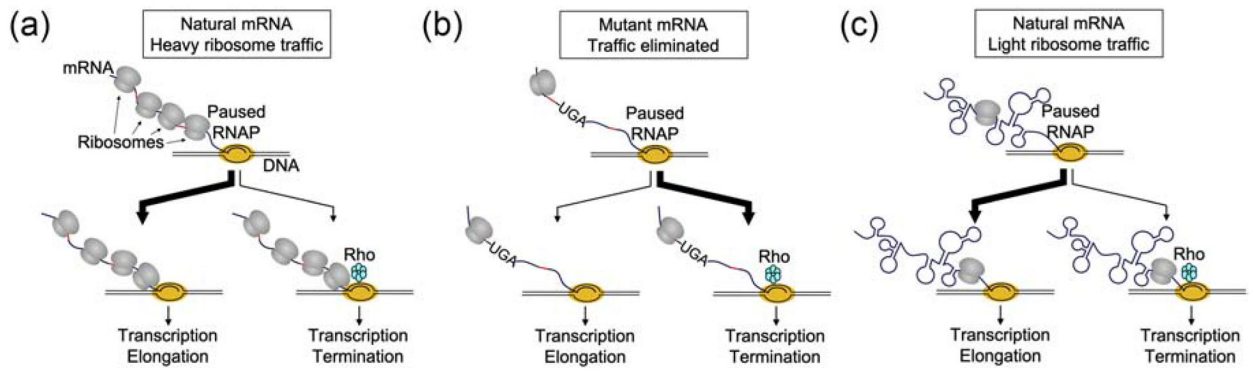


Fig. 2.

Premature transcription termination (PTT) depends on various factors. (A) When RNAP pauses during transcription of a natural mRNA that is highly translated, ribosomes occlude Rho from nascent chain *rut* sites (red) and may facilitate pause escape by “nudging” RNAP forward. Hence, the chances of Rho-dependent PTT are low. (B) When the same gene depicted in panel A contains a nonsense mutation, ribosome traffic is eliminated. This gives Rho access to *rut* sites (red) and PTT becomes favorable. (C) When RNAP pauses during transcription of a natural mRNA that is normally translated with a low initiation rate, other mechanisms must be employed to prevent Rho-dependent PTT. These likely include RNA structures and omission of *rut* sites.