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Point of View

Traffic patrol in the transcription of ribosomal RNA

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Abbreviations: RNAP, RNA polymerase; Pol I, RNA polymerase I; AT, antitermination

Key words: rRNA, transcription, RNA polymerase, transcript elongation, antitermination, growth rate

Synthesis of ribosomal RNA (rRNA) is essential for fast cell growth and rRNA transcription is typically characterized by dense traffic of RNA polymerases along the rRNA genes. However, dense traffic is susceptible to traffic jams which may arise inevitably due to stochastic pausing of the polymerases. Based on recent theoretical and experimental results, we suggest that the "traffic viewpoint" provides a unique perspective towards understanding the control of ribosome synthesis in both bacterial and eukaryotic cells.

Cell growth is intimately linked to ribosome biogenesis, as ribosomes synthesize proteins which account for a large fraction of the cell's biomass. The cellular abundance of ribosomes is adjusted according to the growth state of the cell, and fast cell growth is in general accompanied by strongly increased rates of ribosome synthesis. The importance of ribosome synthesis is most clearly seen for bacteria undergoing rapid exponential growth, where the ribosome concentration is observed to increase linearly with the growth rate.[1-4] In *E. coli* cells growing with a doubling time of 24 min, synthesis of ribosomal RNA (rRNA) accounts for 73% of the total cellular RNA synthesis and engages 68% of all actively transcribing RNA polymerases (RNAPs).[4,5] Likewise, rRNA accounts for 60% of all cellular transcription in fast growing yeast[6] and 35% of all transcription in proliferating mammalian cells.[7] The ribosome content has also been found to be proportional to the growth rate in exponential growth of yeast[8,9] and several other eukaryotic microbes (reviewed in ref. 10). Furthermore, ribosome synthesis is strongly increased upon stimulation of growth by the addition of nutrients or growth factors in bacteria, yeast and mammalian cells.[3,7,9]

Transcription of rRNA plays a central role in ribosome synthesis as it is the major target of regulation. In bacteria, ribosome synthesis is controlled at the level of rRNA transcription in response to the cellular growth status and to nutrient availability,[4,11,12] while the synthesis of ribosomal proteins is adjusted to rRNA synthesis by translational autoregulation.[13] In eukaryotic cells, the synthesis of ribosomal proteins is controlled more directly, but also here transcription of rRNA is the target of numerous control mechanisms (reviewed in ref. 7) and a recent study in yeast suggests that it may be the main target of control, as the deregulation of rRNA synthesis specifically de-represses the synthesis of ribosomal proteins.[14]

Furthermore, transcription of rRNA represents a major bottleneck in the synthesis of ribosomes: The synthesis of ribosomal proteins as well as all other proteins is subject to amplification at the translation level. Every mRNA transcript gives rise to multiple copies of the protein it encodes. In bacteria this amplification can be anywhere between a few-fold and a hundred-fold.[4] For rRNA, which is not translated, there is no such amplification; so for every ribosome the cell produces, it has to make one transcript of the rRNA genes. Together with the huge demand for ribosomes in fast growing cells, this implies that large rates of transcription of rRNA are required at fast growth. As mentioned above, rRNA accounts for 73% of total RNA synthesis in *E. coli* cells growing with a doubling time of 24 min and occupies 68% of all actively transcribing RNAPs (or ~20% of the total cellular RNAPs).[4,5] High rRNA transcription rates are achieved by a combination of multiple copies of the rRNA genes per genome and high transcription rates per gene. There are seven copies of the rRNA genes in the *E. coli* genome. These are located close to the origin of replication, so that the gene copy number per cell can be much higher at fast growth (e.g., 35 at 24 min doubling time) due to overlapping rounds of DNA replication. Additionally the transcription rate per gene, ~70 transcripts/min,[4] is much higher than transcription rates for protein-encoding genes, which are typically only a few transcripts/min.[15,16] The high transcription rate is reflected in the unusually dense packing of RNAPs on rRNA genes as visualized by electron microscopy (Miller spreads, see Figure 1A)[17,18] or by chromatin immunoprecipitation[19] with up to one RNAP (which itself spans ~50 nt during elongation) every ~80–100 nt.[17,18] Likewise, there are hundreds of copies of rRNA genes in eukaryotic genomes, and transcription of these genes is also characterized by high densities of RNAPs (RNA polymerase I, Pol I).[7]

As the dense traffic of RNAPs on rRNA genes is unusual compared to other genes, it is important to consider specific

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physical constraints on rRNA transcription due to the dense traffic. In particular, the high density of elongating RNAPs suggests the possibility that the transcription rate may not be limited by transcript initiation, but by elongation, i.e., by the flow of the traffic of RNAPs along the gene and by 'traffic jams' that may arise from the high density of RNAPs. In a recent theoretical study, we have explored the effects of the elongation dynamics of RNAPs (pausing, premature termination, antitermination) on the transcription rate of rRNA in *E. coli*,[20] based on experimental data for the dynamics of individual and multiple RNA polymerase as measured in vitro and in vivo. One result of this study was that the high transcription rates of rRNA genes that are physiologically required for fast growth (~70 min^{-1}) are only possible in the presence of the ribosomal antitermination (AT) system, which is known to modify the complexes transcribing rRNA and speed up their elongation rate by about two-fold, from ~40–50 nt/s in its absence to ~85–90 nt/s in its presence.[21] Without the AT system, the transcription rate would be limited by transcript elongation to ~40 min^{-1}, which would not be able to provide the number of rRNA transcripts needed at fast growth, because the traffic flow of RNAPs is limited by traffic jams that arise from stochastic pausing of RNAPs[22] during transcription (Figure 1B). We therefore proposed that one function of the ribosomal AT complex is to enable the high transcription rates necessary to maintain the large ribosome content of fast growing cells by preventing these traffic jams through a reduction of transcriptional pausing. Indeed, AT is generally believed to speed up elongation by reducing pausing of RNAP;[23] this idea is quantitatively validated by our calculations.[20]

The nominal function of *E. coli*'s ribosomal AT complex is however to prevent premature termination by the termination factor Rho.[23] That the termination factor would play any role in a process requiring rapid transcription is itself intriguing, and we examined this role from the perspective of RNAP traffic. One clue for us is the observation that antitermination is never 100 percent efficient; some RNAPs fail to assemble a functional AT complex and therefore transcribe more slowly than the antiterminated RNAPs. Given a dense traffic of RNAPs on rRNA genes, these slow RNAPs represent obstacle and results of our computational model suggests that even a small fraction of slow RNAPs can severely reduce the transcription rate by jamming other RNAPs behind.[20] As only slow RNAPs are susceptible to premature termination by Rho, we suggest that Rho can function as a correction mechanism that removes the slow RNAPs to restore the traffic flow (Figure 2). We thus predict that premature termination does not attenuate the transcription rate, but rather increases it by restoring the traffic flow of RNAPs.[20] Comensurate with our picture, data from various AT mutants suggest that sites where Rho loads to the transcript are found immediately downstream of the loading sequences for the AT complex on both the 16S and 23S genes (reviewed in ref. 20), as required for a correction mechanism. Furthermore, rRNA transcription is reduced in Rho mutants (Adhya S, personal communication), consistent with our traffic picture. The 'traffic view' of rRNA transcription thus suggests novel functions for both termination and antitermination in rRNA transcription.

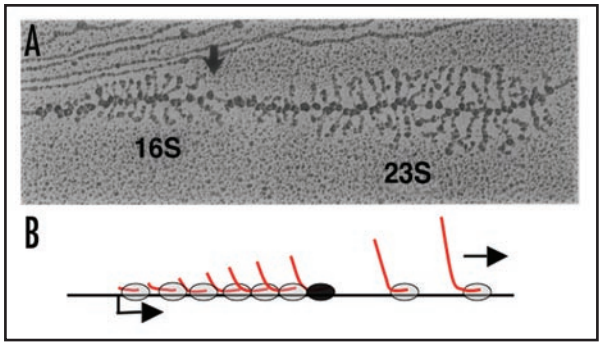

Figure 1. Dense traffic of RNAPs on rRNA genes. (A) Electron micrograph of an rRNA genes (16S and 23S) of *E. coli* growing on rich medium (2.4 doublings per hour); reprinted from ref.17 with permission from American Society for Microbiology. The image shows dense packing of RNAPs on the rRNA genes as well as co-transcriptional processing of transcript. (B) Pausing of an RNAP (black) in dense RNAP traffic induces a 'traffic jam' and slows down trailing RNAPs. If transcription is elongation-limited, these jamming events limit the rate at which the promoter is cleared and can severely reduce the overall transcription rate.

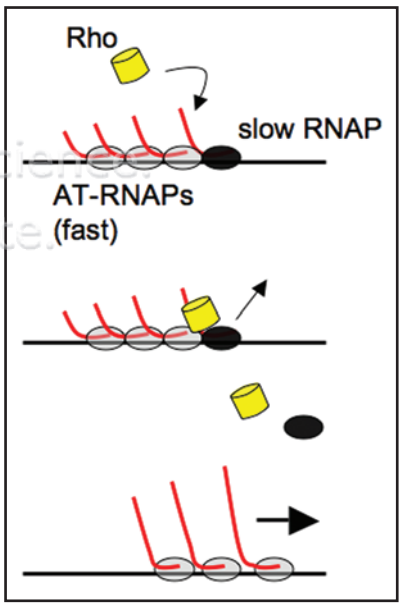

Figure 2. Termination by the Rho protein can function as a correction mechanism for imperfect antitermination: RNAPs that failed to assemble a functional antitermination complex are slow and cause jamming of the faster antiterminated RNAPs. Rho can terminate the slow RNAPs without an antitermination complex, but not the fast antiterminated RNAPs. Premature termination of transcription by a slow RNAP restores the traffic flow of RNAPs along the gene.

It would be interesting to know whether the modulation of RNAP elongation speed is used by the cell to regulate the transcription of the rRNA genes. To our knowledge there is no clear evidence that bacteria make use of this possibility and it is possible that the unavoidable coupling between elongation and (premature) termination impedes the use of elongation-based control. The only case where a modulation of the elongation speed has been observed in bacteria is in an *E. coli* strain with reduced

number of *rrn* operons. This strain had only a very weak growth defect in rich medium and surprisingly exhibited increased elongation speed for rRNA (135 nt/s instead of ~90 nt/s), but not mRNA.[18] While this observation is consistent with the idea of an elongation-limited transcription rate, the mechanism underlying this effect is unknown, and it is also not clear whether this effect is relevant for any physiological situation. We hypothesize that it might be relevant in transitions from slow to fast growth or during outgrowth from stationary phase, when the DNA replication and thus the gene copy number has not yet adapted to the fast growth conditions, but this has not been tested so far.

Given that a high density of RNAPs (Pol I) is also typical for rRNA genes in growing eukaryotic cells, a 'traffic viewpoint' may also be useful outside the bacterial realm, even though molecularly, bacterial and eukaryotic systems are quite different. Indeed, a recent study in mammalian cells (mouse and human) demonstrated that rRNA transcription is regulated at the transcript elongation stage:[24] stimulation by growth factors increased the elongation speed ~5-fold. At the same time, the Pol I density remained essentially unchanged and the transcription rate also increased ~5-fold. These observations indicate that transcription is elongation-limited and that the modulation of the elongation speed is used to regulate the transcription rate. The elongation speed is however not modulated by a modification of the RNAP as for the bacterial antitermination system, but through phosphorylation of UBF, a component of rRNA gene chromatin.[7,24]

Finally, in bacterial and eukaryotic cells, the elongation speed of rRNA is also important for other aspects of ribosome synthesis such as rRNA processing and assembly of ribosomal proteins on the rRNA, which happen co-transcriptionally.[25,26] It is tempting to speculate that the coupling between these processes and elongation functions in both directions. This speculation would raise the intriguing possibility of another layer of feedback of downstream processes in ribosome assembly onto the rRNA transcription rate via elongation control.

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