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LOCALIZATION OF THE BACTERIAL RNA INFRASTRUCTURE

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Abstract

The bacterial RNA network includes most of the same components found in eukaryotes, and many of the interactions that underlie transcription, RNA processing and stability, translation, and protein secretion are conserved. The major difference is that all of these functions take place in a single cellular compartment. In spite of the absence of membrane-bound organelles, or in some cases because of it, key components of the RNA network are localized to specific subcellular spaces or structures to ensure proper processing and regulation. This chapter focuses on what is known about subcellular localization of the bacterial RNA network and what insights localization provides to regulation of the RNA infrastructure of the cell.

INTRODUCTION

In bacteria, as in eukaryotes, most of the RNA infrastructure is dedicated to gene expression and the majority of transcripts are rRNA, tRNA, and mRNA. Although there is little processing of mRNA in bacteria, rRNA and tRNA are produced as longer transcripts that are processed by a variety of endo- and exonucleases. Other bacterial RNAs, such as sRNA, 6S RNA, CRISPR RNA, and tmRNA, are also involved in regulating transcription or translation. Although the enzymatic processes of transcription and translation are the same in bacteria and eukaryotes, the spatial distribution of these processes is very different. In bacteria, transcription and translation both occur in the cytoplasm, and ribosomes typically initiate translation of an mRNA before it is completely transcribed. Therefore, it would not be surprising if the RNA infrastructure was more or less uniformly distributed throughout the bacterial cell. In fact, some components of the gene expression machinery, including ribosomes and RNA polymerase, are widely dispersed. However, some key factors, including the major RNA degradation machinery and translation factors, are localized in cytoskeletal structures, suggesting that the RNA infrastructure and bacterial cell architecture are intimately entwined (Fig. 1). This chapter will describe what is known about localization of the bacterial RNA infrastructure and speculate on the roles localization plays in regulating key RNA processes.

Bacteria are small compared to eukaryotic cells, and their size imposes restrictions on the extent of localization that is possible. Some eukaryotic RNA infrastructure components are localized to regions such as the nucleolus, which are approximately the same size as an entire bacterial cell. In contrast, the bacterial nucleoid occupies most of the cytoplasmic space, and most bacteria have no membrane-bound organelles. Nevertheless, bacteria do localize multicomponent structures and individual enzymes to specific subcellular

addresses.^{1–3} In addition, bacteria have cytoskeletal structures composed of homologs of actin, tubulin, intermediate filaments, and some unique bacterial cytoskeletal proteins.^{2–4} It is not yet known how many functions the bacterial cytoskeleton shares with its eukaryotic counterpart, but it is clear that there is extensive subcellular organization in bacterial cells. RNA localization experiments have been performed predominantly in three bacterial model systems: the Gram-negative species *Escherichia coli*, the Gram-positive species *Bacillus subtilis*, and *Caulobacter crescentus*, a Gram-negative bacterium with an asymmetric cell morphology that facilitates localization studies. As described below, RNA infrastructure components in bacteria are frequently associated with the cytoskeleton or form cytoskeletal structures themselves.

LOCALIZED TRANSCRIPTION AND mRNA

Localization studies of RNA polymerase in *E. coli* and *B. subtilis* indicate that it is found throughout the nucleoid, suggesting that transcription activity is widely dispersed.^{5,6} However, most transcripts that have been observed do not diffuse freely. Golding, et al., used a transcript containing repeated MS2-binding aptamer sequences and an MS2-GFP fusion protein to observe the spatial dynamics of single RNAs in live *E. coli* cells.⁷ They identified three patterns of RNA localization and movement. Most of the transcripts did not move far during the time of the experiment, but moved randomly in a restricted spot, typically near the midpoint or quarter-points of the cell. These positions are significant because the mRNA was expressed from an F plasmid known to be concentrated in a similar pattern, suggesting that the mRNA is not moving far from its cognate gene.⁷ A minority of transcripts diffused throughout the cell, and others moved as chains.⁷ Subsequent imaging of individual mRNAs and their cognate genes in fixed *E. coli* and *Caulobacter crescentus* cells using fluorescence in situ hybridization (FISH) confirmed that the majority of each mRNA is concentrated in one spot near the site of transcription, consistent with limited diffusion.⁸ It has been suggested that mRNAs may be anchored near the site of their transcription by some unknown process.⁸ However, the simplest model to explain these data is that low-mobility mRNA is still tethered to the DNA by RNA polymerase, and the mRNAs diffusing throughout the cytoplasm are completed transcripts. The diffused transcripts are likely to be difficult to observe by FISH, but are evident in live cell studies. This model is attractive because many RNAs must have considerable mobility after transcription. For example, tRNAs, rRNAs, and regulatory RNAs are active at many locations in the cell so they must leave the site of their transcription. In addition, many secreted proteins are transported as they are being translated, so the mRNAs encoding these proteins must be able to move to the membrane. Nevertheless, many mRNAs may be localized near the site of their transcription for a significant portion of their lifetime.

Even though fluorescence experiments indicate that RNA polymerase can be found throughout the nucleoid, some or all of the RNA polymerase may be associated with the cytoskeleton. Experiments using *E. coli* proteins showed that RNA polymerase binds the cytoskeletal protein MreB, both in vitro and in vivo.⁹ MreB is an actin homolog, which polymerizes into filaments that associate with the cytoplasmic membrane and span the length of the cell.^{10–12} MreB is found in many species, but it is not yet known if the association with RNA polymerase is conserved. The interaction between MreB and RNA

polymerase in *E. coli* is intriguing, because both MreB and RNA polymerase are required for correct chromosome segregation. It has been proposed that the force generated from transcription by RNA polymerase anchored to the MreB structure could push newly replicated chromosomes into different daughter cells.⁹ If this model is correct, localized transcription would impact DNA segregation as well as protein synthesis, and would play a crucial role in cell biology.

SPATIAL CONTROL OF RNA DEGRADATION AND PROCESSING

Much of the RNA processing and degradation activities in bacteria are contained in a single complex, the degradosome, which is an integral part of the cytoskeleton (Fig. 2). The degradosome contains RNase E, the RhlB RNA helicase, polynucleotide phosphorylase (PNPase), and enolase.¹³ This complex is responsible for initiating most of the mRNA degradation in bacteria, as well as participating in maturation of tRNA, rRNA, tmRNA, and RNase P RNA.^{13,14} Electron microscopy studies initially showed that RNase E is concentrated near the membrane in *E. coli*,¹⁵ and subsequent experiments with fluorescent protein fusions to RNase E and other degradosome components showed the characteristic pattern of regularly spaced spots or bands indicating a helical structure.^{16,17} The degradosome filaments resemble structures formed by MreB and MinD, a cytoskeletal component in *E. coli*. However, studies with MinD mutants and inhibitors of MreB polymerization indicate that the degradosome structure forms independently of these cytoskeletal elements.^{16,17} A single domain of RNase E, distinct from the catalytic site and the sequences that bind other degradosome components, inserts in the cytoplasmic membrane.¹⁸ Deletion of this domain from RNase E eliminates localization of the degradosome and has severe physiological consequences, discussed below.^{17,19} RhlB localizes in a helical pattern independently of RNase E, suggesting that it also contains a membrane targeting sequence or that it interacts with other cytoskeletal components as well as the degradosome.^{16,17} PNPase is localized through interactions with RNase E and RhlB, and enolase is localized by binding RNase E.^{16,17}

The cytoskeletal structure formed by the degradosome is critical for normal cellular physiology. Cells in which degradosomes are not localized due to a deletion of the membrane targeting sequence of RNase E have severe defects in growth rate, cell division, chromosome segregation, cell morphology, and autoregulation of RNase E expression.^{16,17} Although there are several possible explanations for these phenotypes, the most attractive hypothesis is that degradosome localization to the cytoskeleton is required for normal mRNA degradation and RNA processing activities, and disrupting these processes causes pleiotropic effects.^{16,17,19} Cytoskeletal localization of the degradosome might provide a site for mRNA destruction to facilitate removal of particular mRNA species. In fact, over-expressed transcripts localize in a pattern very similar to that observed for the degradosome.²⁰ A second, nonexclusive explanation would be to sequester the nuclease activity from the site of gene expression to limit unwanted mRNA turnover. The helical structure would then provide a supply of degradosome activity that is not too distant from any point in the cell, but is nonetheless confined from free diffusion. Alternatively, the structure could be for storage of excess degradosome complexes, or could be important for RNA processing but not mRNA degradation.

Other mRNA degradation and RNA processing activities are also localized. Poly(A) polymerase, RNase III and RNase P are localized near the membrane in *E. coli*,^{21,22} and RNase R is localized in a helix-like structure in *C. crescentus*.²³ The importance of localizing these enzymes is not known, but it appears that most ribonucleases are not found distributed throughout the cell and may be sequestered from nontarget RNAs.

EF-Tu IS A COMPONENT OF THE CYTOSKELETON

Translation and transcription occur simultaneously in the bacterial cytoplasm, and there have been hints that translation may be spatially organized. Fusions of fluorescent proteins to ribosomal proteins L1, S2 and L7/L12 accumulate preferentially at the cell poles and near the membrane in *E. coli* and *B. subtilis*.^{5,6,24} However, it is not known what percentage of the fusion proteins are incorporated into ribosomes and how many of the ribosomes are engaged in active translation, so interpretation of these results is complicated. Cryoelectron tomography studies indicate that 70S ribosomes are distributed throughout the cytoplasm, with 15% near the membrane.^{4,25} Therefore, it is likely that translation is widely dispersed in the cell.

On the other hand, EF-Tu, a key translation component and RNA-binding protein is clearly part of the bacterial cytoskeleton. EF-Tu polymerizes and colocalizes with MreB in helical filaments.²⁶ Several results indicate that MreB and EF-Tu form a complex that has a function distinct from protein synthesis.²⁶ The concentration of EF-Tu is much higher than that required for translation, and partial depletion of EF-Tu causes mislocalization of MreB, but does not affect protein synthesis. Conversely, in the absence of MreB, EF-Tu is not localized but translation is not affected. Finally, EF-Tu and MreB bind in vitro and in vivo, and EF-Tu alters the curvature of MreB filaments. Because MreB filaments are dynamic, forming and depolymerizing within the cell, but EF-Tu structures are static, it has been proposed the EF-Tu forms the tracks that control placement of MreB filaments.²⁶ Although the role of EF-Tu in the cytoskeleton appears to be distinct from its interactions with the ribosome and tRNAs, it does suggest an ancient connection between the RNA infrastructure and the cytoskeleton. Consistent with this idea, eEF1A, the eukaryotic homolog of EF-Tu, binds to actin in eukaryotic cells.^{27,28}

LOCALIZATION OF *TRANS*-TRANSLATION COMPONENTS

The tmRNA-SmpB complex is also localized to helices in the cell. tmRNA is a specialized RNA that has properties of both a tRNA and an mRNA.^{29,30} The tRNA like structure is aminoacylated by alanine-tRNA synthetase, and is bound by SmpB and EF-Tu. A distinct part of tmRNA contains an open reading frame that encodes a peptide with multiple proteolytic determinants. The tmRNA-SmpB complex can enter translating ribosomes that have stalled near the end of the mRNA and initiate a reaction known as *trans*-translation. The alanine-charged tmRNA acts like a tRNA to accept the nascent polypeptide. The mRNA is released, and the tag reading frame of tmRNA is used as a message to translate the tag peptide onto the C terminus of the nascent polypeptide. This reaction targets the newly-made protein for rapid degradation, and releases the stalled translational complex. *trans*-Translation is ubiquitous in bacteria, as genes encoding tmRNA and SmpB have been found

in all sequenced bacterial genomes, and in *E. coli* approximately 1 in 250 translation initiation reactions ends in *trans*-translation.^{29,30} FISH, immunofluorescence, and GFP fusion studies showed that the tmRNA-SmpB complex is localized in a helical structure in *Caulobacter crescentus* and *Shigella flexneri*.²³ This structure is not colocalized with MreB and does not require MreB filaments. It is not known if tmRNA-SmpB forms these structures independently or through interaction with another cytoskeletal component. Inactive mutants of tmRNA are still localized, suggesting that localization is not due to interaction with stalled ribosomes. In *C. crescentus*, tmRNA is specifically degraded as a function of the cell cycle. This degradation is accomplished by RNase R, a highly conserved ribonuclease. RNase R is also localized in a helical structure in *C. crescentus*, but the tmRNA-SmpB and RNase R structures do not intersect.²³ Therefore, it is possible that one function of tmRNA localization in *C. crescentus* to prevent inappropriate degradation by RNase R. Localization might also serve to sequester the tmRNA-SmpB from productive translational complexes, or to increase the concentration of tmRNA-SmpB in the vicinity of stalled translational complexes.

SPATIAL CONTROL OF GENE EXPRESSION

Small RNAs that bind the RNA chaperone Hfq are used to silence gene expression in bacteria, and localization is required for at least some of the silencing activity. sRNAs have partial complementarity to target mRNAs, and bind to the mRNA to rapidly shut off gene expression and promote degradation of the mRNA.³¹ Hfq prevents degradation of sRNAs and promotes base pairing with target mRNAs. GFP fusion studies and electron microscopy have shown that Hfq is localized in close proximity to the membrane in *E. coli*.^{6,32} Because sRNAs regulate the expression of many membrane proteins and the mRNAs encoding these proteins are localized to the membrane by cotranscriptional secretion, Hfq localization might be due in part to interactions with target mRNAs. However, silencing by at least one sRNA, SgrS, suggests that localization regulates sRNA-Hfq activity. SgrS binds to the 5' UTR of the *ptsG* mRNA, which encodes the glucose transporter EIICB^{Glc}.³³ Stress caused by excess glucose-6-phosphate induces SgrS to inhibit translation of EIICB^{Glc} and promote degradation of *ptsG* mRNA by RNase E, thereby preventing further accumulation of phosphosugars.^{33,34} Kawamoto, et al., showed that SgrS silencing of *ptsG* was eliminated by mutations that prevent secretion of the encoded protein.³⁵ SgrS activity on *ptsG* could be restored by replacing the membrane targeting sequence of EIICB^{Glc} with the membrane targeting sequence from LacY, an unrelated membrane protein.³⁵ These observations suggest that the *ptsG* mRNA must be near the membrane to interact with SgrS, and therefore it is likely that SgrS-Hfq is localized independently of interactions with the target mRNA. It is not yet known whether sRNA-Hfq complexes are localized to regulate silencing activity, or to facilitate interactions with the degradosome.

CONCLUSION

The field of bacterial cell biology is relatively new, and many specialized RNAs have only recently been discovered, yet several interactions between the RNA infrastructure and the cytoskeleton have already been established. The best data to date suggests that most of the transcription and translation activity is not localized, although some of the machinery may

interact with actin-like proteins. More compelling data indicate that systems that degrade mRNA and resolve stalled translational complexes are localized. The reasons for RNA infrastructure localization are generally not known, but both the degradosome and the tmRNA-SmpB complex might be localized to limit destructive interactions with productive RNAs. It remains to be seen if sequestration is a general rationale for localization. The localization of RNAs to regulate gene expression and the intimate connections between the RNA infrastructure and the cytoskeleton suggest that the role of localization in the RNA infrastructure is just beginning to be understood.

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Figure 1.

Location of RNA infrastructure in bacteria. A schematic cross section of the cell is shown with the membrane at the bottom. Transcription of RNAs is widely dispersed in the cytoplasm, although some RNA polymerase complexes are associated with the cytoskeletal protein MreB. Some mRNA diffuses through the cytoplasm, and some is concentrated in discrete spots. Processing of rRNA, tRNA, and tmRNA is performed by the degradosome, which is a component of the cytoskeleton, and by other nucleases which are also concentrated near the membrane. Translation occurs throughout the cytoplasm, but the sRNA-Hfq and tmRNA-SmpB ribonucleoprotein complexes that regulate translation are associated with the membrane or cytoskeletal structures. The major mRNA degrading activity is in the cytoskeletal degradosome complex, and RNase R, which degrades tmRNA, is also associated with the cytoskeleton.

Figure 2.

Localization of degradosome components. RNase E associates with the cytoplasmic membrane and polymerizes in the absence of other components. Enolase (Eno) localizes by binding to RNase E. RhIB binds RNase E, but can localize independently, suggesting that it also associates with another component of the cytoskeleton (not shown). PNPase binds RNase E and RhIB, and either interaction is sufficient to localize PNPase.