Published in final edited form as:

Curr Opin Microbiol. 2011 April ; 14(2): 155–159. doi:10.1016/j.mib.2011.01.009.

RNA localization in bacteria

Kenneth C. Keiler

Department of Biochemistry and Molecular Biology, Penn State University 401 Althouse Laboratory, University Park, PA 16802 kkeiler@psu.edu

Abstract

Bacteria localize proteins and DNA regions to specific subcellular sites, and several recent publications show that RNAs are localized within the cell as well. Localization of tmRNA and some mRNAs indicates that RNAs can be sequestered at specific sites by RNA binding proteins, or can be trapped at the location where they are transcribed. Although the functions for RNA localization are not yet completely understood, it appears that one function for RNA localization is to regulate RNA abundance by controlling access to nucleases. New techniques for visualizing RNAs will likely lead to increased examination of spatial control of RNAs and the role this control plays in regulation of gene expression and bacterial physiology.

Introduction

The role of RNA localization in controlling gene expression and RNA stability in eukaryotes has been well established, but until recently there were no bacterial RNAs known to localize to specific subcellular sites. Studies in *Caulobacter crescentus* and *Escherichia coli* have now demonstrated that both regulatory RNAs and mRNAs can be concentrated at fixed positions in bacterial cells. tmRNA, a regulatory RNA involved in *trans*-translation, is localized to a helical structure in *C. crescentus* [1**], and some mRNAs are localized to discrete spots in *C. crescentus* and *E. coli* [2**]. Given that bacteria also localize proteins, plasmids, and regions of the chromosome [3–5], the localization of RNA may not be particularly surprising. Nonetheless, the possibility that RNA activity and abundance can be controlled by subcellular localization represents a new paradigm for regulation of RNA, and suggests that many new discoveries of bacterial RNA localization lie ahead.

Why are RNAs localized, and how is RNA localization achieved? Our understanding is far from complete because these questions are just beginning to be addressed, yet it is now reasonable to review what is known about the mechanisms and physiological rationales for localization of RNAs in bacteria, and to speculate about what additional pathways remain to be discovered.

Localization in the life of an RNA

The life cycle of an RNA dictates some aspects of its localization. Because RNAs are tethered to DNA by RNA polymerase during transcription, the movement of the RNA will be restricted until transcription is complete. Many plasmids and regions of bacterial chromosomes are localized by the combined forces of replication, segregation, and packing

© 2011 Elsevier Ltd. All rights reserved.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

of DNA within the cell [5–7], and if a gene is localized the cognate RNA product will be produced at that site. RNAs may also be localized during degradation, because the degradosome and other RNases are localized within bacterial cells [1**,8*]. As discussed below, RNAs can move throughout the cytoplasm after transcription and before degradation, yet some RNAs are localized to specific sites in the cell.

Localization and dynamics of model RNAs

Proteins can diffuse rapidly through the bacterial cytoplasm, and protein localization mechanisms frequently limit diffusion through polymerization and/or interactions with other macromolecules [9]. Likewise, studies with model RNAs, which do not encode proteins and do not have any cellular function, indicate that RNAs can diffuse. Golding and Cox used MS2-GFP fusions to visualize single RNA molecules composed of tandem repeats of an MS2 aptamer sequence [10**]. Most of the RNA molecules moved randomly within a restricted area near the center or quarter points of the cell. These locations are significant, because the RNAs were encoded on an F plasmid, which is known to be localized to the center and quarter points of *E. coli* cells [5]. Therefore, these RNAs may be localized because they are still attached to the DNA by RNA polymerase. Other RNA molecules moved throughout the cell. These data are consistent with restricted mobility of the RNA while it is tethered to the DNA during transcription, followed by release and movement throughout the cell [10**]. Using time-lapse images, Valencia-Burton et al. found that the localization pattern of a model RNA containing eIF4A-binding sequences changed significantly over 2 min periods, suggesting that the RNA was moving within the cell [11**]. These studies indicate that RNAs can diffuse throughout the cell, and are not inherently fixed at the site of their transcription. This conclusion would be consistent with the requirement for many RNAs, such as rRNA, tRNA and other regulatory RNAs, to move throughout the cytoplasm to perform their functions at disparate locations.

Localization of tmRNA - avoiding a premature death

tmRNA, the RNA responsible for trans-translation, is the best characterized example of an endogenous bacterial RNA that is localized. During *trans*-translation, tmRNA enters a translating ribosome and mediates the addition of a peptide tag to the C terminus of the nascent polypeptide [12,13]. *trans*-Translation is a prevalent reaction, terminating ~0.5% of protein synthesis reactions, and tmRNA is one of the most abundant RNAs in bacteria. tmRNA was visualized in fixed *C. crescentus* cells using FISH, and was found to be localized in a helix-like pattern (Fig. 1) [1**]. Observations in live cells using a GFP fusion to the tmRNA-binding protein SmpB confirmed the localization pattern. Mutant studies showed that tmRNA is not localized in the absence of SmpB, but SmpB is localized in cells lacking tmRNA, suggesting that tmRNA is localized by binding to SmpB. It is likely that SmpB tethers tmRNA to an unidentified structure in the cell, because SmpB-tmRNA does not polymerize in vitro. tmRNA-SmpB does not require the cytoskeletal protein MreB for localization, but other cytoskeletal structures have not yet been investigated [1**].

What is the physiological rationale for tmRNA-SmpB localization? The prevalence of *trans*-translation suggests that tmRNA-SmpB must have access to most of the ribosomes in the cell, so either tmRNA-SmpB must be able to diffuse away from the structure to interact with substrate ribosomes or the substrate ribosomes must be moved to the localized tmRNA-SmpB. It is possible that localization plays a role in regulating *trans*-translation activity, either by concentrating tmRNA-SmpB at the sites where it will be used or by sequestering it from elongating ribosomes, but there is not yet any evidence that localization is important for activity. Another possible explanation is that tmRNA is localized to control its degradation. Unlike in *E. coli*, the stability of tmRNA in *C. crescentus* is regulated as a

function of the cell cycle, and tmRNA is specifically degraded by RNase R after the initiation of DNA replication [14,15]. Intriguingly, RNase R is also localized to a helix-like structure in *C. crescentus* [1**]. The RNase R structure and tmRNA-SmpB structures have similar patterns, but are out of phase so they do not appear to intersect (Fig. 1). Mutations that disrupt tmRNA localization also result in degradation of tmRNA, supporting the idea that one role of localization is to limit access to RNase R [1**]. Spatial separation of tmRNA and RNase R could ensure that tmRNA is abundant when it is likely to be needed for *trans*-translation, and allow rapid turnover of tmRNA in response to physiological cues that release tmRNA and let it interact with RNase R. For example, *trans*-translation activity is required for the correct timing of initiation of DNA replication, so tmRNA must be present in G1 phase cells. During G1 phase, tmRNA is localized and sequestered from RNase R. On the other hand, tmRNA must be removed in early S phase for the cell cycle to continue. Proteolysis of SmpB in early S phase releases tmRNA, which delocalizes and is then degraded by RNase R (Fig. 1).

Regulation of nuclease activity could be a general rationale for RNA localization. The major mRNA degrading nuclease activity in bacteria is contained in the degradosome, a complex containing RNase E, the RNA helicase RhlB, polynucleotide phosphorylase (PNPase), and enolase [16]. In *E. coli*, components of the degradosome are localized in a helical structure spanning the length of the cell, and RNase E binds to the cytoplasmic membrane, suggesting that the degradosome is part of the cytoskeleton [8*,17,18]. Other RNases, including RNase R discussed above, are also localized in some species [1**,19]. Therefore, RNAs may be localized either to promote degradation or to limit interaction with nucleases. It is noteworthy that the degradation of some proteins is also regulated by subcellular localization in bacteria. For example, the stability of CtrA in *C. crescentus* is controlled in part by the localization of both CtrA and the ClpXP protease, and localization of the ClpCP protease to the forespore during *Bacillus subtilis* sporulation promotes removal of specific substrates from that compartment [20,21]. Thus, localization appears to be a general mechanism to control stability of proteins and RNAs.

Localization of mRNAs - trapped by translation?

Despite the ability of model RNAs to diffuse, some mRNAs remain localized near the site of their transcription. Montero Llopis et al. used FISH to visualize several endogenous mRNAs and their cognate genes in *E. coli* and *Caulobacter crescentus*, and found that the mRNAs remained close to their cognate DNA even after transcription was likely to be complete [2**]. The localization was confirmed in live cells by inserting MS2-binding sequences in the mRNAs and expressing an MS2-GFP fusion protein. Are these mRNAs localized for regulatory control? Montero Llopis et al. proposed that protein localization and protein assemblies could be controlled by the location of the mRNA, which in turn would be controlled by the position of the gene within the cell [2**]. In principle, this model could be correct for some mRNAs. However, most localized proteins that have been studied are correctly localized when they are expressed from a plasmid or from an ectopic site on the chromosome, indicating that protein localization is not generally encoded by the position of the gene on the chromosome (for example [20,22–25]). Protein complexes such as the ribosome and cytoskeletal filaments are also typically assembled correctly when some components are expressed from ectopic sites, indicating that gene order is not generally critical for macromolecular structures either (for example [25–28]). It is difficult to reconcile these observations with a model in which gene order on the chromosome dictates localization of the cognate mRNAs and proteins. An alternative explanation is that mRNAs are localized because their diffusion is limited by co-transcriptional translation.

Unlike the model RNAs described above, the localized mRNAs encode proteins and are presumably translated by multiple ribosomes during and after transcription. Therefore, translation and the nascent polypeptides could play a significant role in the observed localization pattern. The nascent polypeptides may limit diffusion, either by simply increasing the size of the RNA-protein particle or by interacting with additional macromolecules. For example, cotranslational targeting of nascent polypeptides for secretion links the translocators, nascent polypeptides, ribosomes, mRNA, and DNA [29]. In these complexes the mRNA would have little mobility even after transcription is complete, because the mRNA is tethered to the translocators through the ribosomes. Proteins that begin to oligomerize before they are released from the ribosome would also tie the mRNA to a large macromolecular complex. It would be informative to test the role of translation in the localization of mRNAs to their cognate genes, for example by mutating the translation initiation signals in localized mRNAs to determine if translation limits RNA diffusion.

Even if cotranscriptional translation fixes the location of many mRNAs to the site of their transcription, localization near the cognate gene or to a different site might play a regulatory role for some mRNAs. Localization might be particularly useful for mRNAs that are not co-transcriptionally translated, such as flagellar mRNAs in which translation is repressed by FliB, and mRNAs in which translation is delayed by ribosome stalling or riboswitch mechanisms [30,31]. As noted above, mRNA localization could also provide a mechanism to limit the interaction of an mRNA with the degradosome and other nuclease activities to regulate the abundance of the RNA.

Other possible uses of RNA localization

Eukaryotes use RNA localization to regulate gene expression and cell-type specific inheritance of RNAs, as well as to control degradation [32]. Could bacteria use RNA localization for similar purposes? One possible mechanism for controlling gene expression via RNA localization is through sRNAs (Fig. 2). sRNAs are small RNAs in bacteria that typically act in conjunction with Hfq, an RNA chaperone [33]. Base pairing interactions between the sRNA and a target mRNAs regulate the expression or stability of the mRNA. Electron microscopy and GFP fusion studies have shown that Hfq is concentrated in proximity to the cytoplasmic membrane in *E. coli* [34,35], and the activity of at least one sRNA is correlated with membrane localization of its target mRNA. SgrS is an sRNA that inhibits translation of *ptsG* mRNA and targets the mRNA for degradation by RNase E [36,37]. *ptsG* encodes an integral membrane protein, EIICB^{Glc}, and the protein is secreted co-translationally, targeting the mRNA to the membrane. Mutations in *ptsG* that eliminated the membrane targeting sequences decrease SgrS activity on the mRNA, but SgrS activity was restored when an unrelated membrane targeting sequence from *lacY* was inserted in the *ptsG* gene [38*]. These results indicate that maximal SgrS silencing activity is correlated with localization of *ptsG*, and presumably SgrS, near the membrane. However, it is not clear whether Hfq is actively localized near the membrane to facilitate interactions between sRNAs and mRNAs, or if interactions between sRNAs and mRNAs such as SgrS-*ptsG* are responsible for localizing Hfq-sRNA complexes. In addition, membrane localization of Hfq-sRNA could be due to interactions with the degradosome, where the mRNA target is degraded. Clearly, more work will be required to determine what role localization of Hfq-sRNAs plays in regulating their activity. As this example suggests, RNA localization now needs to be considered as a potential regulatory mechanism for RNA-based processes in bacteria.

Conclusions

It is now clear that bacteria can localize RNAs to specific sites within the cell, and tools to study RNA localization in bacteria have been developed. Early discoveries of protein localization in bacteria led to a rapid appreciation of the widespread and crucial role localization plays in controlling protein activity. A similar path is likely for the new field of RNA localization. In the small number of existing examples, localization of RNAs can regulate their interactions with nucleases and control their stability. Whether protection from nuclease activity is the primary reason for RNA localization or one of many regulatory outcomes remains to be seen.

Techniques for studying RNA localization

Two techniques have been used to visualize RNA in bacterial cells, fluorescence in situ hybridization (FISH), and indirect labeling using a fluorescent RNA binding protein. To visualize RNA with FISH, an oligonucleotide complementary to the target RNA is covalently modified with a fluorescent dye and used to probe fixed bacterial cells (A) [1**]. The fluorescence signal can be increased by using probes for multiple sites in the RNA [2**]. FISH does not require modification of the native RNA. However, because FISH requires cell fixation it cannot be used to observe RNA dynamics and controls for fixation artifacts are important. RNAs can be observed in living cells using RNA binding proteins. RNAs that have specific protein binding partners can be observed by making a fusion of GFP or another fluorescent protein to the binding protein. For example, the location of tmRNA was observed in *C. crescentus* using a fusion of GFP to the tmRNA binding protein SmpB [1**]. RNAs that do not have a specific binding partner can be modified by addition of a sequence that is recognized by an exogenous RNA binding protein (B) [10**,11**,39*,40*]. The MS2 and eIF4A proteins have been used for indirect visualization because they recognize relatively short RNA sequences. Frequently, tandem repeats of the RNA recognition sequence are used to increase the fluorescent signal. For example, individual RNA molecules in live *E. coli* were observed by Golding and Cox using a fusion of the MS2 coat protein to GFP and an RNA containing multiple repeats of a 17 nucleotide RNA aptamer that binds with nanomolar affinity to MS2 [10**]. In an adaptation of this technique, the RNA binding protein eIF4A was split and each half was fused to a split GFP (C) [11**,40*]. The split fusions allow biomolecular fluorescence studies, which reduce the background fluorescence because no signal is produced unless both halves of the split fusion simultaneously bind RNA. Visualizing RNAs with fluorescently labeled proteins has many of the benefits of using GFP variants to observe protein localization, including the ability to observe RNAs in live cells and to measure RNA dynamics. However, modification of the native RNA sequence and binding of exogenous proteins can potentially disrupt the localization or dynamics of the target RNA. Ideally, RNAs should be visualized using both FISH and protein labeling techniques to control for the effects of cell fixation and sequence modification.

Acknowledgments

Thanks to Sarah Ades and Patrick Viollier for helpful discussions. KCK is supported by NIH grants GM68720 and NS71542.

Bibliography

1. Russell J, Keiler K. Subcellular localization of a bacterial regulatory RNA. *Proc Natl Acad Sci U S A*. 2009; 106:16405–16409. [PubMed: 19805312] ** This was the first report of an endogenous

bacterial RNA that is localized. This paper describes the use of FISH and protein labeling to examine the localization of tmRNA and SmpB in a helix-like pattern. RNase R, the nuclease that degrades tmRNA, was found to be in a distinct helix-like pattern.

2. Llopis PM, Jackson AF, Sliusarenko O, Surovtsev I, Heinritz J, Emonet T, Jacobs-Wagner C. Spatial organization of the flow of genetic information in bacteria. *Nature*. 2010; 466:77–81. [PubMed: 20562858] ** In this study, the location of mRNAs and their cognate genes was examined using FISH and protein labeling. mRNAs were found to localize in a spot near the site of their transcription.
3. Shapiro L, McAdams HH, Losick R. Why and how bacteria localize proteins. *Science*. 2009; 326:1225–1228. [PubMed: 19965466]
4. Thanbichler M, Viollier PH, Shapiro L. The structure and function of the bacterial chromosome. *Curr Opin Genet Dev*. 2005; 15:153–162. [PubMed: 15797198]
5. Hiraga S. Dynamic localization of bacterial and plasmid chromosomes. *Annu Rev Genet*. 2000; 34:21–59. [PubMed: 11092821]
6. Berlatzky IA, Rouvinski A, Ben-Yehuda S. Spatial organization of a replicating bacterial chromosome. *Proc Natl Acad Sci U S A*. 2008; 105:14136–14140. [PubMed: 18779567]
7. Viollier PH, Thanbichler M, McGrath PT, West L, Meewan M, McAdams HH, Shapiro L. Rapid and sequential movement of individual chromosomal loci to specific subcellular locations during bacterial DNA. *Proc Natl Acad Sci U S A*. 2004; 101:9257–9262. [PubMed: 15178755]
8. Taghbalout A, Rothfield L. RNaseE and the other constituents of the RNA degradosome are components of the bacterial cytoskeleton. *Proc Natl Acad Sci U S A*. 2007; 104:1667–1672. [PubMed: 17242352] * This paper demonstrates the subcellular localization of the degradosome in a helix-like structure.
9. Rudner DZ, Losick R. Protein subcellular localization in bacteria. *Cold Spring Harb Perspect Biol*. 2010; 2:a000307. [PubMed: 20452938]
10. Golding I, Cox EC. RNA dynamics in live *Escherichia coli* cells. *Proc Natl Acad Sci U S A*. 2004; 101:11310–11315. [PubMed: 15277674] ** This study uses an MS2-GFP fusion protein to observe the localization and dynamics of single RNA molecules containing multiple MS2 aptamers. The data suggested that RNA has restricted mobility while it is tethered to the DNA during transcription, and moves throughout the cell after it is released from RNA polymerase.
11. Valencia-Burton M, Shah A, Sutin J, Borogovac A, McCullough RM, Cantor CR, Meller A, Broude NE. Spatiotemporal patterns and transcription kinetics of induced RNA in single bacterial cells. *Proc Natl Acad Sci U S A*. 2009; 106:16399–16404. [PubMed: 19805311] ** This paper describes the use of a split eIF4A-GFP protein for biomolecular fluorescence studies of the localization and dynamics of model RNAs.
12. Keiler KC. Biology of trans-Translation. *Annu Rev Microbiol*. 2008; 62:133–151. [PubMed: 18557701]
13. Moore SD, Sauer RT. The tmRNA system for translational surveillance and ribosome rescue. *Annu Rev Biochem*. 2007; 76:101–124. [PubMed: 17291191]
14. Keiler K, Shapiro L. tmRNA in *Caulobacter crescentus* is cell cycle regulated by temporally controlled transcription and RNA degradation. *J Bacteriol*. 2003; 185:1825–1830. [PubMed: 12618446]
15. Hong SJ, Tran QA, Keiler KC. Cell cycle-regulated degradation of tmRNA is controlled by RNase R and SmpB. *Mol Microbiol*. 2005; 57:565–575. [PubMed: 15978085]
16. Carpousis AJ. The RNA degradosome of *Escherichia coli*: an mRNA-degrading machine assembled on RNase E. *Annu Rev Microbiol*. 2007; 61:71–87. [PubMed: 17447862]
17. Khemici V, Poljak L, Luisi BF, Carpousis AJ. The RNase E of *Escherichia coli* is a membrane-binding protein. *Mol Microbiol*. 2008; 70:799–813. [PubMed: 18976283]
18. Taghbalout A, Rothfield L. RNaseE and RNA helicase B play central roles in the cytoskeletal organization of the RNA degradosome. *J Biol Chem*. 2008; 283:13850–13855. [PubMed: 18337249]
19. Miczak A, Srivastava RA, Apirion D. Location of the RNA-processing enzymes RNase III, RNase E and RNase P in the *Escherichia coli* cell. *Mol Microbiol*. 1991; 5:1801–1810. [PubMed: 1943711]

20. Kain J, He GG, Losick R. Polar localization and compartmentalization of ClpP proteases during growth and sporulation in *Bacillus subtilis*. *J Bacteriol.* 2008; 190:6749–6757. [PubMed: 18689476]
21. McGrath P, Iniesta A, Ryan K, Shapiro L, McAdams H. A dynamically localized protease complex and a polar specificity factor control a cell cycle master regulator. *Cell.* 2006; 124:535–547. [PubMed: 16469700]
22. Russell J, Keiler K. Screen for localized proteins in *Caulobacter crescentus*. *PLoS One.* 2008; 3:e1756. [PubMed: 18335033]
23. Ebersbach G, Briegel A, Jensen GJ, Jacobs-Wagner C. A self-associating protein critical for chromosome attachment, division, and polar organization in *caulobacter*. *Cell.* 2008; 134:956–968. [PubMed: 18805089]
24. Huitema E, Pritchard S, Matteson D, Radhakrishnan SK, Viollier PH. Bacterial birth scar proteins mark future flagellum assembly site. *Cell.* 2006; 124:1025–1037. [PubMed: 16530048]
25. Jones LJ, Carballido-Lopez R, Errington J. Control of cell shape in bacteria: helical, actin-like filaments in *Bacillus subtilis*. *Cell.* 2001; 104:913–922. [PubMed: 11290328]
26. Sharma D, Cukras AR, Rogers EJ, Southworth DR, Green R. Mutational analysis of S12 protein and implications for the accuracy of decoding by the ribosome. *J Mol Biol.* 2007; 374:1065–1076. [PubMed: 17967466]
27. Fredrick K, Dunny GM, Noller HF. Tagging ribosomal protein S7 allows rapid identification of mutants defective in assembly and function of 30 S subunits. *J Mol Biol.* 2000; 298:379–394. [PubMed: 10772857]
28. Aarsman ME, Piette A, Fraipont C, Vinkenvleugel TM, Nguyen-Disteche M, den Blaauwen T. Maturation of the *Escherichia coli* divisome occurs in two steps. *Mol Microbiol.* 2005; 55:1631–1645. [PubMed: 15752189]
29. Driessen AJ, Nouwen N. Protein translocation across the bacterial cytoplasmic membrane. *Annu Rev Biochem.* 2008; 77:643–667. [PubMed: 18078384]
30. Anderson PE, Guber JW. FlbT, the post-transcriptional regulator of flagellin synthesis in *Caulobacter crescentus*, interacts with the 5' untranslated region of flagellin mRNA. *Mol Microbiol.* 2000; 38:41–52. [PubMed: 11029689]
31. Smith AM, Fuchs RT, Grundy FJ, Henkin TM. Riboswitch RNAs: regulation of gene expression by direct monitoring of a physiological signal. *RNA Biol.* 2010; 7:104–110. [PubMed: 20061810]
32. Martin KC, Ephrussi A. mRNA localization: gene expression in the spatial dimension. *Cell.* 2009; 136:719–730. [PubMed: 19239891]
33. Gottesman S, Storz G. Bacterial Small RNA Regulators: Versatile Roles and Rapidly Evolving Variations. *Cold Spring Harb Perspect Biol.* 2010
34. Azam TA, Hiraga S, Ishihama A. Two types of localization of the DNA-binding proteins within the *Escherichia coli* nucleoid. *Genes Cells.* 2000; 5:613–626. [PubMed: 10947847]
35. Diestra E, Cayrol B, Arluison V, Risco C. Cellular electron microscopy imaging reveals the localization of the Hfq protein close to the bacterial membrane. *PLoS One.* 2009; 4:e8301. [PubMed: 20011543]
36. Vanderpool CK, Gottesman S. Involvement of a novel transcriptional activator and small RNA in post-transcriptional regulation of the glucose phosphoenolpyruvate phosphotransferase system. *Mol Microbiol.* 2004; 54:1076–1089. [PubMed: 15522088]
37. Kimata K, Tanaka Y, Inada T, Aiba H. Expression of the glucose transporter gene, *ptsG*, is regulated at the mRNA degradation step in response to glycolytic flux in *Escherichia coli*. *EMBO J.* 2001; 20:3587–3595. [PubMed: 11432845]
38. Kawamoto H, Morita T, Shimizu A, Inada T, Aiba H. Implication of membrane localization of target mRNA in the action of a small RNA: mechanism of post-transcriptional regulation of glucose transporter in *Escherichia coli*. *Genes Dev.* 2005; 19:328–338. [PubMed: 15650111] * This paper showed that silencing activity of the *SgrS* sRNA is affected by localization of the target mRNA.
39. Golding I, Cox EC. Chapter 8: Spatiotemporal dynamics in bacterial cells: real-time studies with single-event resolution. *Methods Cell Biol.* 2008; 89:223–251. [PubMed: 19118677] * This chapter describes methods for real-time monitoring of RNA localization.

40. Valencia-Burton M, McCullough RM, Cantor CR, Broude NE. RNA visualization in live bacterial cells using fluorescent protein complementation. *Nat Methods*. 2007; 4:421–427. [PubMed: 17401371] * This paper describes the method for observing RNA localization using protein complementation.

Fig. 1.

Regulation of tmRNA by localization. *C. crescentus* G1 phase (swarmer) cells (left) localize tmRNA-SmpB (red) and RNase R (blue) in different helix-like patterns [1**]. After DNA replication initiates (S phase) and the cells differentiate into stalked cells, SmpB is proteolyzed, releasing tmRNA and allowing degradation by RNase R. Before cell division, tmRNA-SmpB is resynthesized and the helix-like patterns are observed again [1**].