Polyribosome-Dependent Clustering of Membrane-Anchored RNA Degradosomes To Form Sites of mRNA Degradation in *Escherichia coli*

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**ABSTRACT** The essential endoribonuclease RNase E, which is a component of the *Escherichia coli* multienzyme RNA degradosome, has a global role in RNA processing and degradation. RNase E localizes to the inner cytoplasmic membrane in small, short-lived clusters (puncta). Rifampin, which arrests transcription, inhibits RNase E clustering and increases its rate of diffusion. Here, we show that inhibition of clustering is due to the arrest of transcription using a rifampin-resistant control strain. Two components of the RNA degradosome, the 3′ exoribonuclease polynucleotide phosphorylase (PNPase) and the DEAD box RNA helicase RhlB, colocalize with RNase E in puncta. Clustering of PNPase and RhlB is inhibited by rifampin, and their diffusion rates increase, as evidenced by *in vivo* photobleaching measurements. Results with rifampin treatment reported here show that RNA degradosome diffusion is constrained by interaction with RNA substrate. Kasugamycin, which arrests translation initiation, inhibits formation of puncta and increases RNA degradosome diffusion rates. Since kasugamycin treatment results in continued synthesis and turnover of ribosome-free mRNA but inhibits polyribosome formation, RNA degradosome clustering is therefore polyribosome dependent. Chloramphenicol, which arrests translation elongation, results in formation of large clusters (foci) of RNA degradosomes that are distinct from puncta. Since chloramphenicol-treated ribosomes are stable, the formation of RNA degradosome foci could be part of a stress response that protects inactive polyribosomes from degradation. Our results strongly suggest that puncta are sites where translationally active polyribosomes are captured by membrane-associated RNA degradosomes. These sites could be part of a scanning process that is an initial step in mRNA degradation.

**IMPORTANCE** Here, we show that RNase E, RhlB, and PNPase act together as components of the multienzyme RNA degradosome in polyribosome-dependent clustering to form puncta on the inner cytoplasmic membrane. Our results support the hypothesis that RNA degradosome puncta are sites of mRNA degradation. We propose that clustering of RNA degradosomes is a pre-RNase E cleavage step in which polyribosomes are scanned in a search for ribosome-free mRNA. This work is part of an emerging view that posttranscriptional events such as tRNA maturation, late steps in ribosome assembly, and mRNA degradation are membrane associated and partitioned from translation in the cytoplasm and transcription in the nucleoid. This separation could protect newly synthesized transcripts from premature destructive interactions with the RNA degradosome. The scanning of ribosomes and polyribosomes could be part of a general mechanism in which defective stable RNA or ribosome-free mRNA is targeted for destruction by the RNA degradosome.

**KEYWORDS** RNA degradosome, mRNA degradation, inner cytoplasmic membrane, polyribosome, rifampin, kasugamycin, chloramphenicol

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Nase E is an essential endoribonuclease with major roles in maturation of stable RNA, degradation of mRNA, and posttranscriptional regulation of gene expression (1–6). Instability of mRNA is important for regulation of gene expression because it permits rapid remodeling of the transcriptome. Degradation involves fragmentation of mRNA by endoribonucleases, principally RNase E, followed by digestion to nucleotides by exoribonucleases and oligoribonuclease (5, 7, 8). tRNA maturation and mRNA degradation are essential functions of RNase E (9–13).

The quaternary structure of RNase E is a homotetramer of 118-kDa monomers. The N-terminal half of the monomer folds into subunits that associate to form the catalytic core of RNase E (14, 15). The C-terminal half is a large noncatalytic region, which is mostly natively unstructured protein (16). Embedded within the noncatalytic region are small linear motifs (SLiMs), also known as microdomains, which are sites of interaction with other macromolecules (4, 17, 18). The noncatalytic region includes sites for interaction with the DEAD box RNA helicase RhlB, the glycolytic enzyme enolase, and the 3’ exoribonuclease polynucleotide phosphorylase (PNPase) (19–23). The multienzyme complex of RNase E, RhlB, enolase, and PNPase is known as the RNA degradosome (24–27). Experimental evidence has shown that RhlB, RNase E, and PNPase act coordinately as components of the RNA degradosome in the processing and degradation of RNA (28, 29). Mutant strains of Escherichia coli in which the RNA degradosome has been disrupted by deletion of part or all of the RNase E noncatalytic region are viable (20, 30). Nevertheless, disruption of the RNA degradosome has been shown to reshape the transcriptome and proteome (31, 32) and to result in defects in processes including initiation of mRNA degradation, small RNA (sRNA)-mediated gene silencing, and turnover of intermediates in mRNA degradation and of hypomodified tRNA (28, 33–35).

Recent work has shown that RNase E, RhlB, and PNPase are localized to the inner cytoplasmic membrane of E. coli (36, 37). RNase E has a 15-residue SLiM, the membrane-targeting sequence (MTS), which is located about 50 residues from the catalytic domain and forms an amphipathic alpha helix upon insertion into the phospholipid bilayer (36–38). The localization of RhlB and PNPase to the inner membrane requires their association with RNase E (36, 37). Quantification of superresolution images of live cells shows that RNA degradosome components are highly enriched on the inner cytoplasmic membrane and that deletion of the MTS results in their localization to the cytoplasm (37). Although it has been suggested that the membrane localization of RNase E preferentially destabilizes mRNA encoding inner-membrane proteins (37), another study found a global slowdown in mRNA degradation when the MTS is deleted but no correlation between changes in stability and the function or cellular location of the encoded proteins (39). The latter study suggests that membrane association of the RNA degradosome has a wide-ranging role in mRNA turnover.

Imaging of live cells by epifluorescence and total internal reflection fluorescence microscopy (TIRFm) has shown that RNase E-yellow fluorescent protein (YFP) forms short-lived clusters on the inner cytoplasmic membrane (36). Although we previously called these clusters foci, we now use the term “puncta” to distinguish small clusters of RNase E in the inner membrane of E. coli from large foci formed by cytoplasmic RNase E in Caulobacter crescentus (40). Rifampin treatment, which inhibits transcription (41, 42), disperses RNase E-YFP puncta. Within a few minutes of treatment, mRNA and precursors of tRNA and rRNA are depleted, resulting in arrest of protein synthesis and inhibition of growth. This arrest is accompanied by the degradation of 23S and 16S rRNA, a brief eclipse in cell viability, and a 50% decrease in cell size due to a terminal cell division (43). Ectopic expression of a hybrid lacZ-tRNAArg5 transcript by bacteriophage T7 RNAP, which is not inhibited by rifampin, restored RNase E-YFP clustering in rifampin-treated cells (36). Photobleaching measurements showed that rifampin treatment relaxes constraints on the diffusion of RNase E-YFP. These results are evidence that the clustering of RNase E-YFP to form short-lived puncta is dependent on the presence of RNA substrate.
Here, we made strains with single-copy chromosomal constructs of RNase E, RhlB, and PNPase tagged with mCherry or monomeric superfolder green fluorescent protein (msfGFP), and we introduced a mutation that results in resistance to the drug rifampin. We show that RNase E, RhlB, and PNPase colocalize in puncta on the inner membrane of *E. coli*. Photobleaching measurements showed that rifampin treatment relaxes constraints on the rate of diffusion of RhlB and PNPase, as was previously shown for RNase E (36). We show that punctum formation and dynamics of RNase E, RhlB, and PNPase are unchanged upon drug treatment of rifampin-resistant strains. These results show that RNase E, RhlB, and PNPase act together as components of the RNA degradosome in clustering to form puncta on the inner cytoplasmic membrane of *E. coli*. Kasugamycin, which is a drug that inhibits the initiation of translation, has effects similar to those of rifampin. This result shows that the clustering of the RNA degradosome to form short-lived puncta depends on the presence of polyribosomes. Treatment with chloramphenicol, which inhibits translation elongation, results in large clusters (foci) of RNase E-mCherry that are distinct from the puncta observed in untreated cells. The formation of foci could stockpile RNA degradosomes as part of a mechanism that protects inactive polyribosomes from degradation. We discuss the implication of our results for the cell biology of mRNA degradation in *Escherichia coli* and related Gram-negative bacteria.

**RESULTS**

We recently constructed and characterized an *E. coli* K-12 strain (SLM001) that is rifampin resistant (43). SLM001 has a mutation in the *rpoB* gene, which encodes the β-subunit of RNA polymerase. The isogenic *rpoB*+ and *rpoB*(DS16Y) strains Kti162 and SLM001 are *E. coli* K-12 derivatives that encode RNase E-mCherry at the *rne* locus under the control of endogenous expression signals (Table 1). The isogenic strain SLM001 grows on LA plates at temperatures ranging from 20 to 43°C with no visible difference...
in colony size or morphology compared to Kti162. In LB cultures at 37°C with vigorous shaking, Kti162 and SLM001 have the same growth rate (doubling time, 21 min).

Inhibition of RNA degradosome clustering by rifampin. mCherry is a monomeric red fluorescence protein that is well suited for pairing with green fluorescence protein (GFP) (44). Figure 1A shows the distribution of RNase E-mCherry in Kti162 (rpoB<sup>+</sup>) before and 30 min after rifampin addition. The puncta, which are conspicuously present (Fig. 1A, top), dispersed after rifampin treatment (bottom). We used line scans parallel to the long axis of the cell to measure average pixel intensity (C) and normalized variance in average pixel intensity (D). The graphs represent 60 to 100 line scans for each measurement. The red horizontal line in each plot marks the median. Statistical significance of the differences between untreated and rifampin-treated cells was calculated using the nonparametric Mann-Whitney test (***, P < 0.0001; *, 0.01 < P < 0.05).
1 h for mCherry to mature, results in a large pool of immature mCherry that continues to mature after the arrest of mCherry synthesis.

The decrease in the normalized variance of average pixel intensity is a quantitative measure of the dispersion of RNase E-mCherry in a population of cells treated with rifampin (Fig. 1D). Inhibition of punctum formation by rifampin is comparable to previously described effects on RNase E-YFP (36). These results show that the clustering of RNase E-YFP on the inner cytoplasmic membrane is not an artifact due to ectopic expression from a plasmid or a tendency of YFP fusion proteins to aggregate (47).

Rifampin had no effect on the formation of RNase E-mCherry puncta in the SLM001 strain (Fig. 1B). Since there is only a small change in average pixel intensity, the proportion of mature mCherry molecules is nearly the same 30 min after the addition of rifampin due to growth in the presence of the antibiotic (Fig. 1C). Quantification of the normalized variance in average pixel intensity for a population of cells confirms that there is at most a small increase that could be due to growth after rifampin addition (Fig. 1D). These results are proof that the dispersion of RNase E clusters by rifampin treatment is due to inhibition of transcription.

To examine the localization and distribution of PNPase and RhlB, we constructed two strains, SLM018 and SLM024, expressing PNPase-msfGFP and RhlB-msfGFP, respectively. msfGFP is a monomeric derivative of superfolder GFP that minimizes aggregation and mislocalization (47). Both strains have growth rates comparable to that of their isogenic wild-type parent. We observed puncta of PNPase-msfGFP and RhlB-msfGFP at the periphery of the cell (Fig. 2A, top). After rifampin treatment, we detected a smooth distribution pattern (Fig. 2A, bottom). We also observed an increase in average pixel intensity (Fig. 2C). The smaller increase in msfGFP pixel intensity compared to mCherry is due to the 3-fold-higher rate of msfGFP maturation (48). There is a decrease in the normalized variance of PNPase-msfGFP and RhlB-msfGFP average pixel intensity after rifampin treatment (Fig. 2D). These results show that the membrane localization and distribution of PNPase-msfGFP and RhlB-msfGFP and the effect of rifampin treatment are the same as with RNase E-mCherry.

We next analyzed SLM027 and SLM029, which are rifampin-resistant rpoB(D516) strains encoding PNPase-msfGFP and RhlB-msfGFP, respectively (Table 1). In contrast to the rpoB+ strains, rifampin treatment did not result in dispersion of PNPase-msfGFP or RhlB-msfGFP (Fig. 2B). There was a small decrease in average pixel intensity and no difference in the normalized variance in average pixel intensity (Fig. 2C and D). These results show that rifampin treatment does not affect the clustering of RhlB-msfGFP and PNPase-msfGFP to form puncta in the rpoB(D516Y) background.

We constructed a fusion protein in which the RNase E sequence from position 568 to 592, corresponding to the MTS, was fused to the N terminus of msfGFP. Figure S1 shows that msfGFP localizes to the inner membrane, as evidenced by the ghost-like epifluorescence image and the localization to the surface of the cell in the TIRF image. As expected, treatment with rifampin resulted in an increase in average pixel intensity. There was, however, no significant change in the normalized variance in pixel intensity. This result shows that the MTS can target msfGFP to the inner membrane. The smooth, uniform distribution shows that the MTS by itself does not promote punctum formation and that membrane attachment does not aggregate msfGFP.

Rifampin inhibition of clustering relaxes constraints on diffusion of the RNA degradosome. Some membrane proteins have been shown to form puncta at the periphery of the cell. In B. subtilis, 65% of more than 200 proteins are localized in patchy patterns (49). Examples include SecA/SecY (50), MreB (51), Mdr (49), and RNase Y (45). The appearance and disappearance of RNase E-YFP puncta in E. coli over a period of seconds give the impression of movement (36). Photobleaching by TIRFm is a powerful method to measure relative rates of diffusion of membrane-associated proteins. Since only the membrane closest to the glass coverslip is excited in TIRFm, the cell is partitioned into illuminated and dark compartments. Rapid diffusion relative to the intrinsic rate of photobleaching results in slow photobleaching, since individual molecules spend only a short time in the illuminated field. In contrast, slow diffusion results in fast photobleaching. TIRFm photobleaching of membrane proteins results in biphasic
curves that can be fitted using an initial high rate ($K_f$) and a low rate ($K_s$). $K_f$ and $K_s$ are not independent. However, $K_f$ can be thought of as the initial rate of photobleaching of molecules in the illuminated field, whereas $K_s$ is related to the rate of diffusion. Under carefully controlled conditions, $K_f$ can be used to measure relative rates of diffusion (36, 52). Previous work showed that rifampin treatment increased the rate of diffusion of RNase E-YFP (36).

We measured photobleaching of RNase E-mCherry in Kti162 and SLM001 before and after treatment with rifampin. Snapshots (Fig. 3A and B) suggest that photobleaching in the rpoB$^+$ strain is slower after treatment with rifampin. This was confirmed by quantification of the rate of photobleaching of a population of cells. The graphs in Fig. 3C and D show quantification of time-lapse videos in which photobleaching is continuous. Whereas rifampin treatment results in slower photobleaching in the rpoB$^+$ strain, there is no effect in the rpoB(D516Y) strain. We observed a similar effect of rifampin on RNase E-
GFP photobleaching (Fig. S2). Table 2 shows the slow diffusion-limited rate of photobleaching \( K_s \), the goodness of fit \( R^2 \), the standard error of the mean (SEM), and the number of measurements \( n \). These results show that the diffusion rate of RNase E-mCherry and RNase E-GFP expressed from the native \( rne \) locus under the control of endogenous regulatory elements increases with rifampin treatment. The \( rpoB(D516Y) \) mutation is validated as a control, since photobleaching is not affected by rifampin.

**FIG 3** Photobleaching of RNase E-mCherry in the \( rpoB^+ \) and \( rpoB(D516Y) \) backgrounds. Cultures of Kti162 and SLM001 expressing RNase E-mCherry in the \( rpoB^+ \) and \( rpoB(D516Y) \) backgrounds, respectively, were grown to an OD\(_{600}\) of 0.5 to 0.6 in LB at 37°C. Images taken from 15-s TIRFm time-lapse videos filmed with no delay and a 100-ms exposure time before and after treatment with 150 \( \mu \)g/ml rifampin for 30 min. (A and B) Photobleaching of Kti162 (A) and SLM001 (B). (C and D) Quantification of TIRFm continuous photobleaching of Kti162 (C) and SLM001 (D). The graphs show the averaged percent normalized fluorescence intensities (blue and red dots), after background subtraction, of two independent fields of cells, before and after treatment with rifampin. Curve fits were performed using a biexponential decay model, where the mCherry rapidly bleaches with an initial intensity of 100 at time zero. Faint blue and red dotted lines show standard deviations.
In TIRFm imaging, PNPase-msfGFP and RhlB-msfGFP cluster in puncta (Fig. 4A and B), which are similar to RNase E-mCherry (Fig. 3). We used TIRFm photobleaching to measure the effect of rifampin treatment on diffusion. The rates of photobleaching of PNPase-msfGFP and RhlB-msfGFP decreased after rifampin treatment (Fig. 4C and E). In the \( rpoB \)\(^{(D516Y)} \) background, there was no change (Fig. 4D and F). Table 2 shows the curve fitting constants obtained from these measurements. The diffusion rates of PNPase-msfGFP and RhlB-msfGFP increase after rifampin treatment, as is the case for RNase E-mCherry and RNase E-GFP. We also measured photobleaching of the MTS-msfGFP fusion protein before and after rifampin treatment (Fig. S3). As there is no effect on MTS-msfGFP, this result shows that the decrease in the rates of photobleaching of PNPase-msfGFP and RhlB-msfGFP after rifampin treatment is specific to components of the RNA degradosome.

### Colocalization of RNA degradosome components

Previous work showed that membrane localization of RhlB depends on a direct protein-protein interaction with RNase E (36), and this is also likely for PNPase (37). Here, we used epifluorescence and TIRF microscopy to examine the colocalization of PNPase and RhlB with RNase E. Since PNPase and RhlB form puncta, we coexpressed RNase E-mCherry with RhlB-msfGFP (SLM025) or PNPase-msfGFP (SLM019). To investigate if the protein pairs colocalized, we fixed cells with 1% formaldehyde and imaged by epifluorescence and TIRF illumination. A merge of artificially colored red/green fields suggests that PNPase-msfGFP and RhlB-msfGFP colocalize with RNase E-mCherry, as evidenced by the yellow color (Fig. 5A and B). Several factors, including alignment of the images, noise, and the limit of resolution of light microscopy, which is about one-quarter the width of an \( E. \) \( coli \) cell, affect colocalization measurements. We therefore applied a pixel-by-pixel analysis of the RNase E-mCherry/PNPase-msfGFP and RNase E-mCherry/RhlB-msfGFP pairs. Figure S4 shows a graphical representation of the correlation in pixel intensity between the mCherry and msfGFP pairs. \( R \) values, which are a measure of the degree of correlation, are shown in the insets of the graphs (Fig. S4). The maximum value for Pearson’s coefficient (\( R = 1.000 \)) indicates perfect correlation (52). Considering previous studies using Pearson’s coefficient in fluorescent-image analyses, \( R \) values in the range of 0.88 to 0.95 are strong support for colocalization of these proteins (52, 53).

We also performed a statistical analysis in which the micrographs were treated with Costes’ randomization function (54) to generate 1,000 scrambled images. Pearson’s correlation coefficients were calculated for each scrambled image compared to the non-randomized image of the partner protein. As expected, the same results were obtained when scrambled mCherry images were compared to the msfGFP image and the scrambled msfGFP images were compared to the mCherry image. \( P \) values, expressed as percentages, are shown in the merged images (Fig. 5). \( P \) values of 100% in the epifluorescence images and 98 and 99% in the TIRFm images offer strong statistical support for the colocalization of RNase E-mCherry, PNPase-msfGFP, and RhlB-msfGFP.

We also analyzed epifluorescence and TIRF images of an RNase E-mCherry/AtpB-msfGFP pair (Fig. S5). AtpB is a subunit of the F\(_{\text{F}}\) ATPase, which is partly localized to the inner cytoplasmic membrane. Membrane-associated AtpB is visible in epifluorescence

### Table 2 Photobleaching measurements

| Strain | Fluorescent protein | \( rpoB \) | Without rifampin | \( K_s \) (1/s) | \( R^2 \) | SEM | With rifampin | \( K_s \) (1/s) | \( R^2 \) | SEM | \( n \) |
|--------|---------------------|----------|-----------------|----------------|-------|-----|---------------|----------------|-------|-----|-----|
| Kti162 | RNase E-mCherry     | +        | 0.4356          | 0.9763         | 0.01106|     | 0.2760        | 0.8223         | 0.02536|     | 3   |
| Kti164 | RNase E-GFP        | +        | 0.2493          | 0.9721         | 0.00655|     | 0.1655        | 0.9302         | 0.00887|     | 3   |
| SLM001 | RNase E-mCherry     | *        | 0.4753          | 0.9955         | 0.01021|     | 0.4375        | 0.9949         | 0.01059|     | 3   |
| SLM018 | PNPase-msfGFP      | +        | 0.03337         | 0.9987         | 0.00023|     | 0.01510       | 0.9997         | 0.00027|     | 3   |
| SLM027 | PNPase-msfGFP      | *        | 0.03356         | 0.9998         | 0.00048|     | 0.03660       | 0.9998         | 0.00040|     | 2   |
| SLM024 | RhlB-msfGFP        | +        | 0.3352          | 0.9993         | 0.01456|     | 0.1024        | 0.9847         | 0.01971|     | 3   |
| SLM029 | RhlB-msfGFP        | *        | 0.2286          | 0.9579         | 0.01946|     | 0.2109        | 0.9519         | 0.00812|     | 2   |

\( K_s \) is the diffusion-limited slow rate constant, \( R^2 \) is the goodness of fit, and SEM is the standard error of the curve fit. \( n \), number of measurements. *, \( rpoB(D516Y) \) allele.
FIG 4 Photobleaching of PNPase-msfGFP and RhlB-msfGFP in the rpoB+ and rpoB(D516Y) backgrounds. (A and B) PNPase-msfGFP (A) and RhlB-msfGFP (B). E. coli cells were grown in LB medium at 37°C and imaged by TIRFm. Bar, 5 µm. (Continued on next page)
(Fig. S5, top) and TIRF (bottom) imaging. Visual inspection of the micrographs in Fig. S5A suggests little overlap of RNase E-mCherry puncta and AtpB-msfGFP puncta. Correlation coefficients of less than 0.80 (Fig. S5B) are consistent with little or no overlap localization of RNase E-mCherry and AtpB-msfGFP.

Our epifluorescence images (Fig. 2 and 5) suggest that in addition to membrane localization, a proportion of PNPase is cytoplasmic. Modeling based on known quaternary structures and protein-protein interactions shows that 1 trimer of PNPase can bind to 1 protomer of RNase E (3:1) (17). Measurements of fluorescent protein levels in chemically fixed cells gave a 5:1 ratio of PNPase to RNase E (55), thus suggesting that there are nearly equivalent levels of RNA degradosome-associated PNPase and free PNPase. This estimate is consistent with absolute protein synthesis rates measured by ribosome profiling, which shows an excess of PNPase synthesis relative to RNase E (4.4:1) (56). The existence of free PNPase is further supported by pulldown experiments in which some, but not all, PNPase copurifies with RNase E (e.g., see Fig. 2 in reference 39).

PNPase-msfGFP photobleaching is 5- to 10-fold slower than RhlB-msfGFP photobleaching (Table 2). We therefore measured in vitro photobleaching of purified RNA degradosomes containing PNPase-msfGFP or RhlB-msfGFP (Fig. S6 and S7). Since there is no difference in the intrinsic sensitivity of the purified complexes to photobleaching in vitro, the slow photobleaching of PNPase-msfGFP must be due to the structure and/or dynamics of the RNA degradosome in vivo. Possible explanations include (i) geometry of the RNA degradosome on the inner cytoplasmic membrane, in which PNPase-msfGFP is further from the glass slide than RhlB-msfGFP, resulting in lower excitation levels, and (ii) an exchange between degradosome-associated PNPase-msfGFP and cytoplasmic PNPase-msfGFP, which would serve as an additional pool of photobleachable molecules. This phenomenon was not investigated further.

Kasugamycin treatment inhibits RNA degradosome clustering. We asked if the formation of RNA degradosome puncta involves a direct interaction with mRNA by treating cells with kasugamycin, which results in inhibition of translation of canonical mRNAs with a 5′ leader containing a Shine-Dalgarno sequence, arrest of growth, and low-level translation of leaderless mRNA (57). Continued transcription results in the degradation of ribosome-free mRNA and the maturation of tRNA and rRNA precursors (43). Figure 6 shows that kasugamycin inhibits formation of RNA degradosome puncta. As expected, there was an increase in average pixel intensity due to maturation of mCherry (Fig. 6C). Statistical support for decreased normalized variance in pixel intensity is quantitative evidence for the inhibition of RNA degradosome clustering in a population of cells (Fig. 6D). Figure 6E shows that the rate of photobleaching of RNase E-mCherry decreased after kasugamycin treatment, which is consistent with an increase in the diffusion rate of the RNA degradosome. These results show that the degradation of ribosome-free mRNA and the maturation of tRNA and rRNA precursors in the presence of kasugamycin is not associated with the formation of RNA degradosome puncta.

We also measured the effect of kasugamycin on the photobleaching of AtpB-msfGFP. Figure S8 shows that kasugamycin treatment has no effect on the rate of photobleaching of AtpB-msfGFP. We therefore conclude that the effect of kasugamycin is specific to the dynamics of the RNA degradosome. The effects of kasugamycin treatment on RNA degradosome clustering and diffusion are very similar to the effects of rifampin treatment. Kasugamycin treatment has been shown to result in the absence of detectable polyribosomes (58). Since mRNA synthesis continues in the presence of kasugamycin (57), these results strongly suggest that the clustering of RNA degradosomes to form puncta depends on the presence of polyribosomes.

**FIG 4 Legend (Continued)**
Red arrows indicate puncta. (C to F) Quantitative analysis of continuous photobleaching as described for Fig. 3. Photobleaching was performed before or 30 min after rifampin treatment (150 μg/ml). (C) SLM018 is PNPase-msfGFP and rpoBΔ. (D) SLM027 is PNPase-msfGFP and rpoBΔ(D516Y). (E) SLM024 is RhlB-msfGFP and rpoBΔ. (F) SLM029 is RhlB-msfGFP and rpoBΔ(D516Y).
Chloramphenicol treatment results in large clusters of RNA degradosomes (foci).

We tested the effect of another protein synthesis inhibitor on the formation of RNA degradosome puncta on the inner cytoplasmic membrane. Chloramphenicol inhibits peptide bond synthesis, thus freezing translation elongation (59). Figure 7A to C shows epifluorescent images of cells expressing RNase E-mCherry in the absence or presence of 25 or 125 μg/ml chloramphenicol. Although cell growth was inhibited on agar plates at the lower concentration, the higher concentration is often used with liquid media. The images reveal large clusters of RNA degradosomes that are distinct from the membrane-associated puncta seen under normal growth conditions (Fig. 7B and C).

**FIG 5** RNase E colocalizes with PNPase and RhlB. (A) *E. coli* strain SLM019 expressing RNase E-mCherry (left) and PNPase-msfGFP (middle) and merged images (right). (B) *E. coli* strain SLM025 expressing RNase E-mCherry (left) and RhlB-msfGFP (middle) and merged images (right). Cells were fixed with 1% formaldehyde and then imaged in epifluorescence (top) and TIRFm (bottom) illumination modes. After image background subtraction and conversion into 8-bit type, an analysis was performed using the ImageJ JACoP plugin (54). Costes’ randomization function was used to generate 1,000 scrambled images. Pearson’s correlation coefficient was calculated for each scrambled image and then compared to the original nonrandomized image. *P* values, expressed as percentages, are shown in the merged images. *P* values that exceed 95% are deemed statistically significant. Bar, 2 μm.
Kasugamycin (Kas) treatment inhibits formation of RNase E puncta and increases diffusion rate. Kti162 strain expressing RNase E-mCherry was grown to an OD₆₀₀ of 0.5 to 0.6 in LB at 37°C. (A and B) Epifluorescence micrographs of RNase E-mCherry showing the distribution of RNase E before (A) and after (B) treatment with kasugamycin for 30 min. Bar, 2 μm. Average pixel intensity (C) and variance in average pixel intensity (D) were determined as described for Fig. 1. Sixty line scans of cells before and 66 line scans of cells after treatment with Kti162 + Kas.

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FIG 6  Kasugamycin (Kas) treatment inhibits formation of RNase E puncta and increases diffusion rate. Kti162 strain expressing RNase E-mCherry was grown to an OD₆₀₀ of 0.5 to 0.6 in LB at 37°C. (A and B) Epifluorescence micrographs of RNase E-mCherry showing the distribution of RNase E before (A) and after (B) treatment with kasugamycin for 30 min. Bar, 2 μm. Average pixel intensity (C) and variance in average pixel intensity (D) were determined as described for Fig. 1. Sixty line scans of cells before and 66 line scans of cells after treatment with Kti162 + Kas.
Average pixel intensity and normalized variance in pixel intensity (Fig. 7D and E) are consistent with the formation of large clusters, which we refer to as foci. The increase in average pixel intensity is partly due to the maturation of mCherry after the inhibition of protein synthesis. However, the higher average pixel intensity at 125 μg/ml chloramphenicol than at 25 μg/ml suggests an additional effect, which could be due to the formation of foci. The increase in the normalized variance of pixel intensity at both concentrations of chloramphenicol is consistent with the localization of a large proportion of RNA degradosomes into a few foci. Taken together, these results show that the inhibition of translation elongation results in the formation of RNA degradosome clusters that are larger and brighter than puncta seen in untreated cells.

FIG 6 Legend (Continued)
kasugamycin were analyzed to generate graphs of average and normalized variance in pixel intensity. Red horizontal lines show means, and the error bars represent standard deviations. Statistical significance was calculated using the nonparametric Mann-Whitney test (***, P < 0.0001; **, 0.001 < P < 0.01).

FIG 7 Chloramphenicol treatment results in focus formation. Kti162 expressing RNase E-mCherry was grown to an OD600 of 0.5 to 0.6 in LB at 37°C. (A to C) Epifluorescence micrographs of RNase E-mCherry. The images show the distribution of RNase E before (A) and after (B and C) treatment with 25 and 125 μg/ml chloramphenicol for 30 min. Bar, 2 μm. Average pixel intensity (D) and normalized variance in average pixel intensity (E) were determined as described in Fig. 1. Red horizontal lines mark the means. Data were collected from 88 to 104 line scans for each measurement. Statistical significance was calculated using the nonparametric Mann-Whitney test (****, P < 0.0001; **, 0.001 < P < 0.01).
DISCUSSION

Epifluorescence and TIRF imaging of cells expressing the RNase E-mCherry and PNase-msfGFP or RhlB-msfGFP shows that these proteins colocalize in puncta on the inner cytoplasmic membrane. Pearson’s correlation coefficients in a pixel-by-pixel comparison of epifluorescence and TIRF images range from 0.88 to 0.96. A statistical analysis employing Costes’ randomization function to generate scrambled images yielded \( P > 98\% \). As a control, analysis of images of the RNase E-mCherry and AtpB-msfGFP, another inner membrane protein, resulted in Pearson’s correlation coefficients of less than 0.80. The statistical analysis together with the AtpB-msfGFP control is important experimental evidence for the association of RNase E, PNase, and RhlB as components of the RNA degradosome in living cells.

Epifluorescence measurements show that rifampin treatment results in the dispersion of puncta containing RNase E, PNase, and RhlB. Photobleaching measurements by TIRFm show that rifampin treatment relaxes constraints on the diffusion of RNase E, PNase, and RhlB. Punctum formation is thus due to clustering of the RNA degradosomes. There are three arguments against the hypothesis that RNA degradosome puncta are an artifact of fluorescence protein aggregation. (i) Punctum formation has been seen with fusions of four different fluorescence proteins (YFP, GFP, mCherry, and msfGFP) to three different components of the RNA degradosome (RNase E, PNase, and RhlB). (ii) With the exception of the MTS-msfGFP control, all fusion proteins in this work were expressed from single-copy chromosomal constructs at the \( \text{rne} \), \( \text{pnp} \), or \( \text{rhlB} \) locus under the control of endogenous expression signals. (iii) The dispersion of puncta by rifampin treatment argues that RNA degradosome clustering involves an additional factor whose presence depends on transcription.

Kasugamycin treatment results in dispersion of RNA degradosome puncta and relaxation of constraints on diffusion. Although this effect is the same as that observed with rifampin, the mechanism is necessarily different. Figure 8A shows a cartoon in which we propose that RNA degradosomes cluster upon binding to a polyribosome to form puncta. Figure 8B and C show the outcome of rifampin and kasugamycin treatment, respectively. In Fig. 8B, polyribosomes and mRNA are absent due to the degradation of mRNA, and there are fewer ribosomes due to the degradation of rRNA during rifampin treatment (43). In Fig. 8C, free ribosomes and mRNA are shown after kasugamycin treatment, since transcription continues and rRNA is not degraded (43, 57). The result of kasugamycin treatment shows that processing of tRNA and rRNA precursors, which continues (43), and degradation of ribosome-free mRNA do not result in the formation of puncta. Although ribosomes are freely diffusible, experimental work has shown that polyribosomes are constrained in a glass-like state (61, 62). Puncta could therefore be the result of the capture of slow-moving polyribosomes by RNA degradosomes. Our results strongly suggest that the puncta are sites of degradation of polyribosomal mRNA.

The inhibition of translation by chloramphenicol results in large clusters (foci) of RNase E-mCherry that are distinct from the puncta observed in untreated cells. The loss of an RNase E-mCherry signal in TIRF mode after chloramphenicol treatment strongly suggests that RNA degradosomes in the foci are not membrane attached. Recent work has shown that nitrogen starvation in \( \text{E. coli} \) triggers a stress response that results in the formation of foci containing RNA degradosomes and the RNA chaperone Hfq (63, 64). By analogy, we propose that the foci formed by chloramphenicol treatment are part of a stress response induced by the inhibition of translation elongation. We recently showed that rRNA is mostly stable after treatment with chloramphenicol, which contrasts with the massive degradation of rRNA after treatment with rifampin (43). The formation of foci could stockpile RNA degradosomes as part of a mechanism that protects inactive polyribosomes from degradation. Future work on characterization of the localization, composition, and dynamics of the chloramphenicol-induced RNA degradosome foci could give new insight into how the mRNA degradation machinery is regulated under stress conditions.

Biological systems are not engineered, they evolve, and conservation of RNase E.
homologues throughout the Proteobacteria emphasizes their importance in evolutionary fitness (4, 17). Protein sequence comparisons have shown that RNase E homologues in the gammaproteobacteria descended vertically from an ancient proto-RNase E that had a large noncatalytic region containing an MTS (18). Clustering of RNA degradosomes on polyribosomes could contribute to selectivity or efficiency in the initiation of mRNA degradation. Since bacteria are continually challenged by changes in the environment, the accuracy and speed with which gene expression is reprogrammed and fine-tuned are critical for survival of a population of cells. When bacterial cells are considered as a system, components such as the RNA degradosome interact with other components involved in the regulation of gene expression. Since mutations that affect RNA degradosome structure, function, or cellular localization could disrupt the correct functioning of other components, there is strong selective pressure to conserve its normal function(s). For this reason, we believe that RNA processing and mRNA degradation involving RNA degradosomes attached to the inner cytoplasmic membrane are conserved features of gammaproteobacterial cell biology.

MATERIALS AND METHODS

Media and strains. Liquid medium (LB) and agar (LA) plates were prepared as described elsewhere (65). Strains used in this work are listed in Table 1. NCM3416 was the parent strain used for *λ*red.
recombination as described elsewhere (36, 66). Briefly, DNA templates encoding C-terminal mCherry and mEGFP fusions were generated by crossover PCR with an frt-cat-frt cassette. The resulting products were transformed into NCM3416/pKD46 and selected at 37°C on LB plates containing 12.5 μg/ml chloramphenicol. Recombinants were colony purified. The constructs were genetically purified by P1 transduction into NCM3416. The cat cassette was removed by transformation with pCP20, which encodes the FLP recombinase (66). Constructs were validated by PCR amplification of genomic DNA, sequencing, and fluorescence microscopy. SLM019 and SLM025 were obtained by P1 transduction into Kts162. The rpoB (D516Y) mutation was selected as a spontaneous mutation of the NCM3416 strain, as described elsewhere (43).

**Microscopy and photobleaching.** TIRFm photobleaching experiments were carried out as described in reference 36. All epifluorescence and TIRFm acquisitions were taken independently and at least in duplicate for each strain and type of experiment. Images were analyzed using ImageJ v.1.38 (National Institutes of Health) (67, 68). Quantitative analyses of TIRFm photobleaching data to determine relative diffusion rates were performed as described in reference 52. The image analysis of colocalization experiments was carried out using ImageJ and the JACoP plugin (54). Graphic processing and two-tailed t tests for the statistical analysis were performed using GraphPad Prism v.8.0 with an alpha level of 5%.

**Affinity purification of native RNA degradosome.** The affinity purification protocol was adapted from procedures described in references 39 and 69. Buffers are described therein.

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**SUPPLEMENTAL MATERIAL**

Supplemental material is available online only.

**TEXT S1**

DOCX file, 0.01 MB.

**FIG S1**

TIF file, 1.2 MB.

**FIG S2**

TIF file, 1.1 MB.

**FIG S3**

TIF file, 0.4 MB.

**FIG S4**

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**FIG S5**

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**FIG S6**

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**FIG S7**

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**FIG S8**

TIF file, 0.4 MB.

**FIG S9**

TIF file, 1.3 MB.

**REFERENCES**

1. Mackie GA. 2013. RNase E at the interface of bacterial RNA processing and decay. Nat Rev Microbiol 11:45–57. https://doi.org/10.1038/nrmicro2930.

2. Saramago M, Barria C, Dos Santos RF, Silva IJ, Pobre V, Domingues S, Andrade JM, Viegas SC, Arraião CM. 2014. The role of RNases in the regulation of small RNAs. Curr Opin Microbiol 18:105–115. https://doi.org/10.1016/j.mib.2014.02.009.

3. Hui MP, Foley PL, Belasco JG. 2014. Messenger RNA degradation in bacterial cells. Annu Rev Genet 48:537–559. https://doi.org/10.1146/annurev-genet-120213-092340.

4. Al-Bara S, Carposious AJ. 2015. RNA degradosomes in bacteria and chloroplasts: classification, distribution and evolution of RNase E homologs. Mol Microbiol 97:1021–1135. https://doi.org/10.1111/mmi.13095.

5. Mohanty BK, Kushner SR. 2016. Regulation of mRNA decay in bacteria. Annu Rev Microbiol 70:25–44. https://doi.org/10.1146/annurev-micro-090914-014515.

6. Mohanty BK, Kushner SR. 2018. Enzymes involved in posttranscriptional RNA metabolism in Gram-negative bacteria. Microbiol Spectr 6:RWR-0011-2017. https://doi.org/10.1128/microbiolspec.RWR-0011-2017.

7. Carposious AJ, Luisi BF, McDowall KJ. 2009. Endonucleolytic initiation of mRNA decay in Escherichia coli. Prog Mol Biol Transl Sci 85:91–135. https://doi.org/10.1016/S0079-6603(08)00803-9.

8. Bechhofer DH, Deutscher MP. 2019. Bacterial ribonucleases and their roles in RNA metabolism. Crit Rev Biochem Mol Biol 54:242–300. https://doi.org/10.1080/10409238.2019.1651816.

9. Li Z, Deutscher MP. 2002. RNase E plays an essential role in the maturation of Escherichia coli tRNA precursors. RNA 8:97–109. https://doi.org/10.1017/s1355838202014929.

10. Ow MC, Kushner SR. 2002. Initiation of tRNA maturation by RNase E is essential for cell viability in E. coli. Genes Dev 16:1102–1115. https://doi.org/10.1101/gad.983502.

11. Penwez T, Hami D, Maples VF, Min Z, Wang BC, Kushner SR. 2008. Intragenic suppressors of temperature-sensitive rne mutations lead to the dissociation of RNase E activity on mRNA and tRNA substrates in Escherichia coli. Nucleic Acids Res 36:35306–35318. https://doi.org/10.1093/nar/gkn476.

12. Hammarlof DL, Bergman JM, Garmendia E, Hughes D. 2015. Turnover of mRNAs is one of the essential functions of RNase E. Mol Microbiol 98:34–45. https://doi.org/10.1111/mmi.13100.

13. Hughes D. 2016. Using the power of genetic suppressors to probe the essential functions of RNase E. Curr Genet 62:53–57. https://doi.org/10.1007/s00294-015-0510-1.

14. Callaghan AJ, Grossmann JG, Redko Yu, Ilag LL, Monciffe MC, Symmons MF, Robinson CV, McDowall KJ, Luisi BF. 2003. Quatermary structure and...
catalytic activity of the Escherichia coli ribonuclease E amino-terminal catalytic domain. Biochemistry 42:13848–13855. https://doi.org/10.1021/bi3035199.

35. Callaghan AJ, Marcia MJ, Stead JA, McDowell KJ, Scott WG, Luisi BF. 2005. Structure of Escherichia coli Rnase E catalytic domain and implications for RNA turnover. Nature 437:1187–1191. https://doi.org/10.1038/nature04084.

36. Callaghan AJ, Arizkii KJ, Ilag LL, Gunter Grossmann J, Chandran V, Kuhnle K, Poljak L, Capporius AJ, Robinson CV, Symmons MF, Luisi BF. 2004. Studies of the RNA degradosome-organizing domain of the Escherichia coli ribonuclease Rnase E. J. Mol Biol 340:965–979. https://doi.org/10.1016/j.jmb.2004.05.046.

37. Marcia MD, Defriso MA, Chandran V, Capporius AJ, Luisi BF. 2006. The RNA degradosome: life in the fast lane of adaptive molecular evolution. Trends Biochem Sci 31:359–365. https://doi.org/10.1016/j.tibs.2006.05.005.

38. Ait-Bara S, Capporius AJ, Quentin V. 2015. Rnase E in the gamma-Proteobacteria: conservation of intrinsically disordered noncatalytic and molecular evolution of microdomains. Mol Genet Genomics 290:847–862. https://doi.org/10.1007/s00438-014-0959-5.

39. Vanzo NF, Li SY, Py B, Blum E, Higgins CF, Raynal LC, Krisch HM, Carpousis AJ. 2007. The RNA degradosome of Escherichia coli: an mRNA-degrading machine assembled on RNase E. J. Mol Biol 367:113–132. https://doi.org/10.1016/j.jmb.2006.12.014.

40. Chandran V, Luisi BF. 2006. Recognition of enolase in the Escherichia coli RNA degradosome. J Mol Biol 358:8–15. https://doi.org/10.1016/j.jmb.2006.03.012.

41. Chandran V, Poljak L, Vanzo NF, Leroy A, Miguel RN, Fernandez-Recio J, Parkinsson J, Burns C, Capporius AJ, Luisi BF. 2007. Recognition and cooperation between the ATP-dependent RNA helicase RhlB and ribonuclease Rnase E. J. Mol Biol 367:113–132. https://doi.org/10.1016/j.jmb.2006.12.014.

42. Chaurasia L, Vaidyalingam B, Callaghan AJ, Luisi BF. 2009. Crystal structure of Escherichia coli polynucleotide phosphorylase core bound to Rnase E and mRNA and manganese: implications for catalytic mechanism and RNA degradosome assembly. J Mol Biol 389:17–33. https://doi.org/10.1016/j.jmb.2009.03.051.

43. Capporius AJ. 2007. The RNA degradosome of Escherichia coli: an mRNA-degrading machine assembled on Rnase E. Annu Rev Microbiol 61:71–87. https://doi.org/10.1146/annurev.micro.61.080706.093440.

44. Capporius AJ, Van Houver G, Ehretsmann C, Krisch HM. 1994. Copuri fer of the polynucleotide phosphorylase with RNase E: evidence for a specific association between two enzymes important in RNA processing and degradation. Cell 76:889–900. https://doi.org/10.1016/0022-8173(94)90363-8.

45. Py B, Higgins CF, Krisch HM, Capporius AJ. 1996. A DEAD-box RNA helicase in the Escherichia coli ribonuclease degradation reaction. Nature 381:169–172. https://doi.org/10.1038/381169a0.

46. Mizzac A, Kaderbini VR, Wei CL, Lin-Chiao S. 1996. Proteins associated with Rnase E in a multisubunit ribonucleoprotein complex. Proc Natl Acad Sci U S A 93:3865–3869. https://doi.org/10.1073/pnas.93.9.3865.

47. Khemici V, Capporius AJ. 2004. The RNA degradosome and poly(A) poly- merase of Escherichia coli are required in vivo for the degradation of small mRNA decay intermediates containing REP-stabilizers. Mol Microbiol 51:777–800. https://doi.org/10.1046/j.1365-2958.2003.03862.x.

48. Khemici V, Poljak L, Toeaes C, Capporius AJ. 2005. Evidence in vivo that the DEAD-box RNA helicase RhlB facilitates the degradation of ribosome-free mRNA by Rnase E. Proc Natl Acad Sci U S A 102:6913–6918. https://doi.org/10.1073/pnas.0511291102.

49. Ow MC, Liu Q, Kushner SR. 2000. Analysis of mRNA decay and rRNA processing in Escherichia coli in the absence of Rnase E-based degradation assembly. Mol Microbiol 38:854–866. https://doi.org/10.1046/j.1365-2958.2000.02016.x.

50. Stead MB, Marshburn S, Mohanty BK, Mitra J, Pena Castillo L, Ray D, van Bakel H, Hughes TR, Kushner SR. 2011. Analysis of Escherichia coli Rnase E and Rnase III activity in vivo using tiling microarrays. Nucleic Acids Res 39:3188–3203. https://doi.org/10.1093/nar/gkq1242.

51. Zhou L, Zhang AB, Wang R, Marcotte EM, Vogel C. 2013. The proteomic response to mutants of the Escherichia coli RNA degradosome. Mol Biol Syst 9:750–757. https://doi.org/10.1093/moleubio/mxs2513a.

52. Lopez PJ, Marchand J, Joyce SA, Dreyfus M. 1999. The C-terminal half of Rnase E, which organizes the Escherichia coli degradosome, participates in mRNA degradation but not rRNA processing in vivo. Mol Microbiol 33:188–199. https://doi.org/10.1046/j.1365-2958.1999.01465.x.

53. Masse E, Escorcia FE, Gottesman S. 2003. Coupled degradation of a small regulatory RNA and its mRNA targets in Escherichia coli. Genes Dev 17:2374–2383. https://doi.org/10.1101/gad.1127103.

54. Kimura S, Waldor MK. 2019. The RNA degradosome promotes tRNA quality control through clearance of hypomodified tRNA. Proc Natl Acad Sci U S A 116:1394–1403. https://doi.org/10.1073/pnas.1814130116.

55. Strahl H, Turlan C, Khalid S, Bond PJ, Kebalo JM, Peyron P, Poljak L, Bouvier M, Hamoen L, Luisi BF, Capporius AJ. 2015. Membrane recognition and dynamics of the RNA degradosome. PLoS Genet 11:e1004961. https://doi.org/10.1371/journal.pgen.1004961.

56. Moffitt JR, Pandy S, Boettiger AN, Wang S, Zhuang X. 2016. Spatial organization shapes the turnover of a bacterial transcriptome. Elife 5:13065. https://doi.org/10.7554/eLife.13065.

57. Khemici V, Poljak L, Luisi BF, Capporius AJ. 2008. The RNases E of Escherichia coli is a membrane-binding protein. Mol Microbiol 70:799–813. https://doi.org/10.1111/j.1365-2958.2008.06454.x.
56. Li GW, Burkhardt D, Gross C, Weissman JS. 2014. Quantifying absolute protein synthesis rates reveals principles underlying allocation of cellular resources. Cell 157:624–635. https://doi.org/10.1016/j.cell.2014.02.033.
57. Muller C, Sokol L, Vesper O, Sauert M, Moll I. 2016. Insights into the stress response triggered by kasugamycin in Escherichia coli. Antibiotics (Basel) 5:5020019. https://doi.org/10.3390/antibiotics5020019.
58. Kaberdina AC, Szafarski W, Nierhaus KH, Moll I. 2009. An unexpected type of ribosomes induced by kasugamycin: a look into ancestral times of protein synthesis? Mol Cell 33:227–236. https://doi.org/10.1016/j.molcel.2008.12.014.
59. Lambert T. 2012. Antibiotics that affect the ribosome. Rev Sci Tech 31:57–64. https://doi.org/10.20506 rst.31.1.2095.
60. Trache A, Meingner GA. 2008. Total internal reflection fluorescence (TIRF) microscopy. Curr Protoc Microbiol Chapter 2:Unit 2A.2.1–2A.2.22.
61. Bakshi S, Siryaporn A, Goulian M, Weisshaar JC. 2012. Superresolution imaging of ribosomes and RNA polymerase in live Escherichia coli cells. Mol Microbiol 85:21–38. https://doi.org/10.1111/j.1365-2958.2012.08081.x.
62. Parry BR, Surovtsev IV, Cabeen MT, O'Hern CS, Dufresne ER, Jacobs-Wagner C. 2014. The bacterial cytoplasm has glass-like properties and is fluidized by metabolic activity. Cell 156:183–194. https://doi.org/10.1016/j.cell.2013.11.028.
63. McQuail J, Switzer A, Burchell L, Wigneshweraraj S. 2020. The RNA-binding protein Hfq assembles into foci-like structures in nitrogen starved Escherichia coli. J Biol Chem 295:12355–12367. https://doi.org/10.1074/jbc.RA120.014107.
64. McQuail J, Carpousis AJ, Wigneshweraraj S. 2021. The association between Hfq and RNase E in long-term nitrogen starved Escherichia coli. bioRxiv https://doi.org/10.1101/2021.04.19.440462.
65. Miller JH. 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
66. Datsenko KA, Wanner BL. 2000. One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. Proc Natl Acad Sci U S A 97:6640–6645. https://doi.org/10.1073/pnas.120163297.
67. Collins TJ. 2007. ImageJ for microscopy. Biotechniques 43:25–30. https://doi.org/10.2144/000112517.
68. Schneider CA, Rasband WS, Eliceiri KW. 2012. NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9:671–675. https://doi.org/10.1038/nmeth.2089.
69. Morita T, Maki K, Alba H. 2005. RNase E-based ribonucleoprotein complexes: mechanical basis of mRNA destabilization mediated by bacterial noncoding RNAs. Genes Dev 19:2176–2186. https://doi.org/10.1101/gad.1330405.
70. Ait-Bara S, Carpousis AJ. 2010. Characterization of the RNA degradation of Pseudoalteromonas haloplanktis: conservation of the RNase E-RhlB interaction in the gammaproteobacteria. J Bacteriol 192:5413–5423. https://doi.org/10.1128/JB.00592-10.