SUPPLEMENTARY INFORMATION FOR

Spatial organization of the flow of genetic information in bacteria

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Includes supplementary figures, supplemental text, image and data analysis, modeling, supplementary tables, construction of plasmids and strains, and FISH probe sequences.
**a** | **b**
---|---
![Diagram](image1)

**c**

![Images](image2)

**d**

![Images](image3)

**e**

![Images](image4)

**f**

![Images](image5)

**g**

![Bar Chart](image6)

**h**

![Graph](image7)
Supplementary Fig. 1. The assembly-defective RNA-binding protein MS2(dFG) is better suited than MS2d to visualize transcripts in C. crescentus. a, Schematic representation of the methodology and genetic background of strain CJW2555. In this strain, a gene encoding an MS2d-mGFP fusion was placed on the chromosome under the control of the xylose-inducible promoter Pxy1 while mcherry transcriptionally fused to 48 MS2 binding sites (bs48) at the 3’ end was encoded from the vanilliacid-responsive promoter Pvan at the chromosomal vanA locus. b, Schematic representation of the methodology and genetic background of strain CJW2560. As in (a) except that Pxy1 expresses MS2(dFG)-mGFP, instead of MS2d-mGFP. c, Top panels, fluorescent images of CJW2555 cells producing MS2d-mGFP and mcherry-bs48 mRNA by growing in the presence of xylose and vanillic acid for 1.5 h. Bottom panels, fluorescent images of control CJW2780 cells producing MS2d-mGFP only (i.e., there is no inserted mcherry-bs48 construct in this strain). Images were acquired using the GFP (left) and mCherry (right) filter sets. MS2d-mGFP displayed a largely diffuse signal in the cytoplasm in control CJW2780 cells. However, fluorescent foci appeared in some CJW2555 cells when MS2d was co-produced with mcherry-bs48 mRNA. These foci were found internally or at cell poles. Fluorescent mCherry signal was detected in the cytoplasm in CJW2555 cells, consistent with production of mCherry protein. d, As in (c), except that the top panels shows CJW2560 cells producing MS2(dFG)-mGFP and mcherry-bs48 mRNA, and the bottom panels show control CJW2556 cells producing MS2(dFG)-mGFP alone (i.e., there is no inserted mcherry-bs48 construct). MS2(dFG)-mGFP displayed a largely diffuse signal in the cytoplasm in control CJW2556 cells. However, fluorescent foci appeared in some CJW2560 cells when MS2(dFG)-mGFP was co-produced with mcherry-bs48 mRNA. These foci were found internally and not at the cell poles. Fluorescent mCherry signal was detected in the cytoplasm in CJW2560 cells, indicating mCherry production. e, Time-lapse microscopy of CJW2555 cells producing MS2d-mGFP and mcherry-bs8 mRNA after 1.5 h growth in liquid culture at 30°C in the presence of xylose and vanillic acid. The agarose pad also contained vanillic acid (and no xylose). Images were taken at 30-sec intervals for 30 min at room temperature; only selected images from this time-lapse sequence are shown (h:min:sec). These experiments showed that polar MS2d-mGFP foci (arrow) are long lived. f, Time-lapse microscopy of CJW2560 cells growing on an agarose padded-slide containing vanillic acid to maintain expression of mcherry-bs48 mRNA. Prior to microscopy, the cells were grown in a liquid culture in the presence of xylose and vanillic acid for 1.5 h to produce MS2(dFG)-mGFP and mcherry-bs48 mRNA after 1.5 h growth in liquid culture at 30°C in the presence of xylose and vanillic acid. The agarose pad also contained vanillic acid (and no xylose). Images were taken at 30-sec intervals for 30 min at room temperature; only selected images from this time-lapse sequence are shown (h:min:sec). These experiments showed that polar MS2d-mGFP foci (arrow) are long lived. g, Time-lapse microscopy of CJW2560 cells growing on an agarose padded-slide containing vanillic acid to maintain expression of mcherry-bs48 mRNA. Prior to microscopy, the cells were grown in a liquid culture in the presence of xylose and vanillic acid for 1.5 h to produce MS2(dFG)-mGFP and mcherry-bs48 mRNA after 1.5 h growth in liquid culture at 30°C in the presence of xylose and vanillic acid. The agarose pad also contained vanillic acid (and no xylose). Images were taken at 30-sec intervals for 30 min at room temperature; only selected images from this time-lapse sequence are shown (h:min:sec). These experiments showed that polar MS2d-mGFP foci (arrow) are long lived. h, Western blot of protein extracts from cells producing either MS2d-mGFP (CJW2555) or assembly-defective MS2(dFG)-mGFP (CJW2560), in the presence or absence of mcherry-bs48 mRNA. CJW2560 cells were grown in the presence of 0.3% xylose for 5 h to produce high levels of MS2(dFG)-mGFP, and without (lane 1) or with (lane 2) 0.5 mM vanillic acid for 5 h to express mcherry-bs48 mRNA. CJW2555 cells were grown under identical conditions to produce MS2d-mGFP in the absence (lane 3) or presence (lane 4) of mcherry-bs48 mRNA. SDS-PAGE followed by Western blot analysis using anti-GFP antibodies revealed that MS2d-mGFP, unlike MS2(dFG)-mGFP, tends to make stable aggregates when co-produced with mcherry-bs48 mRNA. Asterisk shows a minor degradation product. i, Quantification of GFP foci in CJW2560 cells producing MS2(dFG)-mGFP and mcherry-bs48 mRNA after 1.5 h of xylose and vanillic acid induction before (blue) and after (crimson) treatment with rifampicin (25 μg/ml) for 20 min. Foci were detected using spotFinder. N = 3 experiments; ≥164 cells for each experiment without rifampicin and ≥179 cells for each experiment with rifampicin.
Supplementary Fig. 2. Validation of the lacO method. a, Visualization of \textit{groESL-lacO}_{120} mRNA-expressing CJW2966 cells using lacO-FITC LNA probe before (left) or after treatment with DNase I (middle) or RNase A (right). b, RNA FISH microscopy of \textit{groESL-lacO}_{120} mRNA-expressing CJW2966 cells using lacO-FITC or the complementary probe, lacO-Rev-FITC. c, Visualization of \textit{groESL-lacO}_{120} mRNAs in CJW2966 cells using groEL-Cy3 and lacO-FITC probes. d, Decay fit of the \textit{groESL-lacO}_{120} mRNA signal (detected with the lacO-Cy3 probe) in CJW2966 cells after rifampicin addition. Cells were grown at 30°C (blue) or 42°C (red) for 15 min before rifampicin addition. Error bars indicate standard deviation from 2 independent experiments (>245 cells for each time point).
Supplementary Fig. 3. Visualization of endogenous creS mRNA by FISH. a, Visualization of native creS mRNA in wild-type CB15N cells by RNA-FISH using 38 Cy3-labeled DNA probes that hybridize in tandem to the creS mRNA sequence. Arrows show wild-type cells with a pattern of fluorescent signal similar to that of ΔcreS cells (see panel c). b, Representative fluorescent intensity profiles of individual wild-type cells exhibiting either one (left), two (center), or zero (right) polar accumulation(s). The red dashed line represents the average background fluorescence determined from the signal intensity obtained from ΔcreS cells treated under identical conditions (see panel c). c, Visualization of background fluorescence in ΔcreS cells (negative control) due to unspecific probe binding and cellular autofluorescence using conditions as in (a). d, Representative fluorescent intensity profiles of individual ΔcreS cells. The red dashed line represents the average background fluorescence in ΔcreS cells.
Supplementary Fig. 4. Limited dispersion of mRNA is common. a, Visualization of divJ-lacO\textsubscript{120} mRNA in CJW2968 cells by RNA FISH using the lacO-Cy3 probe. b, Representative intensity profiles of divJ-lacO\textsubscript{120} mRNA (hybridized with the lacO-Cy3 probe) along the cell length in individual CJW2968 cells. The red dashed line corresponds to the measured background fluorescence. c, divJ-lacO\textsubscript{120} mRNA dispersion inside cells was determined by plotting the distribution of FWHM values for each peak obtained from intensity profiles of CJW2968 cells. d-f, Same as (a-c) for ompA-lacO\textsubscript{120} mRNA in CJW3093 cells. g-i, Same as (a-c) for mcherry-lacO\textsubscript{120} mRNA in CJW3097 cells after 10 min of induction with 0.5 mM vanillic acid. j-l, Same as (a-c) for flagellin fljK-lacO\textsubscript{120} mRNA in CJW3364 cells.
Supplementary Fig. 5. divJ-bs48 mRNA foci colocalize with divJ gene loci. 

**a.** Schematic representation of the methodology and genetic background used to simultaneously visualize divJ mRNA and gene locus. In strain CJW2783, divJ is transcriptionally tagged with bs48, allowing visualization of the resulting mRNA using MS2(dlFG)-mCherry produced from Pxyl at the chromosomal xylX locus. This strain also contains a lacO_{240} array inserted nearby the divJ-bs48 gene, which can be used to visualize this gene locus by DNA binding of LacI-CFP also produced from Pxyl. 

**b.** Inverted fluorescence images of CJW2783 cells grown in the presence of xylose for 1h to induce the synthesis of both MS2(dlFG)-mCherry and LacI-CFP. The foci corresponding to MS2(dlFG)-mCherry-labeled divJ-bs48 mRNA (middle) colocalize with LacI-CFP foci representing chromosomal loci (left). The right panel displays processed images from cellTracker and spotFinder analysis, showing cell outlines and identified mRNA foci (red), gene loci (green), and overlap (yellow). Arrows indicate mRNA foci appearing at or near corresponding gene loci.
**Supplementary Fig. 6.** Calculated mRNA distribution profiles show a largely uniform spatial distribution inside cells, regardless of mRNA size or ribosome occupancy. Calculated mRNA distribution profiles in a 3-µm virtual cell. Profiles were calculated with Eq.[6] (see Supporting Information) for (a) 1-kb mRNA (common size) and (b) 20-kb mRNA (illustrating long polycistronic transcripts), either free of ribosome (blue) or saturated with ribosomes (red). The dotted line delineates the source of mRNA (site of transcription) located at the 1.5-µm position. The model assumes a rate of transcription of 0.06 s\(^{-1}\) and a rate of mRNA degradation of 0.004 s\(^{-1}\) (which corresponds to a half-time of 3.5 min). These values resulted in a total of 15 mRNAs of interest per cell.
Supplementary Fig. 7. Comparison of dispersion and intensity distributions for groESL and groESL-lacO_{120} after heat shock. a, The distribution of groESL-lacO_{120} mRNA dispersion (based on FWHM values) in heat shock-treated CJW2966 cells using the lacO-Cy3 probe (red) is similar to that of natural groESL mRNA dispersion in wild-type, heat shock-treated cells using a groEL-Cy3 probe (green). Overlapping distribution is in yellow. b, Similarly, the distribution of groES-lacO_{120} mRNA levels (maximal fluorescence intensities) in heat shock-treated CJW2966 cells using the lacO-Cy3 probe (red) is, after normalization by the mean fluorescence intensity, similar to that of natural groESL mRNA levels in wild-type, heat shock-treated cells using the groEL-Cy3 probe (green).
Supplementary Fig. 8. Mobility of L1-GFP is affected by rifampicin treatment. a, Quantification of fluorescence remaining in unbleached cellular regions after 10-s photobleaching of cell poles in the absence of rifampicin (n = 14 cells) or after 30 min (n = 22 cells), 1 h (n = 34 cells), and 2 h (n = 18 cells) of rifampicin treatment. Error bars represent the standard deviation. b, Methylene blue staining of total RNA extracted from untreated cells, and after rifampicin treatment for 30 min, 1 h or 2 h.
Visualization of mRNA in live *C. crescentus* cells. A method to visualize mRNA in living eukaryotic cells was first developed in yeast, using a GFP-tagged RNA-binding protein, MS2, to label an array of MS2 binding sites transcriptionally fused to the mRNA of interest\(^1\). This approach was adapted for *E. coli*, notably by increasing the number of MS2 binding sites to amplify the bound fluorescent signal and by using a dimeric variant of MS2 (MS2d)\(^2\). While this method has shown great value in quantifying transcriptional bursting\(^3\), it resulted in persistence of the MS2d-GFP signal throughout the cell’s lifetime\(^2,3\), inconsistent with the typically short half-life of bacterial mRNAs\(^4\). Since MS2 is a bacteriophage coat protein that is capable of self-assembly into a phage capsid, we suspected that immortalization of the fluorescent signal may be caused by self-aggregation of MS2d-GFP when mRNA binding brings subunits in close proximity. To test this idea, we attempted to visualize chromosomally expressed *mcherry* mRNAs tagged with 48 MS2 binding sites (*bs48*) in *C. crescentus* using either MS2d (Supplementary Fig. 1a) or an assembly-defective MS2 [MS2(dlFG)] mutant\(^5\) (Supplementary Fig.1b), which is commonly used in eukaryotic mRNA studies. Each MS2 variant was fused to a monomeric version of GFP (mGFP). *mcherry-bs48* and the mGFP-tagged MS2 variant were each produced from the chromosome under inducible promoters *Pvan* and *Pxyl*, respectively (Supplementary Fig. 1a-b).

Synthesis of MS2d-mGFP in *mcherry-bs48* mRNA-expressing cells generated bright fluorescent foci with long lifespans (Supplementary Fig. 1c, e) in 60% of cells (n=1527). Conversely, MS2(dlFG)-mGFP gave weak, short-lived fluorescent foci in 16% of cells (n=559; Supplementary Fig.1d) that appeared and disappeared over time (Supplementary Fig. 1f). Using the assembly-capable MS2d-mGFP also caused inconsistent results with respect to the localization of fluorescent foci. When *mcherry-bs48* mRNA and MS2-mGFP were produced, the cells displayed non-polar and/or polar fluorescent foci (Supplementary Fig. 1c). Polar localization became predominant with increased MS2d-mGFP levels (Supplementary Fig. 1g; n = 3 experiments, ≥101 cells for each time-point). On the other hand, GFP foci formed from assembly-defective MS2(dlFG)-mGFP almost exclusively exhibited non-polar localization (Supplementary Fig. 1d), and the localization pattern did not change significantly between 1.5 h and 3 h.
induction of MS2(dlFG)-mGFP synthesis (Supplementary Fig. 1g; n = 3 experiments, ≥106 cells for each time point). FISH experiments described in the main text (Supplementary Fig. 3g) confirmed that the polar localization of MS2d-mGFP foci was artefactual, possibly because aggregates tend to accumulate at the cell poles\textsuperscript{6,7}. Western blot analysis confirmed that MS2d-mGFP, unlike MS2(dlFG)-mGFP, had the capability of forming in vivo aggregates when mcherry-bs48 mRNAs were expressed (Supplementary Fig. 1h). The fluorescent foci generated in some cells when both MS2(dlFG)-mGFP and mcherry-bs48 mRNA were produced corresponded to MS2(dlFG)-mGFP-bound mRNA signal as they were virtually absent in mRNA-depleted cells after treatment with the transcriptional initiation inhibitor rifampicin (Supplementary Fig. 1i). While using MS2(dlFG)-mGFP to label mRNAs was clearly the better choice for qualitative visualization of mRNA in \textit{C. crescentus}, the significant background fluorescence caused by unbound MS2(dlFG)-mGFP masked some bs48-tagged mRNAs, preventing us from quantifying the spatial distribution of mRNA. The signal-to-noise ratio could not be sufficiently improved by varying the expression level of MS2(dlFG)-mGFP. This is likely due to the poor binding capability of MS2(dlFG) to its target RNA sequences when fused to a fluorescent protein. Consistent with this interpretation, it has been shown in vivo using a blue-white colony assay\textsuperscript{8} that unlike MS2d-GFP, a GFP fusion to MS2(dlFG) is unable to repress the translation of the MS2 replicase fused to β-galactosidase fusion\textsuperscript{2}. Because of these caveats, we turned to RNA FISH for our analysis.

**Visualization of endogenous mRNAs in wild-type cells using RNA FISH and multiple DNA probes**

A single LNA probe of 17 nt complementary to native mRNA in wild-type CB15N cells typically did not provide enough sensitivity for visualization (except for the naturally abundant \textit{groESL} mRNA; Fig. 1a). Therefore, to enhance detection of native mRNA, we used multiple Cy3-labeled DNA probes that bind in tandem to the target mRNA, as previously described\textsuperscript{9-11}. We visualized \textit{creS} mRNA because its gene locus is in close proximity to the origin and thus expected to be polarly localized. A \textit{creS} deletion strain was used to assess nonspecific probe binding. Hybridization of 38 Cy3-labeled DNA
probes of 25 to 30 nt of length (see list below) to the creS mRNA sequence showed accumulation of fluorescent signal to one or both cell poles in many wild-type cells (Supplementary Fig. 3a and b, left and center graphs). A fraction of wild-type cells displayed an uneven but largely dispersed signal (Supplementary Fig. 3a, arrows; and Supplementary Fig. 3b, right graph) that was similar to the unspecific signal observed in all ΔcreS cells hybridized under identical conditions (Supplementary Fig. 3c and d, all 3 graphs). For quantification and assessment of the signal-to-noise ratio, we determined the average background signal (red dotted line in Supplementary Fig. 3b and d) due to nonspecific probe binding and cell autofluorescence by calculating the mean of the fluorescence intensity in ΔcreS cells (n=2098 cells) normalized by the cell area.

Fluorescence intensity profiles along the cell length clearly showed that the polar signal in wild-type cells was well above background (Supplementary Fig. 3b), in striking contrast to the signal of ΔcreS cells (Supplementary Fig. 3d). The polar creS mRNA peaks in wild-type cells were diffraction-limited (FWHM=0.37±0.14 μm; n=338 peaks), confirming that native creS mRNAs display limited dispersion from their site of synthesis, which further validates the lacO tagging method (Fig. 2d). It should be noted that comparatively to the 38-probe approach, the lacO method gives a higher signal-to-noise ratio. In the lacO method, one single LNA probe hybridizes up to 120 equivalent sites whereas in the multiprobe method, 38 distinct probes hybridize to 38 different sites. Thus, there are more binding sites in the lacO method. Additionally, the background fluorescence is about 6 times higher when using 38 DNA probes relative to one single LNA lacO probe because increasing the number of probes to improve the fluorescence signal also has the detrimental effect of increasing unspecific binding.

To expand and generalize our findings, we used the multi-probe approach to examine the localization of LacZ-encoding mRNA in wild-type MG1665 E. coli cells during non-induced or induced conditions. We used 48 Cy3-labeled probes complementary to the lacZ coding region. We showed that after induction with IPTG, lacZ containing mRNA is localized as 1 or 2 spots in wild type cells (Fig.3a), while non-induced cells did not show an accumulation of lacZ mRNA signal (Fig.3c). We determined the fluorescence background by calculating the mean of the average fluorescence intensity from non-induced cells (n=1011 cells). Intensity profiles along the
cells length showed an accumulation of fluorescence in one (Fig. 3b right) or two (Fig. 3b left) peaks that were well above background, as opposed to non-induced cells (Fig. 3d). Measurements of the FWHM of these RNA spots showed that they are diffraction limited (Fig.3 e). Quantification of the percentage of induced cells with RNA spots is shown in Table S2.

Assessment of ribosomal RNA integrity after rifampicin treatment
Total RNA was extracted as previously described from exponentially growing _C. crescentus_ cultures immediately after addition of rifampicin, and after treatment for 30 min, 1 h, or 2 h. Five micrograms of total RNA from each sample were loaded into a formaldehyde agarose gel, and the RNA was transferred to a nylon membrane using a downward capillarity transfer system. Ribosomal RNA bands were detected by methylene blue staining (Supplementary Fig. 8b).

IMAGE AND DATA ANALYSIS

Outlining cells and computing fluorescence intensity profiles (MicrobeTracker):
Cells were outlined in phase images using custom-made MATLAB-based program named MicrobeTracker, which will be described in detail in a manuscript in preparation. Briefly, individual cells were identified from phase images by thresholding and using an edge detection routine. The identified cells were then outlined with a continuous contour, which was adjusted using a version of the Point Distribution Model (PDM)\textsuperscript{17}, using wild-type CB15N cells as a training set. The resulting outline was smoothed using Fourier transform keeping only the first 16 modes. The outline was then used to determine the centerline (skeleton) of the cell. A series of perpendiculars was placed at every pixel along the centerline, thus creating a mesh and splitting the cell into segments along its length.

To obtain fluorescence intensity profiles, the background fluorescence intensity was first computed by averaging fluorescence intensity in cell-free areas of the image and was then subtracted from the image. After applying the cell mesh, the intensity of each segment was integrated and divided by the area of the segment. All operations were
performed in MATLAB using MATLAB standard routines and the Image Processing Toolbox.

Measurements of background cellular fluorescence: A sample of wild-type CB15N cells (which lack the lacO array) was included in each RNA FISH experiment that used the lacO probe. This sample, which was treated under the same conditions (including sample preparation and camera settings) as for the strains that contain the lacO120 array, served as a negative control to assess unspecific binding of the lacO probe and cellular autofluorescence. This background fluorescence was estimated by determining the mean of the fluorescence intensity normalized by the cell area.

Detecting mRNA/DNA Fluorescence Foci (SpotFinder)
To identify fluorescence foci, we developed another custom-built MATLAB-based program named SpotFinder. This program is based on the idea that foci are equivalent to round, diffraction-limited spots on uneven background. It uses band-pass spatial filtering adjusted to detect only the objects close to diffraction-limited spot sizes. Filtered images were processed using morphological opening in order to eliminate non-spherical features. A histogram of integrated spot intensities was computed and compared to a histogram obtained from control cells. From this comparison, a threshold was calculated such that spots above this threshold include no more than 5% of false positives. Importantly, SpotFinder was only used to find the position of fluorescent spots and construct a mask to be applied to the original image. The integrated fluorescence of each spot was calculated from the original image. SpotFinder was also used to quantify the number of mRNA foci in cells after RNA FISH (see Table S2).

mRNA half-time measurements by FISH
Cell samples at different time points after rifampicin (100 μg/ml) addition were immediately fixed with 4% formaldehyde. RNA FISH was performed as described in the method section. Fluorescence intensity in the cell population was calculated for each time point after rifampicin addition using MicrobeTracker. The background fluorescence obtained from wild-type cells lacking a lacO120 array was subtracted from each value.
The corrected values were plotted versus time and fitted using a least squares fit with an exponential function \( I = I_0 \exp(-\lambda t) + I_b \) where \( I_0 \) is the initial intensity, \( I_b \) is the background intensity, and \( \lambda \) is the decay coefficient. Note that the fluorescent intensity values at time 0 and 2 min were similar, which presumably reflects the time for rifampicin to enter the cells and to inhibit transcription. The 0-min values were therefore excluded to allow better curve fitting. The calculated decay coefficients \( \lambda \) for \( groESL-lacO_{120} \) mRNA under normal growth and after heat shock were 0.21 and 0.23, respectively. The R-square coefficients were 0.9999 and 0.9997, respectively. The calculated half-times using the decay coefficients were 3.48±0.15 min without heat shock and 3.01±0.3 min after heat shock.

**mRNA half-time measurements by real-time PCR**

The samples of CB15N or CJW2966 exponentially growing cultures at different time points following rifampicin (100 \( \mu \)g/ml) addition were centrifuged for 30 s and the pellets were immediately resuspended in Trizol (Sigma). Total RNA was then extracted as previously described\(^{12}\) and cDNA was obtained for each sample using High capacity cDNA reverse transcription kit (Applied Biosystems 4368814). The efficiency test for \( groESL \) and 16s-specific primers and the amplification of the products were performed following the Power SYBR Green PCR protocol (Applied Biosystems) using a 7900HT ABI PRISM Real Time PCR System. The efficiency for \( groESL \) and 16S-specific primers was determined to be 89.7% and 89.9%, respectively. The relative amount of \( groESL \) and \( groESL-lacO_{120} \) mRNAs at each time point was calculated by comparing the threshold cycle (Ct) values obtained using the software analysis tool. The relative decrease in mRNA level between time points after rifampicin addition was calculated using the Pfaffl method\(^{18}\) using the equation \( F = (E_{groESL})^{\Delta Ct}/(E_{16s})^{\Delta Ct} \) where \( F \) is the relative difference between time points, \( E_{groESL} \) and \( E_{16s} \) are the efficiencies for \( groESL \) and 16S specific primers, respectively. The normalized values were plotted against time and fitted as described above. The calculated decay coefficients \( \lambda \) for \( groESL \) and \( groESL-lacO_{120} \) mRNAs under normal growth conditions were 0.15 and 0.17, respectively, and after heat shock were 0.14 and 0.16, respectively. The calculated half-times using the decay coefficients were 4.9 ± 1.6 min and 4.35 ±1.1 min for \( groESL \) and
**groESL-lacO_{120}** mRNAs under normal growth, and 5.1 ± 0.6 min and 4.2 ± 0.7 after heat shock, respectively. The differences in half-time values were found to be not statistically different using the one-way ANOVA test from MATLAB Statistics Toolbox (**p-value** 0.703).

Note that the small difference between half-time values obtained by RNA FISH and real-time PCR likely comes from the way the samples were collected. In the FISH method, the samples were immediately fixed, which stops mRNA decay, whereas in the real-time-PCR method, the samples were first centrifuged before cell lysis and RNA extraction, which introduced a delay that was difficult to determine with precision.

**MATHEMATICAL MODELING**

**mRNA intensity profiles obtained for a standard Transcription-Diffusion-Degradation model**

We wanted to examine what the spatial profile of mRNA would be if the full-length transcripts were freely diffusible. In the following, we used standard assumptions:

1) Full-length mRNA transcripts diffuse throughout the cell with a diffusion coefficient $D$.

2) The rate of degradation of mRNA is proportional to mRNA concentration, $C$, with rate constant $k_{deg}$, consistent with the exponential decay of mRNA signal observed in rifampicin-treated cells (Supplementary Fig.2d). We also assume that $k_{deg}$ is spatially uniform in the cytoplasm. Although results presented in this study show patchy RNase E pattern (Fig.6a), those patches are spread around DNA region without particular localization. The mRNA of interest can be degraded at any of these “degradation points”. To simplify the model, we substitute actual RNase distribution with the effective uniform one. Using a multi-peak distribution of RNase E does not change significantly mRNA profiles and most importantly, does not change the degree of mRNA localization (data not shown).

3) We assume a simple 1D geometry with reflective boundary conditions at the cell poles located at $x=0$ and $x=3\mu$m and a point source of mRNA at mid cell $x_0=1.5\mu$m with constant rate $k_{tr}$. 

17
Under these conditions, the spatiotemporal dynamic of the mRNA concentration \( C(x,t) \) is symmetric around the mid-cell position \( x_0 \) and is described by the equation:

\[
\frac{\partial C(x,t)}{\partial t} = D \frac{\partial^2 C}{\partial x^2} - k_{\text{deg}} \cdot C(x,t) \tag{1}
\]

with a reflective boundary conditions at \( x=l \):

\[
\frac{\partial C(x=l,t)}{\partial x} = 0 \tag{2}
\]

and a constant influx of transcript at \( x=x_0 \):

\[
- \frac{\partial C(x=x_0,t)}{\partial x} = \frac{k_{\text{tr}}}{2D} \tag{3}
\]

At steady state the solution reads

\[
C(x) = k_{\text{tr}} \cdot \lambda \cdot \left[ \frac{e^{x/\lambda} + e^{-x/\lambda}}{2 \cdot D \cdot \left\{ e^{x_0/\lambda} - e^{-x_0/\lambda} \right\}} \right] \quad \text{if } x \leq x_0 \tag{4}
\]

\[
C(x) = k_{\text{tr}} \cdot \lambda \cdot \left[ \frac{e^{(l-x)/\lambda} - e^{(-l-x)/\lambda}}{2 \cdot D \cdot \left\{ e^{x_0/\lambda} - e^{-x_0/\lambda} \right\}} \right] \quad \text{if } x > x_0
\]

where \( \lambda = (D/k_{\text{deg}})^{1/2} \) characterizes the width of the mRNA spot around the point source.

Due to the diffraction limit, the measured signal is a convolution of the mRNA concentration profile from Eq.4 with a point spread function (PSF), which we approximate as:

\[
PSF(x,s) = I_0 \cdot \exp\left[ -\frac{(x-s)^2}{2w^2} \right] \tag{5}
\]

where \( I_0, w, x \) and \( s \) are normalization coefficient, width, coordinate of observation point, and coordinate of point source in image plane, respectively. The \( w \) value was obtained by fitting experimentally-determined fluorescence profiles of 175nm-sized red fluorescent beads. The resulting profile is:

\[
I(x) = A \cdot \int C(s) \cdot PSF(x,s) \, ds \tag{6}
\]

For the transcription rate, we use \( k_{\text{tr}}=0.06 \text{ s}^{-1} \), which corresponds to approximately 1 transcript per 17 s (this parameter does not influence the spatial distribution of mRNA) and \( k_{\text{deg}}=0.004 \text{ s}^{-1} \), which corresponds to the measured 3.5 min half-time of \textit{groESL-lacO120} mRNA (Supplementary Fig. 2d).

To estimate the diffusion coefficient of mRNA, we consider mRNA as an ideal chain and calculate gyration radius as \( R_G = a \cdot (N/6)^{1/2} \) where \( a \) is a monomer size and \( N \) is a number of monomers\(^19\). We supposed an “effective monomer size” \( a=1 \text{ nm} \) (\( \sim 3 \) nucleotides), which corresponds to the persistence length of single stranded RNA\(^20\).

Considering chains with \( N=333 \) (corresponds to a 1-kb mRNA), 2100 (for the 6.3 kb \textit{groESL-lacO} mRNA) or 6660 (for a 20-kb mRNA), this results in \( R_G \) of 8 nm, 19 nm, and 33 nm for free mRNA, respectively. In the case of RNA maximally loaded with...
ribosomes, we considered \( a = 20 \) nm, implying that persistence length is determined by the ribosome to ribosome distance with value obtained from cryo-electron tomograms\(^{21}\). This gives: \( R_G \) of 34 nm for a 1-kb mRNA, 60 nm for the 6.3-kb \textit{groESL-lacO} mRNA (\( a = 20 \) nm, \( N = 38 \) and \( a = 1 \) nm, \( N = 566 \) for translated and untranslated portions of the \textit{groESL-lacO} mRNA, respectively), and 150 nm for a 20-kb transcripts.

We used value reported for the diffusion coefficient of GFP molecule within bacterial cell, \( D_{GFP} = 6 \) \( \mu \text{m}^2\text{s}^{-1} \)\(^{22}\), as an estimate a viscosity of the bacterial cytoplasm. Assuming that Stocks radius \( R_S \) and \( R_G \) of chain are connected as \( R_G = (2/5)^{1/2} R_S \approx 0.63 R_S \) (as it would be for solid sphere), and that \( D \) equals to \( kT/(6\pi \eta R_S) \), we estimated diffusion coefficient as \( D = D_{GFP}(0.63 R_{GFP}/R_G) \), where \( R_{GFP} = 2.4 \) nm\(^{23}\). This gives estimates \( D \) of 1 \( \mu \text{m}^2\text{s}^{-1} \) for 1-kb free mRNA; 0.5 for 6.3- kb free \textit{groESL-lacO} mRNA; 0.3 for 20-kb free mRNA; 0.3 for 1-kb mRNA maximally loaded with ribosomes; 0.1 for 6.3- kb \textit{groESL-lacO} mRNA maximally loaded with ribosomes (in this case, only the 2.3-kb \textit{groESL} portion was considered occupied by ribosomes since the \textit{lacO} array is non-coding); and 0.06 for a long 20-kb mRNA maximally loaded with ribosomes.

Fig. 4a and Supplementary Fig. 6a-b represent expected spatial profiles defined by Eq.6 for these different values of \( D \), assuming a transcription rate of 0.06 \( \text{s}^{-1} \) and a mRNA degradation rate of 0.004 \( \text{s}^{-1} \) (corresponding to half-time of 3.5 min). These values resulted in a total of 15 mRNAs per cell. These calculated profiles are essentially uniform throughout the entire cell in sharp contrast with the experimentally observed profiles (Figs. 2a, 2d, 4c and Supplementary Fig 4b, e, h, k). This discrepancy suggests that constraints limit the diffusion of transcripts inside cells.

**Experimental determination of the apparent diffusion coefficient \((D_a)\) for \textit{groESL-lacO120} mRNA in heat shocked-treated cells**

We determined an apparent diffusion coefficient of mRNA by fitting the Transcription-Diffusion-Degradation model described above to mRNA profiles experimentally obtained in individual cells (see Fig. 4c for examples).

Eq. 6 was fitted to fluorescence mRNA profiles of individual cells using the least square fitting routine \textit{lsqcurvefit} from MATLAB. For each cell, cell length \( l \) was determined from corresponding phase image, and \( A, \lambda, \) and \( x_0 \) were obtained by fitting.
Since two-peak fluorescence distributions were also observed in experiments (which corresponded to cells with two segregated \textit{groESL-lacO}_{120} gene locus, see Fig. 4c, left for example), we also tried to fit each mRNA profile with a model that included two sources of mRNA (each with an independent intensity $A$ and a source location $x_0$ but with the same $\lambda$). To decide whether the one-source or two-source model describes the data best, we assessed the sensitivity of the best fit to the changes in parameters values by calculating the mean relative error $\epsilon_{av}=(\Sigma(\delta a_i/a_i)^2)/n_p$, where $n_p$ is a number of free parameters in the fitting ($n_p=3$ and 5 for one-source and two-source models, respectively), $\delta a_i$ is a confidence interval for the parameter estimate, and $a_i$ is a value of parameter with summation over all parameters. The model with the highest sensitivity to changes in the parameters was chosen (fluorescence profiles that gave $\epsilon_{av}>0.5$ for both one-source and two-source models were excluded from the analysis).

This procedure was applied to each fluorescence profiles resulting in $\lambda=0.37\pm0.1$ $\mu$m averaged from two independent experiments ($n=1,717$ and 974 individual cells). The relative accuracy of individual $\lambda$ estimates ($\delta \lambda/\lambda$) ranged from 0.14 to 0.8 for more than 80% of analyzed cells. Taking into account the measured degradation rate $k_{deg}=0.004$ s$^{-1}$ (from Supplementary Fig. 2d), $\lambda=0.37$ $\mu$m gives an estimate for $D_a$ of 0.0005$\pm$0.0003 $\mu$m$^2$s$^{-1}$.

**SUPPLEMENTARY TABLES**

**Table S1.** Strain and plasmid table

| Strain  | Relevant Genotype or Description | Reference or Source |
|---------|---------------------------------|---------------------|
| **C. crescentus strains** | | |
| CB15N   | Synchronizable variant of CB15 (also NA1000) | 14 |
| CJW2555 | CB15N \textit{vanA}::pHL23Pvan-mcherrybs48tm \textit{xylX}::pBGentPxyLM2dmsGFP | This study |
| CJW2556 | CB15N \textit{xylX}::pBGentPxyLM2(dFG)mGFP | This study |
| CJW2560 | CB15N \textit{vanA}::pHL23Pvan-mcherrybs48tm \textit{xylX}::pBGentPxyLM2(dFG)mGFP | This study |
| CJW2780 | CB15N \textit{xylX}::pBGentPxyLM2dmGFP | This study |
| CJW2781 | CB15N \textit{xylX}::pHPV543::pBGentPxyLM2(dFG)-mcherry | This study |
| Strain | Description |
|--------|-------------|
| CJW2783 | CB15N \( divJ::pHL23\)divJc-bs48-3′UTR::pBAp-LacOxylX::pHPV543::pBGentPxylMS2(dlFG)mcherry | This study |
| CJW2966 | CB15N \( groESL::pHL23\)groESL-lacO_{120}tm | This study |
| CJW2967 | CB15N \( creS::pHL23\)creS-lacO_{120}tm | This study |
| CJW2968 | CB15N \( divJ::pHL23\)divJ-lacO_{120}tm | This study |
| CJW2969 | CB15N \( groESL::pHL23\)groESL-lacO_{120}tm \( xylX::pHPV543\) (\( Psyl\ lacI-cfp\)) | This study |
| CJW3093 | CB15N \( ompA::pHL23\)ompA-lacO_{120}tm | This study |
| CJW3097 | CB15N \( vanA::pHL23\)Pvan-mcherry-lacO_{120}tm | This study |
| CJW3099 | CB15 \( divD308(Ts)::pDW110\) divE309(Ts) | This study |
| CJW3100 | CB15N \( rne::pHL23\)rne-mgfp | This study |
| CJW3102 | CB15N \( Cori::Cori(tetO)pGENT\)xylX::pHPV472creS::pHL23creS-lacO_{120}tm | This study |
| CJW3364 | CB15N \( fljK::pHL23\)fljK-lacO_{120}tm | This study |
| CJW3365 | CB15N \( rplA::pL1\)GFPC-1 | This study |
| LS3812 | CB15N \( ΔcreS\) | 24 |
| MT16 | CB15N \( Cori::Cori(tetO)pGENT\)xylX::pHPV472 carrying a translational fusion of \( lacI-cfp\) and \( tetR-yfp\) under the control of \( Psyl\) | 25 |
| PC6340 | CB15 \( divD308(Ts)::pDW110\) divE309(Ts) carrying a ts mutation in \( ftsA\) and \( parE\) | 26 |
| PV3153 | CB15N xylX::pHPV543 carrying \( lacI-cfp\) under the control of \( Pxyl\) | P. Viollier |

**E. coli strains**

| Strain | Description |
|--------|-------------|
| DH5α | Cloning strain | Invitrogen |
| DL2875 | MG1655 \( lacIq\) \( lacZ\) \( χ\) cynX::(tetO)250 (Gent^R) | 27 |
| MG1655 | \( F′\lambda\) \( ilvG\) \( rfb-50\) rph-1 | K. Gerdes |
| SM10 | M294::RP4-2 (Tc::Mu); for plasmid mobilization | 28 |
| S17-1 | M294::RP4-2 (Tc::Mu)(Km::Tn7); for plasmid mobilization | 28 |

**Plasmids**

| Plasmid | Description |
|---------|-------------|
| pBAp | Integration plasmid (Apramycin^R) | This study |
| pBAp-LacO | pBAp carrying an array of 240 \( lacO\) sites | This study |
| pBGent | Integration plasmid (Gent^R) | 29 |
| pBGent-LacO_{240} | pBGent carrying the \( lacO_{240}\) array | This study |
| pBGentPxylMS2dmGFP | pBGent carrying \( ms2d-mgfp\) under control of \( Pxyl\) | This study |
| pBGentPxylMS2(dlFG)mGFP | pBGent integration vector carrying \( ms2-mgfp\) under control of \( Pxyl\) | This study |
| pBGentPxylMS2(dlFG)mCherry | pBGent carrying \( ms2-mCherry\) under control of \( Pxyl\) | This study |
| pBGS18 | Integrating plasmid (Kan^R) | D. Alley |
| pCR2.1-TOPO | Amp^R Kan^R PCR cloning vector | Invitrogen |

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21
| Plasmid Name          | Description                                                                 | Reference |
|----------------------|-----------------------------------------------------------------------------|-----------|
| pCT119 dlFG          | Amp<sup>+</sup> plasmid producing MS2 with deletion of the FG loop, rendering it assembly-defective | 30        |
| pGFPC-1              | Integration plasmid (Spec/StrepR) to create GFP C-terminal fusions, integrated at the site of interest | 12        |
| pH22                 | Integration plasmid (Kan<sup>R</sup>)                                      | 31        |
| pH23creS-lacO<sub>120</sub>tm | pH23 carrying the 3’-end of cre<sub>S</sub> transcriptionally fused to lacO<sub>120</sub>, with an additional E. coli trpA terminator | This study |
| pH23divJc-bs48-3’UTR  | pH23 carrying 3’end of div<sub>J</sub> transcriptionally fused to bs48 and div<sub>J</sub> 3’UTR to create div<sub>J</sub>-bs48-3’UTR under native promoter | This study |
| pH23divJ-lacO<sub>120</sub>tm | pH23 carrying the 3’-end of div<sub>J</sub> transcriptionally fused to lacO<sub>120</sub>, with an additional E. coli trpA terminator | This study |
| pH23fljK-lacO<sub>120</sub>tm | pH23 carrying the 3’end of flj<sub>K</sub> transcriptionally fused to lacO<sub>120</sub>, with an additional transcriptional E. coli trpA terminator | This study |
| pH23groESL-lacO<sub>120</sub>tm | pH23 carrying the 3’-end of groESL transcriptionally fused to lacO<sub>120</sub>, with an additional E. coli trpA terminator | This study |
| pH23ompA-lacO<sub>120</sub>tm | pH23 carrying the 3’-end of ompA transcriptionally fused to lacO<sub>120</sub> and E. coli trpA terminator | This study |
| pH23Pvan-mcherrybs48tm | pH23 carrying mcherry-bs48 under control of Pvan | This study |
| pH23Pvan-mcherry-lacO<sub>120</sub>tm | pH23 carrying mcherry under the control of Pvan and transcriptionally fused to lacO<sub>120</sub> and E. coli trpA terminator | This study |
| pH23rne-mgfp         | pH23 carrying the 3’-end of rne translationally fused to mgfp               | This study |
| pH32                 | Integration plasmid (Kan<sup>R</sup>)                                      | 31        |
| pH32Pvan-mcherry     | pH32 carrying mcherry under the control of Pvan                             | This study |
| pH45Ω aaC4           | Amp<sup>+</sup> Apramycin<sup>R</sup> cloning vector                       | 32        |
| pIG-BS48-I           | pKS vector with an array of 48 MS2 binding sites                            | 3         |
| pIG-E133-2cTG        | ColE1-based expression vector (Kan<sup>R</sup>) carrying ms2d-gfp           | 2         |
| pKS                 | Amp<sup>+</sup> cloning vector                                              | Stratagene |
| pKScreS-R            | Coding sequence, 5’UTR and 3’UTR of cre<sub>S</sub> cloned from the C. crescentus genome | N. Ausmees |
| pKSdivJ              | Coding sequence and 5’UTR of div<sub>J</sub> cloned from the C. crescentus genome | H. Lam    |
| Plasmid Name | Description | Source |
|--------------|-------------|--------|
| pKSgroEL480  | pKS carrying a 3'-end fragment of groEL cloned from the *C. crescentus* genome. | This study |
| pKS(pleD3'UTR)tm | pKS carrying the 3'UTR of pleD fused to *E. coli trpA* terminator | This study |
| pKS(lacO)120tm | pKS carrying lacO120 fused to *E. coli trpA* terminator | This study |
| pKSmCherry-N2 | pKS carrying mcherry in the N-2 frame | P. Angelastro |
| pKSmgfp-N1 | pKS carrying mgfp in the N-1 frame | P. Angelastro |
| pLAU38 | Amp<sup>R</sup> Kan<sup>R</sup> pUC18 derivative plasmid with an array of 120 lacO sequences | This study |
| pLAU43 | Amp<sup>R</sup> Kan<sup>R</sup> pUC18 derivative plasmid with an array of 240 lacO sequences | This study |
| pL1-GFPC-1 | PGFPC-1 carrying a 3’-end fragment of rplA cloned from the *C. crescentus* CB15N genome | This study |
| pNJH17 | Integrating vector (Kan<sup>R</sup>) carrying *ftsZ-mcherry* under the control of *Pvan* | N. Hillson |
| pRW431 | Amp<sup>R</sup> and Kan<sup>R</sup> cloning vector carrying 500bp region that includes *Pxyl* | R. Wright |

**Table S2.** Percentage of cells with fluorescent spots after RNA FISH

| mRNA         | Number of cells | Cells with spots (%) | Cells without spots (%) | Cells with 1 spot (%) | Cells with 2 spots (%) | Cells with > 2 spots (%) |
|--------------|----------------|----------------------|-------------------------|-----------------------|------------------------|--------------------------|
| groESL-lacO<sub>120</sub> (normal growth) | 1508 | 79.3±1.3 | 20.7±1.3 | 56.6±0.8 | 23.9±0.07 | 0.9±0.3 |
| groESL-lacO<sub>120</sub> (heat shock) | 2364 | 95.3±2.2 | 4.7±2.2 | 41.3±3.7 | 37.4±1.9 | 16.5±0.5 |
| lacZ         | 995 | 93.3±3  | 6.75±3 | 30.8±7.4 | 51.9±5.7 | 10.6±4.7 |

The foci were identified using SpotFinder (described above). The errors correspond to the standard deviation for at least 2 experiments. The *groESL-lacO<sub>120</sub>* mRNA signal was visualized in *C. crescentus* CJW2966 strain after growth at 30°C (normal growth) and after heat shock at 42°C for 15 min using the lacO probe, whereas the native *lacZ* mRNA signal was visualized in *E. coli* MG1655 after IPTG induction for 20 min using 48 probes complementary to the *lacZ* coding sequence.

**CONSTRUCTION OF PLASMIDS**
**pBAp**: Plasmid pBGST18 was digested with MluI to remove the kanamycin resistance-encoding cassette. The apramycin resistance-encoding cassette was removed from pH45ΩaacC4 with BamHI (blunted) and cloned into the prepared pBGST18 vector to create pBAp.

**pBAp-LacO**: An XbaI/Nhel fragment containing the lacO\textsubscript{240} array was cut out of plasmid pLAU43 and cloned into pBAp digested with XbaI. The kanamycin resistance cassette was removed from the resulting plasmid with an NsiI digest and re-ligated to create pBAp-LacO.

**pBGentPxyI\textsubscript{MS2}dm\textsubscript{GFP}**: MS2d was cut out of plasmid pIG-E133-2\textsubscript{cTG} using enzyme BamHI and subcloned into pKS, digested with the same enzyme. MS2d was then PCR amplified with forward primer 5’TAGACTAGTGGATCCCCATATGGCTTCT3’, and reverse primer 5’ACTGCAGAAGGGGGATCCGTAGAT3’, adding an NdeI site at the 5’ end and a PstI site at the 3’ end, and sequence verified. An EcoRV/NdeI PxyI fragment from pRW431, an NdeI/PstI MS2d fragment, and a PstI/XbaI mGFP fragment were cloned into pBGent digested with PstI (blunted) and XbaI.

**pBGentPxyI\textsubscript{MS2}m\textsubscript{Cherry}**: The 0.8kb HindIII/BamHI PxyI\textsubscript{MS2} fragment from pBGentPxyI\textsubscript{MS2}m\textsubscript{GFP}, and the 0.8kb BamHI/XbaI mCherry fragment from pKS\textsubscript{mCherry-N2} were cloned into pBGent vector digested with HindIII/XbaI.

**pBGentPxyI\textsubscript{MS2}m\textsubscript{GFP}**: A 0.4kb MS2 fragment was PCR amplified from pCT119 with forward primer 5’TAGACTAGTGGATCCCCATATGGCTTCT3’, and reverse primer 5’ACTGCAGAAGGGGGATCCGTAGAT3’, adding an NdeI site at the 5’ end and a PstI site at the 3’ end, and sequence verified. An EcoRV/NdeI PxyI fragment from pRW431, an NdeI/PstI MS2 fragment, and a PstI/XbaI mGFP fragment were cloned into pBGent restricted with PstI (blunted) and XbaI.

**pHL23creS-lacO\textsubscript{120tm}**: Plasmid pHL32creS was digested with Stul and XbaI and the 800 bp fragment containing the 3’-end of creS was gel purified. Plasmid pKSlacO\textsubscript{120tm}
was digested with NheI and HindIII and the 4.5 Kb \(lacO_{120\text{tm}}\) fragment was gel purified. A triple digestion was set between pH23 digested with EcoRV and HindIII, \(creS\) 3’end digested with StuI and XbaI, and \(lacO_{120\text{tm}}\) digested with NheI and HindIII. The correct construct was verified by restriction analysis and sequencing.

**pHL23divJ-lacO_{120\text{tm}}:** plasmid pKSdivJ was digested with NotI and SmaI, and the 2 Kb fragment was gel purified (\(divJ\) 3’end). In parallel, the pKS\(lacO_{120\text{tm}}\) was digested with NheI, blunted with Klenow and digested with HindIII. The 4.5 Kb \(lacO_{120\text{tm}}\) fragment was gel purified. A triple ligation was set between pH23 digested with NotI and HindIII, the \(divJ\) fragment (NotI and SmaI ends), and the \(lacO_{120\text{tm}}\) array (Blunt and HindIII). The correct construct was verified through restriction analysis and sequencing.

**pHL23divJc-bs48-3’UTR:** 300bp of the \(divJ\) 3’ UTR was cloned out of the genome with forward primer 5’TTTAAGCTTACGCCCGACCGGCGGCGAAGTCTC3’ and reverse primer 5’TTTGTGACATTCTGACCGGACACCAAGG3’, adding a HindIII and SalI site, respectively, and sequence verified. A 1.4kb 3’ \(divJ\) fragment was cut out of pKSdivJ with NotI/BamHI(blunted) and ligated to an XbaI(blunted)/HindIII \(bs48\) fragment from pIG-BS48-I, and the HindIII/SalI \(divJ\) 3’UTR fragment, in the vector backbone pH23 restricted with NotI and SalI.

**pHL23fljK-lacO_{120\text{tm}}:** A 600 bp containing the 3’-end of \(fljK\) was PCR amplified from *C. crescentus* genomic DNA using primers fljK-SacI For (5’ATATGAGCTCTGAGAACGCTGCAAA3’) and fljK-NheI Rev (5’CGCGCGTAGCTTTAAGGACAGGCGCTCA). The PCR product was digested using enzymes SacI and NheI. The 4.5kb \(lacO_{120\text{tm}}\) fragment was cut out from plasmid pKS\(lacO_{120\text{tm}}\) using enzymes NheI and HindIII. The two fragments were inserted into plasmid pH23 cut with SacI and HindIII. The correct construct was verified by restriction analysis and sequencing.

**pHL23groEL-lacO_{120\text{tm}}:** The 1.2Kb 3’-end \(groEL\) fragment was cut out of plasmid pKSgroEL480 using enzymes SacI and XbaI. The 4.5Kb \(lacO_{120\text{tm}}\) fragment was cut out
of plasmid pKS lacO120tm using enzymes NheI and HindIII. The two fragments were gel purified and inserted into plasmid pHL23 digested with enzymes SacI and HindIII. The correct construct was verified through restriction analysis and sequencing.

**pHL23ompAlacO120tm:** A 800 bp fragment containing the 3’-end of *ompA* was PCR amplified using *C. crescentus* genome DNA as template and primers ompA-Sacl-up (5’ GCGCGAGCTCCAACCTCATCTTCGACAT3’) and ompA-NheI-down (5’ GCGCGCTAGCTTATTGGAAGTTGATCGAG). The PCR product was gel purified and was digested using enzymes SacI and NheI. The 4.5kb *lacO120tm* fragment was cut out from plasmid pKS lacO120tm using enzymes NheI and HindIII. The two fragments were inserted into plasmid pHL23 cut with SacI and HindIII. The correct construct was verified by restriction analysis and sequencing.

**pHL23PvanCHY lacO120tm:** The 1.8 kb fragment containing the inducible promoter *Pvan* fused to *mcherry* was cut out of plasmid pHL32Pvan-mcherry using enzymes XbaI and EcoRV and was gel purified. Plasmid pKS lacO120tm was cut using enzyme NheI and the cohesive ends were blunted using Klenow. This plasmid was then digested with HindIII, and the 4.5 kb *lacO120tm* fragment was gel purified. A triple ligation was set between fragments Pvan-mcherry and *lacO120tm* to insert into plasmid pHL23 digested with enzymes XbaI and HindIII. The correct plasmid was verified by restriction analysis and sequencing.

**pHL23PvanmCherrybs48tm:** pKS divJ3’UTRtm was digested with HindIII and Sall to remove the *divJ* 3’UTR fragment, both ends blunted and re-ligated to create pKStm. pHL23bs48tm was created by cutting out the terminator from pKStm with SmaI/Kpnl, cutting out a bs48 fragment with BamHI/HindIII (blunted), and cloning into pHL23 restricted with BamHI / Kpnl. A mcherry fragment was cloned by PCR from pNJH17 with Ndel/EcoRV at the ends, and cloned to *Pvan* fragment cut out of pNJH17 with XbaI/Ndel into a pHL32 vector restricted with XbaI/EcoRV. Then the *Pvan-mcherry* fragment was cut out with XbaI/EcoRV and cloned to bs48tm cut with XbaI(blunted)/Kpnl into pHL32 restricted with XbaI/Kpnl.
pHL23rne-mgfp: The 3’ end of rne was PCR amplified from *C. crescentus* genomic DNA using primers rne(800)-SacI (5’GCGCGAGCTCTACGACAAGGACCTGGAC) and rne(stop)-KpnI (5’ATATGGTACCCCCGGCGCCACCA). The 800bp amplicon was then digested with enzymes SacI and KpnI. Fragment mgfp was cut out from plasmid pKS-mgfp N1 using enzymes KpnI and XbaI. The 900 bp mgfp fragment was gel purified. Both fragments were inserted into plasmid pHL23 digested with enzymes SacI and XbaI.

pHL32Pvan-mcherry: A DNA fragment of the mcherry coding sequence was amplified by PCR using pNJH17 as a template and primers mCherrybs48FOR (5’AAACATATGGTGAGCAAGGGCGAGGAGGAT) and Cherrybs48REV (5’AAAGATATCTTACTTGTACAGCTCGTCCAT), which add NdeI and EcoRV sites at the ends. This PCR product and a XbaI/NdeI DNA fragment of pNJH17 containing Pvan were triple ligated into a pHL32 vector cut with XbaI/EcoRV.

pKSlacO120tm: The 4.5 kb lacO120 fragment was cut out of plasmid pLAU38 using enzymes BamHI and SalI. The 4.5 Kb fragment lacO120 array was gel purified. To add the trpA additional terminator, the plasmid pKS(pleD3’UTR)tm was digested with enzymes Sal I and KpnI. The enzymes were heat inactivated and a triple ligation was set between pKS (digested with BamHI and KpnI), the lacO120 fragment (digested with BamHI and SalI) and the digestion mixture of pKS(pleD3’UTR)tm (the trpA terminator is too small to gel purify it). The correct constructs were verified by restriction analysis and sequencing.

pKS(pleD3’UTR)tm: The 3’UTR region of pleD was PCR amplified from *C. crescentus* genomic DNA using primers DivK3UTR FOR (5’CCGAAGCTTGAGCGGGCGCCCAG) and DivK 3UTR REV (5’ACGCGTCGACGACTGATAGCCGTCGGATA). The 350
bp fragment was inserted into plasmid pKS digested with EcoRV and treated with antarctic phosphatase. The \( trpA \) transcriptional terminator was added by PCR amplification using the primers DivK 3'UTR FOR and DivK 3'UTR ttm (5'CGGGGTACCAAGAAAAAAAAGCCCGCTCATTAGGCGGCCACGCGTCGACG ACT). The 400 bp fragment was inserted into plasmid pKS digested with EcoRV and treated with phosphatase. The correct clone was verified by sequencing.

**pKSgroEL480:** The 3’end of \( groEL \) was PCR amplified from \( C. \ crescentus \) genomic DNA using primers groEL480-For-SacI (GCGCGAGCTCATGATCGCCAAGGCCAT) and groEL1644Rev-XbaI (GCGCTCTAGACCTTAGAAGTCCATGT). The 1.2kb amplicon was inserted into cloning plasmid pKS digested with EcoRV. The correct construct was verified by restriction analysis and sequencing.

**pL1-GFPC-1:** The 3’end of \( rplA \) was PCR amplified from CB15N genomic DNA using primers L1-Forward (ATATAAGCTTGAGATCTCGGTCAACCT) and \( L1 \) (no stop)-Rev (ATATGGTACCAGCGCCGATCGAGCTGATGT). The 700 bp fragment was cut with enzymes HindIII and KpnI and inserted into pGFPC-1 digested with the same enzymes. The correct construct was verified by restriction analysis and sequencing.

**CONSTRUCTION OF STRAINS**

**CJW2555:** pHL23PvanmCherrybs48tm was integrated into the \( vanA \) locus of CB15N by transformation and the \( vanA::pHL23PvanmCherrybs48tm \) locus was moved by \( \Phi CR30 \) phage transduction into CJW2780.

**CJW2556:** pBGentPxylMS2mGFP was integrated into the \( xylX \) locus of CB15N by transformation.

**CJW2560:** pHL23PvanmCherrybs48tm was integrated into the \( vanA \) locus of CB15N by transformation and the \( vanA::pHL23PvanmCherrybs48tm \) locus was moved by \( \Phi CR30 \) phage transduction into CJW2556.
CJW2780: pBGentPxylMS2dmGFP was integrated into the $xylX$ locus of CB15N by transformation.

CJW2781: S17-1 cells carrying pBGentPxylMS2mCherry were mated to CJW1215.

CJW2783: pHL23divJc-bs48-3′UTR was integrated into the $divJ$ locus of CB15N as the only copy of $divJ$ in the genome and ΦCR30 phage lysate of CJW2781 was transduced into the resulting strain to make CJW2783.

CJW2966: Plasmid pHL23groELlacO120tm was introduced into wild-type CB15N cells by conjugation with S17-1 E. coli strain. Integration occurred at the $groESL$ gene locus.

CJW2967: Plasmid pHL23creSlacO120tm was introduced into wild-type CB15N cells by conjugation with S17-1 E. coli strain. Integration occurred at the $creS$ gene locus.

CJW2968: Plasmid pHL23divJlacO120tm was introduced into wild-type CB15N cells by conjugation with S17-1 E. coli strain. Integration occurred at the $divJ$ gene locus.

CJW2969: A ΦCR30 transducing phage lysate carrying $xylX$:pHPV543 (Kan$^R$) was obtained from strain CJW1215. $xylX$:pHPV543 was transduced into strain CJW2966. The synthesis of LacI-CFP was verified by microscopy after induction with 0.3% xylose.

CJW3093: Plasmid pHL23ompAlacO120tm was introduced into CB15N cells by conjugation with S17-1 E. coli strain. Integration occurred at the $ompA$ gene locus.

CJW3096: A ΦCR30 phage lysate carrying $groESL$:pHL23groESLlacO120tm was obtained from strain CJW2966. $groESL$:pHL23groESLlacO120tm was transduced into strain CJW3101. The resulting strain requires xylose for growth.

CJW3097: Plasmid pHL23Pvan-mcherrylacO120tm was introduced into CB15N cells by conjugation with S17-1 E. coli strain. Integration occurred at the $vanA$ gene locus.
CJW3099: A ΦCR30 phage lysate carrying rne::pHL23rne-mgfp was obtained from strain CJW3100. rne::pHL23rne-mgfp was transduced into strain CJW2673.

CJW3100: Plasmid pHL23rne-mgfp was introduced into CB15N cells by conjugation with S17-1 *E. coli* strain. Integration occurred at the rne gene locus.

CJW3102: A ΦCR30 phage lysate carrying creS::pHL23creSlacO120tm was obtained from strain CJW2967. creS::pHL23creSlacO120tm was transduced into strain CJW1059.

CJW3364: Plasmid pHL23fljK-lacO120tm was introduced into CB15N cells by electroporation. Integration occurred at the fljK gene locus.

CJW3365: Plasmid pL1-GFPC-1 was introduced into CB15B cells by conjugation with SM10 *E. coli* strain. Integration occurred at the rplA gene locus.

**List of DNA probes used for RNA FISH of endogenous mRNAs**

For creS mRNA

5’ [Cy3]ATCAAGGCAGATCTGGCGCTGGGCCTG

5’ [Cy3]GCGAGGCGGGCGGAAACCTCGCCTTCCT

5’ [Cy3]GCGTCGGATTCGCCGAGCGCTTCCTGGGTCTG

5’ [Cy3]CAGCAGGGCGTTGCGCAGGCGGTCGATCT

5’ [Cy3]AGGCGTCGAGGCTGGAGACCTTCAGGT

5’ [Cy3]AGGTGCTCGATGCGGGCGGTGGCGTCG

5’ [Cy3]CTGGGCCTGGACGCGCAGGCCCTTCGACGT

5’ [Cy3]GCGTCGGATACGCGCAGCCGCGCGTCGATCT

5’ [Cy3]AGGCGGCGTGCTGGACGCGAGCGCGCGTCGACCTACG

5’ [Cy3]GCGTCGGATACGCGCAGCCGCGCGTCGACCTACG

5’ [Cy3]AGGCGGCGTGCTGGACGCGAGCGCGCGTCGACCTACG

5’ [Cy3]GCGTCGGATACGCGCAGCCGCGCGTCGACCTACG
5’ [Cy3]ACGCGCTTCTTCAGCGTCGCGGCTTCCTC
5’ [Cy3]ATAGGCCGGGCCAGGTCCAGCCCGGCCT
5’ [Cy3]CAGCTGGGCTTTCAAGATCGGTTCATG
5’ [Cy3]CGTTTTCGACGGCCTGAACGCGGGCGCT
5’ [Cy3]CGGATCGTGCGACCGCTGTCGGCCTGATGC
5’ [Cy3]GCGGTTGGCCTCGACCTGCGCTTTCCA
5’ [Cy3]TCTCCAGGCCGCTCTGCAAGAGCGGAGAT
5’ [Cy3]CATCTCTTCCAGCTTGTGCGGCGACCCGT
5’ [Cy3]CTGGAAGTCGGCGAGACGGGCCCCAGATCT
5’ [Cy3]GCGCGCGGCTTCCACCGCCTTTCTGCT
5’ [Cy3]AGGGCGCGCTCCAGGGCAGCATTTGAGAT
5’ [Cy3]TCAGCCTCTTTCTCCAGGCCCGGAGAT
5’ [Cy3]CGGTGTCGACGCCGGCTGACGCTGGC
5’ [Cy3]CAGCTGGTCGGCGCTTCGATGGCCGT
5’ [Cy3]TTGAGGGCCTTTTCTTGCGCCACAGCCT
5’ [Cy3]CGATCTTCGCTCCGACGTGCGCCCGGAA
5’ [Cy3]TTTCCGAGGTACGAGCTCGATAGGCTG
5’ [Cy3]TTGCCGGCTTCAGGGCCTTTCGGCCA
5’ [Cy3]AGCAGGGCCATCTGCAAGCGTGAGCGGT
5’ [Cy3]TTAGGCCGCTCGCCGCGCCACGTCGCCGCT

For lacZ mRNA
5’ [Cy3]GCCAGTGAATCCGTAATCATGGTCA
5’ [Cy3]GGTTTTCCCAGTCACGACGTTGTAA
5’ [Cy3]CTTCCAGGGCCATCTGCAAGCGTGAGCGGT
5’ [Cy3]CTGCAAGGGCATTAGTGGGTAAC
5' [Cy3]TTTCGCTATTACGCCAGCTGGCGAAA
5' [Cy3]ATTCCAGGCTGCAGCAACTGTTGGGAA
5' [Cy3]TTCTGGTGCCGGAACCCAGGCAAAG
5' [Cy3]AAGATCGCACTCCAGCCAGCTTTCC
5' [Cy3]AGTGGAGGGGACGACGACAGTATC
5' [Cy3]TTGGTGTACGATGGCGCATCCTGAAC
5' [Cy3]ACGGCGGATTGACCGTAATGGGATA
5' [Cy3]AGTAAACCAACCCGTCCGGATTCTCGT
5' [Cy3]CCTGTAGCCAGCTTTTATCATCAAAT
5' [Cy3]CGCCATCAAAATAATTCGCGTCTG
5' [Cy3]CGTTGCACGACAGTGAACGCGGAA
5' [Cy3]AAACGACTGCTCCTGGCGTAACCGA
5' [Cy3]CGCGTAAAATCGCGCTCAGGTCAAA
5' [Cy3]AGCACCATCACCAGCGAGCGGTGTTT
5' [Cy3]TCTTCCAGATAACTGCGCTCAGGAC
5' [Cy3]GTCCGGTTATGCAGCAACGAGACGT
5' [Cy3]GAATGCGAAATGAAATCGCTGAT
5' [Cy3]GTACAGCGCGCTGAAATATCATCATT
5' [Cy3]AACTCGCCGCACTCTGAACTTCAG
5' [Cy3]CTGCCATAAAGAAACTGTACCACG
5' [Cy3]TCGATAATTTCCACGCGAGGGCA
5' [Cy3]TAGTGTGACGCGATCGGCATAACCA
5' [Cy3]CCACAGTTTCGGGTTTTCGACGTTC
5' [Cy3]CGCACGATAGAGATTCGGGATTTGC
5' [Cy3]ATCGCAGGGCTCTGCTTCAATCAGC
5' [Cy3]ATTTTCAATCCGCACCTCGCGGA
5' [Cy3]ATTCACGGCTGCTGTCGGACGC
5' [Cy3]GGATGATGGCTCAGGACGTATAACGC
5' [Cy3]TGCTGCTCATCCATGACCTGACC
5' [Cy3]TGCTTCATCCACGGATATCGTGCA
5' [Cy3]AATGCGAACAGCGACGGCGTTAA
5' [Cy3]ACAGCGTGTACCACAGCGGATGGT
5' [Cy3]TTCTCCACCACATACAGGGCCTAG
5' [Cy3]TGGCACCATGCCGCTGGGTTCAATA
5' [Cy3]TAGCCAGCGGATCATCGGTACAGC
5' [Cy3]CATTCGCGTTCGCCGTCGTTCATC
5' [Cy3]ATCACACTCGGGTGGATTACGTACGC
5' [Cy3]ATTAGCGCGGCTGGGCTGATTCATTC
5' [Cy3]AGATTTGATCCAGCGATACAGCGC
5' [Cy3]TTTATACTGCACCGGGCGGGAAGGA
5' [Cy3]AATAATATCGGTTGGCCTGGGCTG
5' [Cy3]GTCTTCATCCACGGCGCGTCATC
5' [Cy3]TGACCATTTCGGCACAGCCGGGA
5' [Cy3]TCTCTCCAGGTAGCGAAAGCCATTT

**List of LNA probes used for FISH**

lacO: 5' [Cy3] or [FITC]AATTGTTATCCGCTCAC
lacO Reverse: 5’ [FITC]GTGAGCGGATAACAATT
tetO: 5’ [Alexa488]CTCTATCACTGATAGGGA
groESL: 5’[Cy3]GTCGGAGGAGAAATAGACGTCTTTA

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