Supplementary Material

Absolute quantitative measurement of transcriptional kinetic parameters *in vivo*

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Supplementary Methods

Construction of a new chromosomal *lacZ* reporter strain.

We used a plasmid, pKDT_P $_{Ltet\text{-}O1}$-cat as a template (1). The cassette "*Km^r:rrnBT:P$_{Llac\text{-}O1}$-cat*" from this plasmid was amplified using the forward primer "Lac_integration_for" (GCATTTACGTTGACACCATCGAATGGCGCAAAACCTTTCGCGGTATGTGTAGGCTGGAGCT GCTTC) and reverse primer "rbs1_ptet_lacZ_integration_rev" (CGTTGTAAAACGACGGCCAGTGAATCCGTAATCATGGTCATGGTACCTTTCTCCTCTTTAAT GAATTCGGTCAGT). The forward primer was designed to contain 47 bases from the *lacI* promoter region followed by a 19 base region that hybridizes to the pKDT plasmid upstream of the kanamycin cassette. The reverse primer was designed to contain three components, the first 22 bases of the primer corresponding to the 5'UTR region of the *cat* gene in the pKDT plasmid, a synthetic ribosome binding site (RBS) sequence, and the first 35 bases of the *lacZ* coding region to facilitate integration of the construct into the chromosome. Hence, the PCR products amplified from the primers contain the region containing -14 to - 61 bases relative to transcription start site of *lacI* gene, kanamycin resistance gene, *rrnB* terminator, P$_{Ltet\text{-}O1}$ promoter, 5'UTR, the synthetic RBS, and first 35 bases of the *lacZ* coding region. The PCR products were purified and transferred to NCM3722 that expresses TetR from a constitutive promoter (P$_{con}$-tetR)(2). The result strain was named NMK80.

Fluorescence *in situ* hybridization

We followed the procedure described in ref (3).

- Labeling the probes

1.Probe Design. DNA oligonucleotide probes were designed using the software available on the Biosearch technologies website (See Supplementary Table for the sequences used). The oligonucleotides with 3' amine modifications (to facilitate labeling of the probes with fluorescent dyes with NHS ester modification) were ordered from the same company. The length of each probe was 20 nucleotides long. Approximately, 20 to 25 probes are chosen for each section of mRNA molecules (i.e., head, body and tail).

2. Probe labeling. To label the probes with fluorescent dyes, ATTO 647N (Sigma #18373), ATTO 488 (Sigma #41698), and 6-TAMRA (ThermoFischer #C6123), equal volumes of 100 µM oligo solutions were combined to a final volume of 720 µl. For example, to label probes used for the head of the *lacZ* mRNA molecules, we took 28.8 µl of each of the 25 oligo solutions to give a final volume of 720 µl. To the solution, 40 µl of 1 M sodium bicarbonate solution was added (Solution 1). In another tube, 1 mg of the fluorescent dye was dissolved in 2 µl of dimethyl sulfoxide (Fisher Scientific, #BP231), and 25 µl of 0.1 M sodium bicarbonate solution was added to the solution (Solution 2). Then, the Solution 1 and Solution 2 were mixed and incubated overnight at 37 °C in the dark.

3.Washing. 787 µl of the combined solution was transferred to 2 ml eppendorf tube, and 47 µl of 3 M sodium acetate solution (pH 5.2, autoclaved) (Fisher Scientific, #BP333) was added. Then, 1180 µl of 100% ethanol was added. The solution was incubated at -70 °C for at least 3 hours (up to overnight). Then, it was centrifuged in a bench-top microcentrifuge at 13000 g for 30 minutes. The supernatant was discarded and any remaining liquid was removed with Kimwipes. The pellet was dissolved in 45 µl of water. To this, 5 µl of 3 M sodium acetate solution (pH 5.2) and 125 µl of ethanol was added, mixed

thoroughly and placed at -80 °C for at least 3 hrs. This washing procedure was repeated two times more to remove free dyes. Finally, 500 µl 1X Tris-EDTA (pH 8.0) (Fisher Scientific, #BP2473) was added to the pellet of probes to yield the probe concentration of 3 µM (the concentration was measured using a spectrophotometer). Below, we will call this solution the probe stock solution. Then, the tube was wrapped in aluminum foil and stored at -20 °C.

- Sample collection

300 µl of 37% formaldehyde was added to 15 ml conical tubes prior to sample collection. At the times indicated, 2.7 ml of cultures was added to the tubes. After 30 minutes of incubation at room temperature, cells were spun down at 1000 g for 8 minutes. The supernatant is removed and the pellet was resuspended in 1 ml of PBS buffer (ThermoFischer,# AM9625) and transferred to 1.5 ml eppendorf tubes. The cells were spun down 900 g for 8 minutes. The cells were washed again using the PBS buffer. Following the second wash, cells were resuspended in 300 µl of water and 700 µl of 100% ethanol. Cells were incubated at room temperature at least for 1hr or overnight at 4°C.

- Hybridizing mRNAs with probes

Cells were spun down at 800 g for 8 minutes and resuspended in 1 ml of 40% wash buffer (2X SSC buffer and 40% w/v formamide). Then, cells were spun down at 1000 g for 7 minutes and resuspended in the 50 µl hybridization buffer; 10 ml of hybridization buffer contains 1 g of dextran sulfate (Sigma, #D8906), 3530µl of formamide, 10 mg of *E. coli* tRNA (Sigma, #R4251), 1 ml of 20X SSC, 40 µl of 50 mg/ml BSA (Ambion, #AM2616), and 100 µl of 200 mM ribonucleoside vanadyl complex (New England Biolabs, #S1402S). (After the preparation, the hybridization buffer was filter sterilized, aliquoted, and stored at -20 °C.) To the 50 µl hybridization buffer containing cells, 2 µl of the probe stock solution was added (because the concentration of the stock solution was initially 3 µM, the final concentration of the probes after added to the hybridization buffer is 120 nM) and the mixture was incubated at 30 °C overnight.

- Washing and preparing cells

Cells were spun down at 900 g at 8 minutes and resuspended in 1 ml of the 40% wash buffer. This procedure was repeated three times. The cells were finally resuspended in 10 µl of 2X SSC buffer.

- Imaging

3 µl of cell sample was placed on a coverslip 30×40, #1.5 coverslip. An agarose pad or 30×30 mm coverslip was placed on the top of the cells. Cells were then imaged using an inverted epi fluorescent (Olympus IX83P2Z) using a cooled cSMOS camera (Andor Neo). A 60× or 100× oil immersion phase contrast objective was used for magnification. The microscope and camera were controlled using the Metamorph software (Molecular Devices). The best focal plane for imaging (z- position) was first determined using the phase contrast image. Then, varying the z- position at spacing of 300 nm, nine fluorescence images (at different z-positions) were acquired. The exposure time for each image was 2s. The intensity of the excitation light was adjusted such that fluorescent foci are clearly visible while minimizing photobleaching. Each sample was imaged at several locations to obtain the images of at least 1000 cells.

Supplementary Note 1

We obtained the number of *lacZ* mRNAs per cell from fluorescence images as described in Supplementary Figure S1. For the head of the mRNA, 25 probes covering the first 935 nucleotides (nt) were used. Then we determined the intensities of individual fluorescence spots. From the histogram of the intensities, we determined the fluorescence intensity of one full-length head of one *lacZ* mRNA molecule, I_T. To examine the dynamics of mRNA expression after transcriptional activation, we used the intensity to characterize the increase in the number of the head after mRNA induction (Figure 3). By fitting the region of the linear increase, we obtained T_{head}. (Performing a similar analysis, we obtained T_{body} and, T_{tail}).

In a control experiment, we found that even with the first ~17 probes (which cover the first 605 nucleotides), a fluorescent spot from a single *lacZ* mRNA molecule was visible in the experimental set-up used. This leads to uncertainty in determining a complete, full-length head of the mRNA. To further elaborate this point, we consider that after transcription was initiated, a RNA polymerase synthesized the first 605 nucleotides. When this incomplete piece of the mRNA was hybridized using our original 25 probes, 17 probes will be hybridized to the first 605 nucleotides and emit strong enough fluorescence to be identified as a single head of a single mRNA. While we tried our best to reject any spots whose intensity is significantly less than I_T, it is possible that incomplete transcripts whose lengths are between 605 and 935 nucleotides might have been identified as a full-length head.

In this note, we will estimate an error in determination of T_{head} due to the uncertainty in identification of a full-length head. To estimate the error, we reformulate this problem in the following way. Consider Y_1 nucleotide long mRNAs and its expression was activated (assume no induction lag, i.e., T_{lag}=0). Assume that the number of the mRNA per cells were plotted against time similarly to Figure 3 and the linear region of induction kinetics (corresponding to the dashed lines in Figure 3) of the Y_1 nucleotide long mRNAs follows

$$m_1 = a \cdot (t - \frac{Y_1}{b}), \tag{1}$$

where m_1, a, and b are the mean number of Y_1 nucleotide long mRNAs per cells, transcription rate and transcription elongation speed. The X intercept, the time at which the full-length (Y_1 nucleotide long) mRNAs begins to appear, is $T_{Y_1} = \frac{Y_1}{b}$.

Now, assume that with a probability of p, premature termination occurs, producing mRNAs whose length is Y_2. If we were able to distinguish these transcripts from the original Y_1 nucleotide long transcripts and followed the induction kinetics, the linear region of induction kinetics of the Y_2 nucleotide long mRNA would follow

$$m_2 = a \cdot (t - \frac{Y_2}{b}) \cdot p \ , \tag{2}$$

where m_2 is the mean number of Y_2 nucleotide long mRNAs per cell.

Now, assume we cannot distinguish them; in the measurement, a Y_1 nucleotide long transcript as well as a Y_2 nucleotide long transcript are counted as one mRNA. If so, the linear region of the induction kinetics would look like

$$m = a \cdot (t - \frac{Y_1}{b}) \cdot (1-p) + a \cdot (t - \frac{Y_2}{b}) \cdot p \,, \tag{3}$$

or alternatively put,

$$m = a \cdot \left[t - \frac{1}{b} \cdot \left((1-p) \cdot Y_1 + p \cdot Y_2 \right) \right] \,, \tag{4}$$

where m is the total mean number of transcripts per cell.

The X intercept is $\frac{1}{b} \cdot \left((1-p) \cdot Y_1 + p \cdot Y_2 \right)$. This is different from $T_{Y_1} = \frac{Y_1}{b}$ (in the case that cells only produce Y_1 nucleotide long mRNAs) by $\frac{p}{b} \cdot (Y_1 - Y_2)$. Hence, this occasional appearance of Y_2 nucleotide long mRNAs results in an error of $p \cdot (1 - \frac{Y_2}{Y_1})$.

Now, we apply this formula to estimate the error in our situation. In our situation, $\frac{Y_2}{Y_1} \approx \frac{605}{935}$ and p is approximately the probability of finding transcribing RNA polymerase between 605 and 935 nucleotide, which is $\approx \frac{(935-605)}{935} \cdot 100\%$. Hence the error is estimated to be about 12%. It means the actual value of T_{head} may differ by 12% from what we obtained in the main text.

Supplementary Note 2

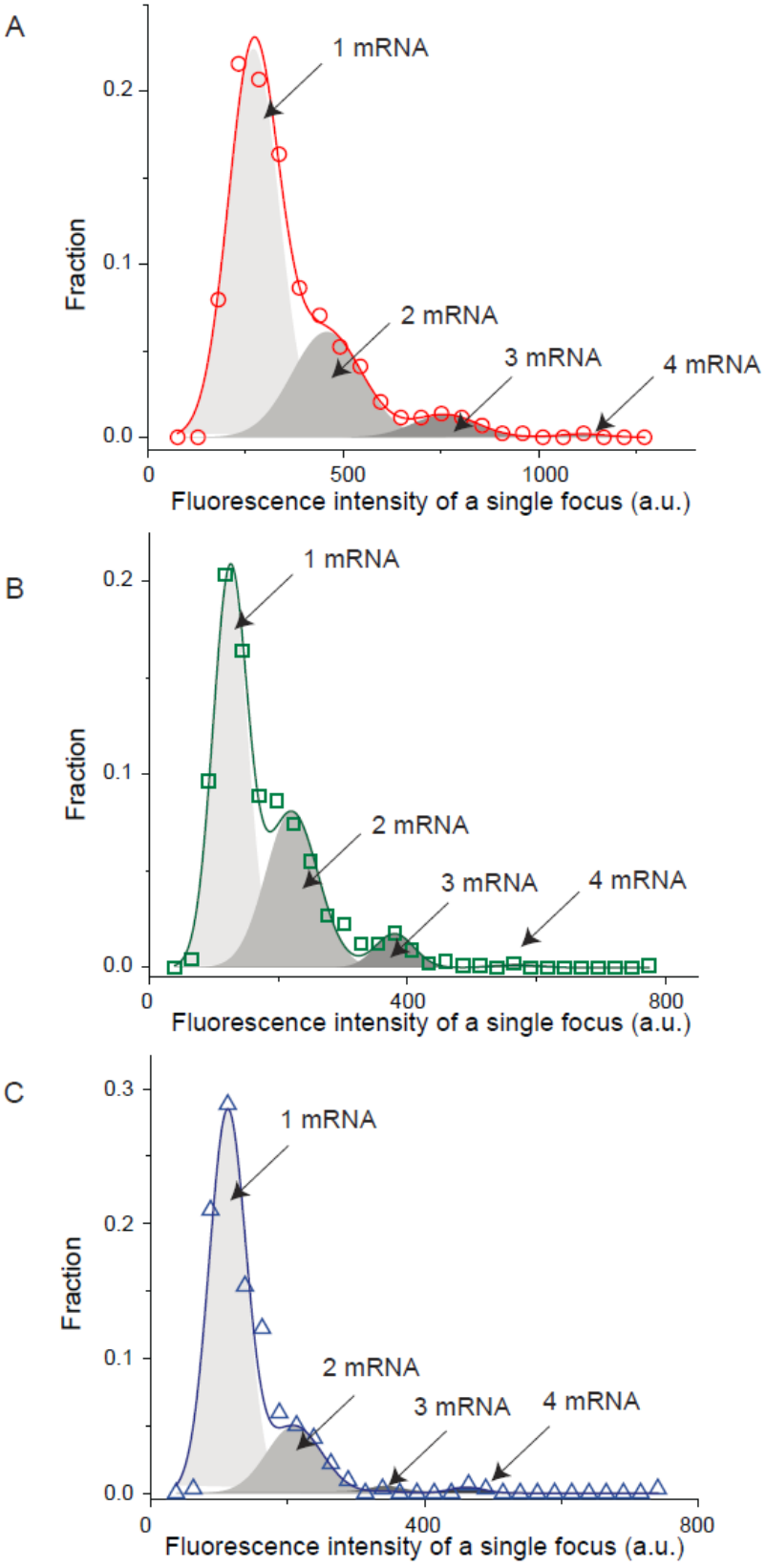
For transcription, the recruitment of RNA polymerases (RNAPs) to a promoter is generally assumed to be the rate-limiting step (4,5), i.e., once RNAPs are recruited and initiate transcription, elongation proceeds smoothly. Define Δt as the time it take for RNAPs to complete synthesis of the tail segment of a mRNA molecule after completion of the synthesis of the head segment. Without premature termination, all RNAPs completing the synthesis of the head at t will complete the synthesis of the tail at $t+\Delta t$. Hence, the transcription rate of the head, $\alpha_{head}(t)$, is the same as the transcription rate of the tail after Δt, i.e., $\alpha_{head}(t) = \alpha_{tail}(t+\Delta t)$. For example, in Fig. 3A, we see that the slope of the head is the same as the slope of the tail after 44 sec (=T_{tail} - T_{head}), indicating all RNAPs completing the synthesis of the head also complete the synthesis of tail (no premature termination).

If RNAPs dissociate before completing the tail segment, $\alpha_{head}(t) > \alpha_{tail}(t+\Delta t)$. The difference $\alpha_{head}(t)$ - $\alpha_{tail}(t+\Delta t)$ will yield the rate of the RNAP dissociation rate, or alternatively put, the premature termination rate. As an example, we use the transcriptional kinetics shown in Supplementary Figure S2B. The slope of the head and tail is 1.8 /min and 1.3 /min (see the Table). From the difference of the slopes, we obtain that the rate of premature termination is 0.5 /min.

In cases of mutants with slow elongation (e.g., due to frequent RNAP pausing (6)), it is possible that transcription elongation, not initiation, may be the rate-limiting step. Such a scenario is realized if Δt becomes long and transcription initiation is frequent. Then, RNAPs will queue, leading to slow-moving

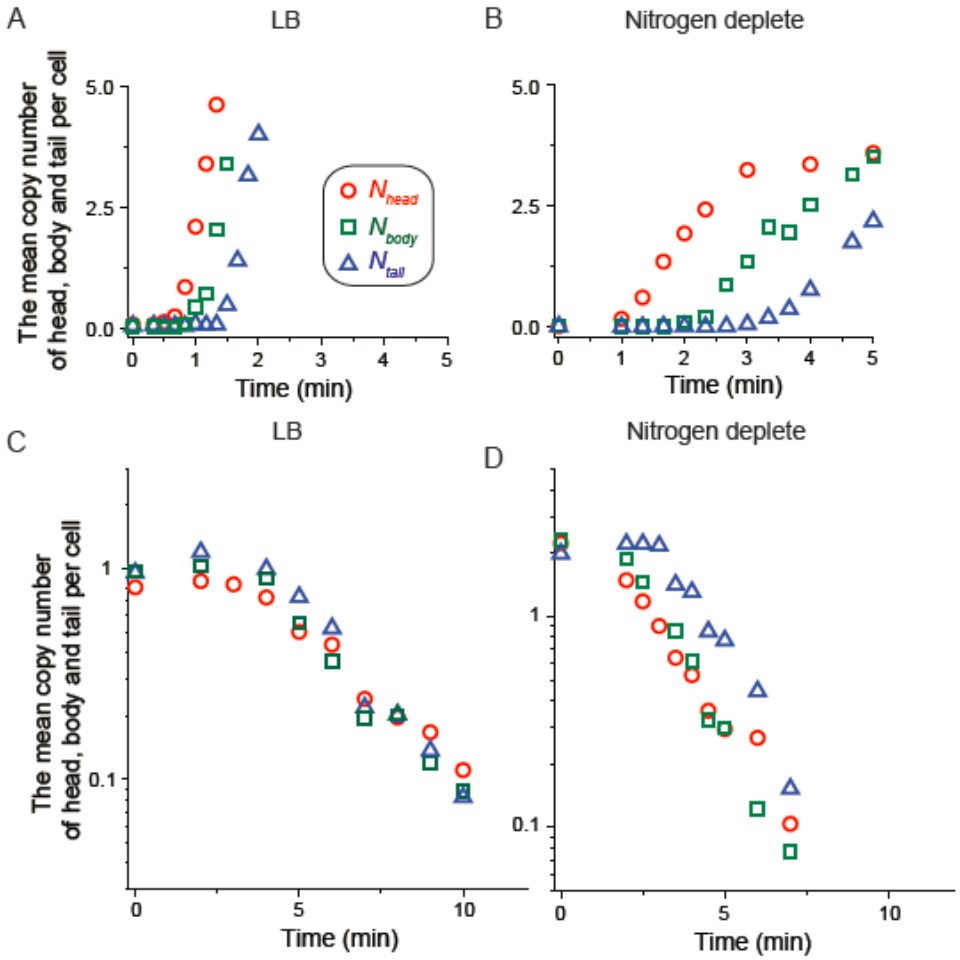
‘traffic jams’ of RNAPs (6). Upon transcription induction, until the traffic jams are established, the rate of transcription will be position-dependent. However, after the traffic jams are established, the dynamics of transcription becomes steady. Then, without premature termination, the transcription rate of the head will the same as the transcription rate of the tail after Δt. With premature termination, the rates will be different and the rate of premature termination can be obtained from the difference.

Supplementary Figure S1



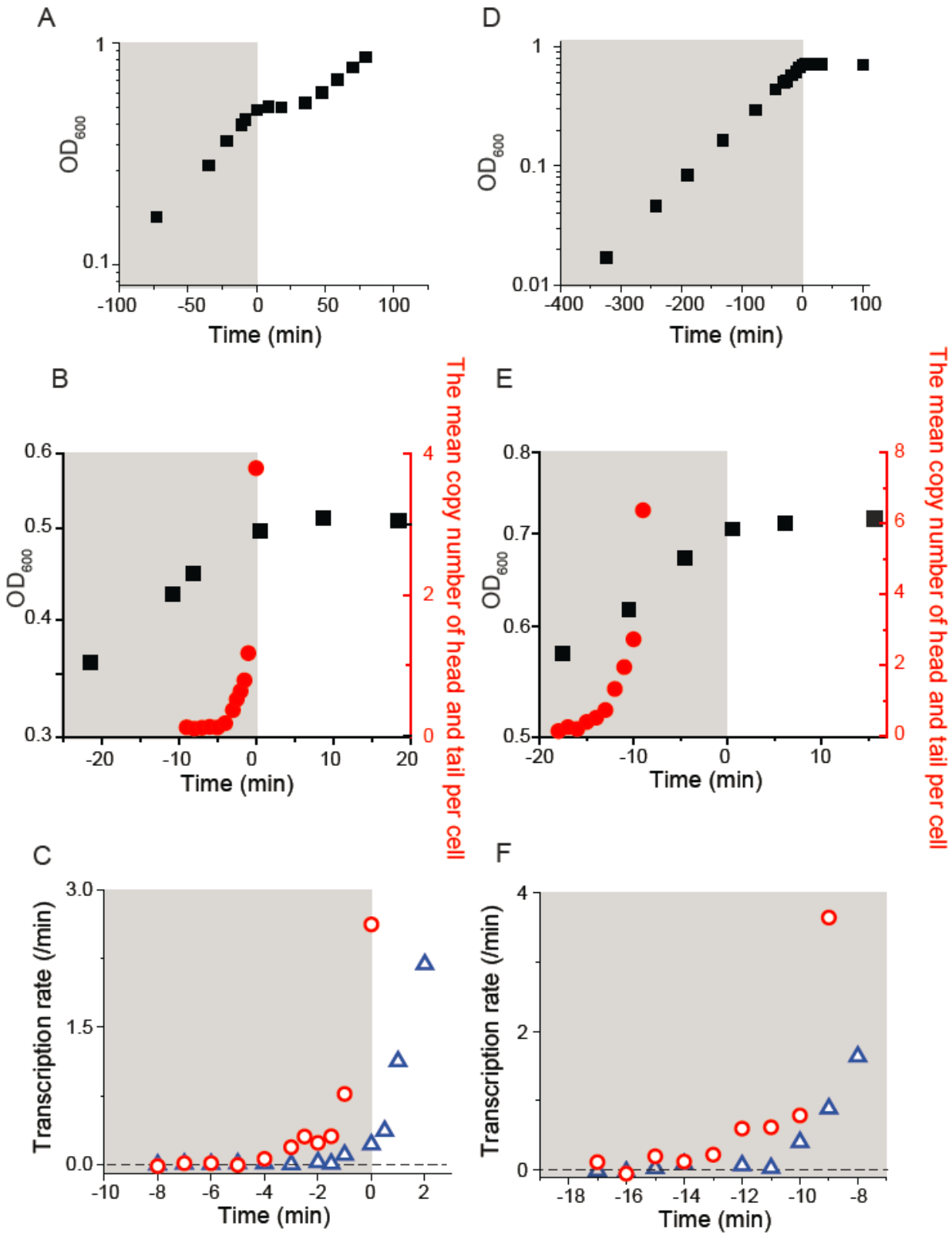
Caption: We induced the expression of *lacZ* mRNAs at very low levels and collected the samples after the expression reached the steady state. As described in the main text, mRNAs were visualized as distinct fluorescence foci. Each focus may represent more than 1 mRNA molecule, which becomes evident when we quantified the intensities of the individual fluorescence foci and drew the histogram for the head (A), body (B), and tail (C). In each histogram, we observed distinct peaks, corresponding to 1 mRNA molecule, 2 mRNA molecules, 3 mRNA molecules, and 4 mRNA molecules. Denoting the value at the center of the first peak as I_T, the centers of subsequent peaks were located approximately at $2\times I_T$, $3\times I_T$, and, $4\times I_T$. In other samples, the value I_T was used to convert the intensities of fluorescence foci to the total copy number of mRNA molecules at the foci. Please see (3) for the details.

Supplementary Figure S2



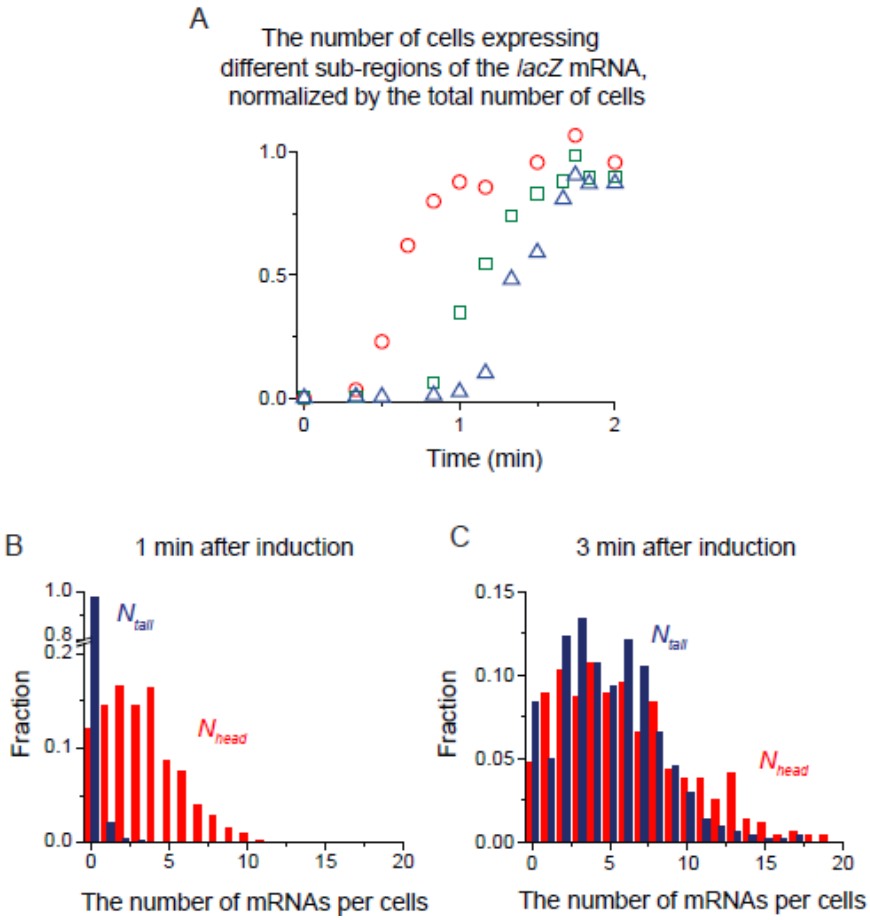
Caption: In the main text, we described that we cultured cells in a minimal medium with glycerol and ammonium as carbon and nitrogen sources, and characterized dynamics of *lacZ* mRNA expression (Figure 3). Here, we cultured cells in a Luria-Bertani (LB) medium and in a minimal medium without ammonium (nitrogen-deplete condition), and repeated the procedure (see the main text and the caption of Figure 3 for details). The increase of N_{head} (red circles), N_{body} (green squares), and N_{tail} (blue triangles) against time after induction by aTc is plotted in the panel A (for cells growing in LB) and in the panel B (for cells under the nitrogen-deplete condition). The decrease of N_{head}, N_{body}, and N_{tail} after inhibition of the mRNA synthesis by adding rifampicin is plotted in the panel C (for cells growing in LB) and in the panel D (for cells under the nitrogen-deplete condition).

Supplementary Figure S3



Caption: (A,B,C). Cells were cultured in a minimal medium with glucose and glycerol as carbon sources (ammonium as the sole nitrogen source). The growth curve is plotted in the panel A. Cells initially grew on glucose (shared region) and their growth stopped abruptly; the onset of the growth stop defines the time zero. After some time, cells began to grow on glycerol. We zoomed in on the growth curve near the region where the growth stopped and plotted the copy number of the head of *glpK* mRNA molecules (adopted from Figure 4A) together in the panel B. The direct comparison reveals that the transcription of *glpK* precedes the growth stop. Dividing the difference in the copy number between two consecutive points by the difference in time, we estimated the transcription rate for the head (red circles) and for the tail (blue triangles), and plotted them in the panel C. (D,E,F). Cells were cultured in a minimal medium with a limited amount of ammonium as the nitrogen source (glycerol as carbon source). The growth curve is plotted in the panel D. The onset of the growth stop due to depletion of ammonium defines the time zero. We zoomed in on the growth curve near the region where the growth stopped and plotted the copy number of the head of *amtB* mRNA molecules (adopted from Figure 4B) together in the panel E. The direct comparison reveals that the transcription of *amtB* precedes the growth stop. Dividing the difference in the copy number between two consecutive points by the difference in time, we estimated the transcription rate for the head (red circles) and for the tail (blue triangles), and plotted them in the panel F.

Supplementary Figure S4



Caption: Upon the induction of the *lacZ* mRNA, expression of mRNA involves significant cell-to-cell variation; see image sequences in Figure 1B. Analyzing the image sequences, we obtained how the number of cells expressing the head of the mRNA (red circle), the body (green square) and the tail (blue triangle) changes over time and normalized them to the total number of cells in the panel A. Then, we plotted the distribution of the number of mRNA copy per cell in 1 min after induction (panel B) and 3 mins after induction (panel C).

Supplementary Table

A. Sequences of oligonucleotide probes used for *lacZ* mRNA molecules

Probes that bind to the head	**Probes that bind to the body**	**Probes that bind to the tail**
GTGAATCCGTAATCATGGTC	TTCTGCTTCAATCAGCGTGC	TTTACCTTGTGGAGCGACAT
TCACGACGTTGTAAAACGAC	ACCATTTTCAATCCGCACCT	GTAGTTCAGGCAGTTCAATC
TGCAAGGCGATTAAGTTGGG	TAACGCCTCGAATCAGCAAC	TTGCACTACGCGTACTGTGA
TATTACGCCAGCTGGCGAAA	ATGCAGAGGATGATGCTCGT	AGCGTCACACTGAGGTTTTC
ATTCAGGCTGCGCAACTGTT	TCTGCTCATCCATGACCTGA	ATTTCGCTGGTGGTCAGATG
AAACCAGGCAAAGCGCCATT	TTCATCAGCAGGATATCCTG	ACCCAGCTCGATGCAAAAAT
AGTATCGGCCTCAGGAAGAT	CACGGCGTTAAAGTTGTTCT	CGGTTAAATTGCCAACGCTT
AACCGTGCATCTGCCAGTTT	AGCGGATGGTTCGGATAATG	CTGTGAAAGAAAGCCTGACT
AATGGGATAGGTCACGTTGG	TTCATCCACCACATACAGGC	TTGTTTTTTATCGCCAATCC
GAACAAACGGCGGATTGACC	TGCCGTGGGTTTCAATATTG	GTGCACGGGTGAACTGATCG
ATGTGAGCGAGTAACAACCC	GATCATCGGTCAGACGATTC	ACTTACGCCAATGTCGTTAT
TAGCCAGCTTTCATCAACAT	GATCACACTCGGGTGATTAC	CACTGCAACAACGCTGCTTC
AATAATTCGCGTCTGGCCTT	ATACAGCGCGTCGTGATTAG	CGCATCAGCAAGTGTATCTG
TTGCACCACAGATGAAACGC	GGATCGACAGATTTGATCCA	AATAAGGTTTTCCCCTGATG
TTCAGACGGCAAACGACTGT	CGCGTACATCGGGCAAATAA	CATCAATCCGGTAGGTTTTC
CGCGTAAAAATGCGCTCAGG	AAGCCATTTTTTGATGGACC	CAACGGTAATCGCCATTTGA
TCCTGATCTTCCAGATAACT	TATTCGCAAAGGATCAGCGG	AGTTTTCTTGCGGCCCTAAT
GAGACGTCACGGAAAATGCC	AAGACTGTTACCCATCGCGT	GTCAAAACAGGCGGCAGTAA
TGTGTAGTCGGTTTATGCAG	TGCCAGTATTTAGCGAAACC	GGAAGACGTACGGGGTATAC
GGCAACATGGAAATCGCTGA	CCTGTAAACGGGGATACTGA	GTGGGCCATAATTCAATTCG
CACATCTGAACTTCAGCCTC	TAATCAGCGACTGATCCACC	TGATGTTGAACTGGAAGTCG
CACCCTGCCATAAAGAAACT	GGGTTGCCGTTTTCATCATA	TCAGTTGCTGTTGACTGTAG
CTCATCGATAATTTCACCGC	TCGGCGTATCGCCAAAATCA	AGATGGCGATGGCTGGTTTC
ACGTTCAGACGTAGTGTGAC	TTCATACAGAACTGGCGATC	ATTCAGCCATGTGCCTTCTT
GCACGATAGAGATTCGGGAT	TGGTGTTTTGCTTCCGTCAG	AATCCCCATATGGAAACCGT
	ACGGAACTGGAAAAACTGCT	GAATTCCGCCGATACTGACG
	TATTCGCTGGTCACTTCGAT	ACACCAGACCAACTGGTAAT
	GTTATCGCTATGACGGAACA	

B. Sequence of the *lacZ* gene, with the probe-binding sequences underlined

Head

ATGACCATGATTACGGATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGG
CGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAG
AGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCTTTGCC
TGGTTTCCGGCACCAGAAGCGGTGCCGGAAAGCTGGCTGGAGTGCGATCTTCCTGAGGCCG
ATACTGTCGTCGTCCCCTCAAACTGGCAGATGCACGGTTACGATGCGCCCATCTACACCAAC
GTGACCTATCCCATTACGGTCAATCCGCCGTTTGTTCCCACGGAGAATCCGACGGGTTGTTA
CTCGCTCACATTTAATGTTGATGAAAGCTGGCTACAGGAAGGCCAGACGCGAATTATTTTTG
ATGGCGTTAACTCGGCGTTTCATCTGTGGTGCAACGGGCGCTGGGTCGGTTACGGCCAGGAC
AGTCGTTTGCCGTCTGAATTTGACCTGAGCGCATTTTTACGCGCCGGAGAAAACCGCCTCGC

GGTGATGGTGCTGCGCTGGAGTGACGGCAGTTATCTGGAAGATCAGGATATGTGGCGGATG
AGCGGCATTTTCCGTGACGTCTCGTTGCTGCATAAACCGACTACACAAATCAGCGATTTCCA
TGTTGCCACTCGCTTTAATGATGATTTCAGCCGCGCTGTACTGGAGGCTGAAGTTCAGATGT
GCGGCGAGTTGCGTGACTACCTACGGGTAACAGTTTCTTTATGGCAGGGTGAAACGCAGGTC
GCCAGCGGCACCGCGCCTTTCGGCGGTGAAATTATCGATGAGCGTGGTGGTTATGCCGATCG
CGTCACACTACGTCTGAACGTCGAAAACCCGAAACTGTGGAGCGCCGAAATCCCGAATCTCT
ATCGTGC

Body

GGTGGTTGAACTGCACACCGCCGACGGCACGCTGATTGAAGCAGAAGCCTGCGATGTCGGT
TTCCGCGAGGTGCGGATTGAAAATGGTCTGCTGCTGCTGAACGGCAAGCCGTTGCTGATTCG
AGGCGTTAACCGTCACGAGCATCATCCTCTGCATGGTCAGGTCATGGATGAGCAGACGATGG
TGCAGGATATCCTGCTGATGAAGCAGAACAACTTTAACGCCGTGCGCTGTTCGCATTATCCG
AACCATCCGCTGTGGTACACGCTGTGCGACCGCTACGGCCTGTATGTGGTGGATGAAGCCAA
TATTGAAACCCACGGCATGGTGCCAATGAATCGTCTGACCGATGATCCGCGCTGGCTACCGG
CGATGAGCGAACGCGTAACGCGAATGGTGCAGCGCGATCGTAATCACCCGAGTGTGATCAT
CTGGTCGCTGGGGAATGAATCAGGCCACGGCGCTAATCACGACGCGCTGTATCGCTGGATCA
AATCTGTCGATCCTTCCCGCCCGGTGCAGTATGAAGGCGGCGGAGCCGACACCACGGCCACC
GATATTATTTGCCCGATGTACGCGCGCGTGGATGAAGACCAGCCCTTCCCGGCTGTGCCGAA
ATGGTCCATCAAAAAATGGCTTTCGCTACCTGGAGAGACGCGCCCGCTGATCCTTTGCGAAT
ACGCCCACGCGATGGGTAACAGTCTTGGCGGTTTCGCTAAATACTGGCAGGCGTTTCGTCAG
TATCCCCGTTTACAGGGCGGCTTCGTCTGGGACTGGGTGGATCAGTCGCTGATTAAATATGA
TGAAAACGGCAACCCGTGGTCGGCTTACGGCGGTGATTTTGGCGATACGCCGAACGATCGCC
AGTTCTGTATGAACGGTCTGGTCTTTGCCGACCGCACGCCGCATCCAGCGCTGACGGAAGCA
AAACACCAGCAGCAGTTTTTCCAGTTCCGTTTATCCGGGCAAACCATCGAAGTGACCAGCGA
ATACCTGTTCCGTCATAGCGATAACGAGCTCCTGCACTGGATGGTGGCGCTGGATGGTAAGC
CGCTGGCAAGCGGTGAAGTGCCTCTGG

Tail

ATGTCGCTCCACAAGGTAAACAGTTGATTGAACTGCCTGAACTACCGCAGCCGGAGAGCGC
CGGGCAACTCTGGCTCACAGTACGCGTAGTGCAACCGAACGCGACCGCATGGTCAGAAGCC
GGGCACATCAGCGCCTGGCAGCAGTGGCGTCTGGCGGAAAACCTCAGTGTGACGCTCCCCG
CCGCGTCCCACGCCATCCCGCATCTGACCACCAGCGAAATGGATTTTTGCATCGAGCTGGGT
AATAAGCGTTGGCAATTTAACCGCCAGTCAGGCTTTCTTTCACAGATGTGGATTGGCGATAA
AAAACAACTGCTGACGCCGCTGCGCGATCAGTTCACCCGTGCACCGCTGGATAACGACATTG
GCGTAAGTGAAGCGACCCGCATTGACCCTAACGCCTGGGTCGAACGCTGGAAGGCGGCGGG
CCATTACCAGGCCGAAGCAGCGTTGTTGCAGTGCACGGCAGATACACTTGCTGATGCGGTGC
TGATTACGACCGCTCACGCGTGGCAGCATCAGGGGAAAACCTTATTTATCAGCCGGAAAACC
TACCGGATTGATGGTAGTGGTCAAATGGCGATTACCGTTGATGTTGAAGTGGCGAGCGATAC
ACCGCATCCGGCGCGGATTGGCCTGAACTGCCAGCTGGCGCAGGTAGCAGAGCGGGTAAAC
TGGCTCGGATTAGGGCCGCAAGAAAACTATCCCGACCGCCTTACTGCCGCCTGTTTTGACCG
CTGGGATCTGCCATTGTCAGACATGTATACCCCGTACGTCTTCCCGAGCGAAAACGGTCTGC
GCTGCGGGACGCGCGAATTGAATTATGGCCCACACCAGTGGCGCGGCGACTTCCAGTTCAAC
ATCAGCCGCTACAGTCAACAGCAACTGATGGAAACCAGCCATCGCCATCTGCTGCACGCGG

AAGAAGGCACATGGCTGAATATCGACGGTTTCCATATGGGGATTGGTGGCGACGACTCCTG
GAGCCCGTCAGTATCGGCGGAATTCCAGCTGAGCGCCGGTCGCTACCATTACCAGTTGGTCT
GGTGTCAAAAATAA

C. Sequences of oligonucleotide probes used for *glpK* mRNA molecules

Probes that bind to the head	**Probes that bind to the tail**
GGTCGAGCGCAACGATATAT	TTTCACGCACAACTGACCAA
GCATCGTGATCCATTACGAC	CATAGGTGTTCTTCGCCATC
GCTGCGACACGCTAATGATA	TTCATCAGCATAAAGCAGCC
AACCTGGTTTTGGGTAGATT	TTTTCTGATTTCACCGCTTT
CAGATTTCCATTGGGTCGTG	CATAGTTCACTTCGCCAGTC
AACTGATATCGGCTTTCGCC	ATAAACACCGCACCTTCCAA
CCGATAGCTGCAATTTGATC	AACTTCATTTCATCGCGCAG
GGTTTCACGCTGGTTCGTAA	ATATTCGGAATCGTAGGCGT
GTTTCTTTTTCCCAGACAAT	ATTGGTGTTTTGCACTTTGG
TGGCGTTATAGATAGGCTTG	AAATGCCGGAACCACATACA
CAGATTTCTGCGGTACGACG	CGAGTCAGACCGAAAATCGC
ACCGTCACGTTTTAAATGCT	CGCGTATAATGTGGTTAGCG
TGCTGCGGATATAATCTTCT	TAAGCAATAGACTCCAGCGT
TACGGGTCAATCACCAGACC	TGCAGACGGATACCAGAGTC
CTTCACTTTGGTGCCAGAAA	GAAATTGTTTGCTACTGCGC
CTTCCACATGGTCGAGGATC	TATCGGACTGGAACTGCATC
CAATTCACCACGACGTGCAC	CGTCGAGGTTCTGCCAGAAG
CACGTATCAACCGTACCAAA	TCAATCACCGCTTTCTCTTG
CCTGAGTCATTTTCCAGATA	TTTCGATGCCTGGACGGAAC
GTAATCGGTCACATGGACAC	GCGTAACGGTAATTACGCTC
ACATGGTACGAGAGGCGTTG	GTTTAACCGCTTTTTTCCAG
TCCAGGGTATGGATGTTGAA	
ACTTCCAGCATTTTGTCGTC	
AGCATCTCGCGCGGAATATC	
GGAAGAACGACGCACTTCTG	
TGTTAGTCTGACCGTATACT	
CGGAGATTGGAATACGCGTG	

D. Sequence of the *glpK* gene, with the probe-binding sequences underlined

Head

ATGACTGAAAAAAAAATATATCGTTGCGCTCGACCAGGGCACCACCAGCTCCCGCGCGGTCGT
AATGGATCACGATGCCAATATCATTAGCGTGTCGCAGCGCGAATTTGAGCAAATCTACCCAA
AACCAGGTTGGGTAGAACACGACCCAATGGAAATCTGGGCCACCCAAAGCTCCACGCTGGT
AGAAGTGCTGGCGAAAGCCGATATCAGTTCCGATCAAATTGCAGCTATCGGTATTACGAACC
AGCGTGAAACCACTATTGTCTGGGAAAAAGAAACCGGCAAGCCTATCTATAACGCCATTGTC
TGGCAGTGCCGTCGTACCGCAGAAATCTGCGAGCATTTAAAACGTGACGGTTTAGAAGATTA
TATCCGCAGCAATACCGGTCTGGTGATTGACCCGTACTTTTCTGGCACCAAAGTGAAGTGGA
TCCTCGACCATGTGGAAGGCTCTCGCGAGCGTGCACGTCGTGGTGAATTGCTGTTTGGTACG

GTTGATACGTGGCTTATCTGGAAAATGACTCAGGGCCGTGTCCATGTGACCGATTACACCAA
CGCCTCTCGTACCATGTTGTTCAACATCCATACCCTGGACTGGGACGACAAAATGCTGGAAG
TGCTGGATATTCCGCGCGAGATGCTGCCAGAAGTGCGTCGTTCTTCCGAAGTATACGGTCAG
ACTAACATTGGCGGCAAAGGCGGCACGCGTATTCCAATCTCCGGGATCGCCGGTGACCAGC
AGGCCGCGCTGT

Tail

TTGGTCAGTTGTGCGTGAAAGAAGGGATGGCGAAGAACACCTATGGCACTGGCTGCTTTATG
CTGATGAACACTGGCGAGAAAGCGGTGAAATCAGAAAACGGCCTGCTGACCACCATCGCCT
GCGGCCCGACTGGCGAAGTGAACTATGCGTTGGAAGGTGCGGTGTTTATGGCAGGCGCATC
CATTCAGTGGCTGCGCGATGAAATGAAGTTGATTAACGACGCCTACGATTCCGAATATTTCG
CCACCAAAGTGCAAAACACCAATGGTGTGTATGTGGTTCCGGCATTTACCGGGCTGGGTGCG
CCGTACTGGGACCCGTATGCGCGCGGGGCGATTTTCGGTCTGACTCGTGGGGTGAACGCTAA
CCACATTATACGCGCGACGCTGGAGTCTATTGCTTATCAGACGCGTGACGTGCTGGAAGCGA
TGCAGGCCGACTCTGGTATCCGTCTGCACGCCCTGCGCGTGGATGGTGGCGCAGTAGCAAAC
AATTTCCTGATGCAGTTCCAGTCCGATATTCTCGGCACCCGCGTTGAGCGCCCGGAAGTGCG
CGAAGTCACCGCATTGGGTGCGGCCTATCTCGCAGGCCTGGCGGTTGGCTTCTGGCAGAACC
TCGACGAGCTGCAAGAGAAAGCGGTGATTGAGCGCGAGTTCCGTCCAGGCATCGAAACCAC
TGAGCGTAATTACCGTTACGCAGGCTGGAAAAAAGCGGTTAAACGCGCGATGGCGTGGGAA
GAACACGACGAATAA

E. Sequences of oligonucleotide probes used for *amtB* mRNA molecules

Probes that bind to the head	**Probes that bind to the tail**
GTTTTTATCGTCGCTATCTT	CGCGTTTTCCTATCAGATAC
AAGCATCGCCAGTGAAGCAA	TTTAAACGCCTCTTTACCGA
AACGCATTGTCGGCTTTATC	TGAAGACCATCGGCAGGTTG
ACCAGCGCAGTACAAATCAT	ATATAGAGAATGGCAGTCCC
CCCCGGAATAGTCATAAACA	GGCGTTAAAGCCAAACCAAC
TCAACCCACCGTAAAACAGG	CCACAGTATTCACAAATGCC
AGCATCGACAGCACGTTTTT	AAGATCCAGCCAAGAATTGC
CAAATGTCACCGTCACCTGC	CAGCAGTGAAGGCTTACCAC
ACCCAGAGAATACAGACCAG	TACCACGCCGATAATCAACG
CGCCAGCGAGTAACCGTAAA	AGCGTTTGAGCATGGTAACG
GAAGAAGTTGTTGCCCTCAC	AAGACATCGCAGGGATCATC
GCATCAACCAGTTAATGTTG	AGCCGACAATGCCACAAACG
GCCGTCAGTTCGATGTTTTT	GCAAAAATCCCGGTCATGAT
TACTGATAAATGCTGCCCAT	ATCGTCACACCTTCAGCGAA
AGGCAAACGATCCCTGAAAC	AGCTGTACCAGCAACTGATG
ACTATCAAGCCGACGGTAAT	GATCGTAATGGCGATGCTTT
TGAGAAGCGGATTCGTTCCG	TAAATGCCACAACACCGGAC
CCACCACGAAAATCAACACA	TCAGATCCGCCAATTTGTAG
ATGTAAGAGAGCGTCAGCCA	TACGCGTTATAGGCATTCTC
CCACACCATATGCGCAATCG	
GTGAGAAGCCAGCAAACCAC	
ATTGCGGCGTTAATGTGCAC	

F. Sequence of the *amtB* gene, with the probe-binding sequences underlined

Head

ATGAAGATAGCGACGATAAAAACTGGGCTTGCTTCACTGGCGATGCTTCCGGGACTGGTAAT
GGCTGCACCTGCGGTGGCCGATAAAGCCGACAATGCGTTTATGATGATTTGTACTGCGCTGG
TGCTGTTTATGACTATTCCGGGGATTGCCCTGTTTTACGGTGGGTTGATTCGCGGCAAAAACG
TGCTGTCGATGCTGACGCAGGTGACGGTGACATTTGCACTGGTCTGTATTCTCTGGGTGGTTT
ACGGTTACTCGCTGGCGTTTGGTGAGGGCAACAACTTCTTCGGCAACATTAACTGGTTGATG
CTGAAAAACATCGAACTGACGGCGGTGATGGGCAGCATTTATCAGTATATCCACGTGGCGTT
TCAGGGATCGTTTGCCTGCATTACCGTCGGCTTGATAGTTGGGGCGCTGGCGGAACGAATCC
GCTTCTCAGCTGTGTTGATTTTCGTGGTGGTATGGCTGACGCTCTCTTACATTCCGATTGCGC
ATATGGTGTGGGGCGGTGGTTTGCTGGCTTCTCACGGTGCGCTGGATTTCGCGGGTGGCACC
GTGGTGCACATTAACGCCGCAATCGCCGGTCTGGTGGGCGC

Tail

GTATCTGATAGGAAAACGCGTGGGCTTCGGTAAAGAGGCGTTTAAACCGCACAACCTGCCG
ATGGTCTTCACCGGGACTGCCATTCTCTATATCGGTTGGTTTGGCTTTAACGCCGGGTCAGCG
GGCACGGCGAATGAAATCGCGGCACTGGCATTTGTGAATACTGTGGTCGCAACGGCGGCGGC
AATTCTTGGCTGGATCTTCGGTGAATGGGCGCTGCGTGGTAAGCCTTCACTGCTGGGGGCG
TGTTCTGGCGCGATTGCCGGTCTGGTCGGCGTGACGCCAGCCTGCGGCTACATTGGGGTTGG
CGGCGCGTTGATTATCGGCGTGGTAGCTGGTCTGGCGGGCTTGTGGGGCGTTACCATGCTCA
AACGCTTGCTGCGGGTGGATGATCCCTGCGATGTCTTCGGTGTGCACGGCGTTTGTGGCATT
GTCGGCTGTATCATGACCGGGATTTTTGCCGCCAGCTCGCTGGGCGGCGTGGGCTTCGCTGA
AGGTGTGACGATGGGCCATCAGTTGCTGGTACAGCTGGAAAGCATCGCCATTACGATCGTCT
GGTCCGGTGTTGTGGCATTTATCGGCTACAAATTGGCGGATCTGACGGTTGGTCTGCGTGTA
CCGGAAGAGCAGGAGCGAGAAGGGCTGGATGTCAACAGCCACGGCGAGAATGCCTATAAC
GCGTAA

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