Supplementary Material 4
(Edouard Bertrand)

Probe sequences for FISH:

Intronic pool of probes:

I1:
5' A T* AATTCTTCTTACAG T* TAAA T GGGATGG T* GCAAGCGC T AGAACATAC T* A

I2:
5' A T* GGCCCTTCT T* CAGGGCCCATCGAGCAA T* TGGGACCGTGC T* A

I3:
5' A T* CTCGAGATTAGGGCCC T* TCTTCAGGGCCCA T* CGAGATTAGG T* A

I4:
5' A T* TATAGGAGGTTATGGGAGAG T* GAAAAATAGTAAAAAAAGG T* AAAAGAGAAATCT T* A

I5:
5' A T* CCCTATTTATTCCAA T* AATATCGTGGTTATTACAGA T* CAGTCAATATAGG AG T* A

I6
5' A T* TATCACTTATCACGAAAA T* ATTTCAGTTTTTTTTTTTT T* CCAAATTTCAAGCCC T* A

I7
5' A T* AGTACATGAGACTTAG T* AACAGTAGCAAA T* AAAAGAAGAATATCACTATC T* A

I8
5' A T* GATCTAAACATATAATA T* AGCAACAAAAAGAA T* GAAGCAATCGATGTT T* A

Exonic pool of probes:

HA1:
5' A T* CCGCATAGTCAGGAACA T* CGTATGGGTAAAAGA T* GttaattaaACTAGT T* A

HA2:
5' A T* GGATAGGATCC T* GCATAGTCCGGGACG T* CATAGGGATAGC T* A

HA3:
5' A T* gcgcgcctcaGCAC T* GAGCAGCGTAATC T* GGAACGTCATAT T* A

E1:
5' A X CAGATCTTTGAAGA X AAAGACATTGTTAAT X CAGTAAATTTTCGGAATTC X A

BoonRep_E2
5' A X CTGGCAATTCCT X ACCTTCCAATAAT X CCAAAGAAGCACCACCAC X A

MS2_bis
5' A T* GTCGACCTGCAGACA T* GGGTGATCCTCA T* GTTTTCTAGGCAAT T* A

T* correspond to amino-modified Ts that are conjugated to fluorophores.

Hybridization

Cells were grown until mid-log phase (OD600 of 0.6), and fixed for 30 minutes at room temperature by adding formaldehyde to a final concentration of 4%. Cells were then spheroplasted and processed for in situ hybridization as previously described (Long, R.M., Elliott, D.J., Stutz, F., Rosbash, M., and Singer, R.H.(1995). Spatial consequences of defective processing of specific yeast mRNAs revealed by fluorescent in situ hybridization. RNA *1*,1071–1078.).

Coverslips with cells attached were stored in 70% ethanol and rehydrated in 1xSSC, 30% formamide. Hybridization was done overnight at 37% °C in 1X SSC, 30% formamide, 10% Dextran sulfate, 2 mM Vanadyl-ribonucleoside complex, 0.2 mg/ml RNAse-free BSA, 2 mg/ml E. Coli tRNAs, and 10 ng of each Cy3-labelled oligo probe.

Coverslips were then washed twice with 1 x SCC, 30% formamide for 30 min at 37°C and twice 1 hour in the same solution. Coverslips were finally rinsed in PBS for 2 minutes and mounted in glycerol 90%; PBS; p-phenylenediamine 1mg/ml; 0.1 mg/ml DAPI.

Detection and automated counting of single RNA molecules

A semi-automated procedure was used to detect and automatically count single mRNA molecules that are visible as bright, individual spots. 3D stacks (planes taken every 200 nm in Z) of control and strains expressing the various reporters were taken with a CCD camera (CoolSnap HQ, Roper Scientific), on an upright microscope equipped for fluorescent imaging (DMRA, Leica microsystems), with a 100X objective, NA 1.4 (pixel size, 67 nm). Image analysis was performed with the Spot function of Imaris 3.0 (Bitplane), as described in the main text, and this allowed the calculation of the total number of mRNA, as well as their position in the image. The number of cells in the image was then calculated by counting the number of nuclei stained in Dapi, and this was used to calculate the average number of mRNA per cell. In each experiment, the data were derived from four images taken in different area of the coverslips, totalizing from 110 to 358 cells.

To obtain the distribution of the number of mRNA per cell within the population, each cell had to be identified separately. This was done by merging the Dapi image with the autofluorescence taken in the green channel. Using the Spot function of Imaris, each nucleus was identified as a spot, and this spot was grown until it filled the volume of the cell. A channel containing all enlarged spots with a different pseudo-color for each was created with Imaris, The pseudo-color of each mRNA in the same image thus indicated the cell it belonged to, and this information was used to calculate the number of mRNA for each cell in the image.