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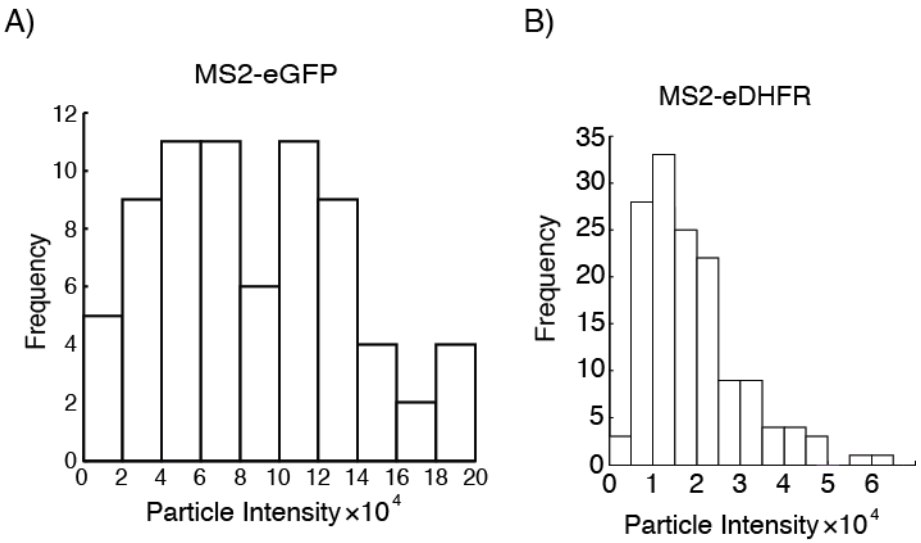
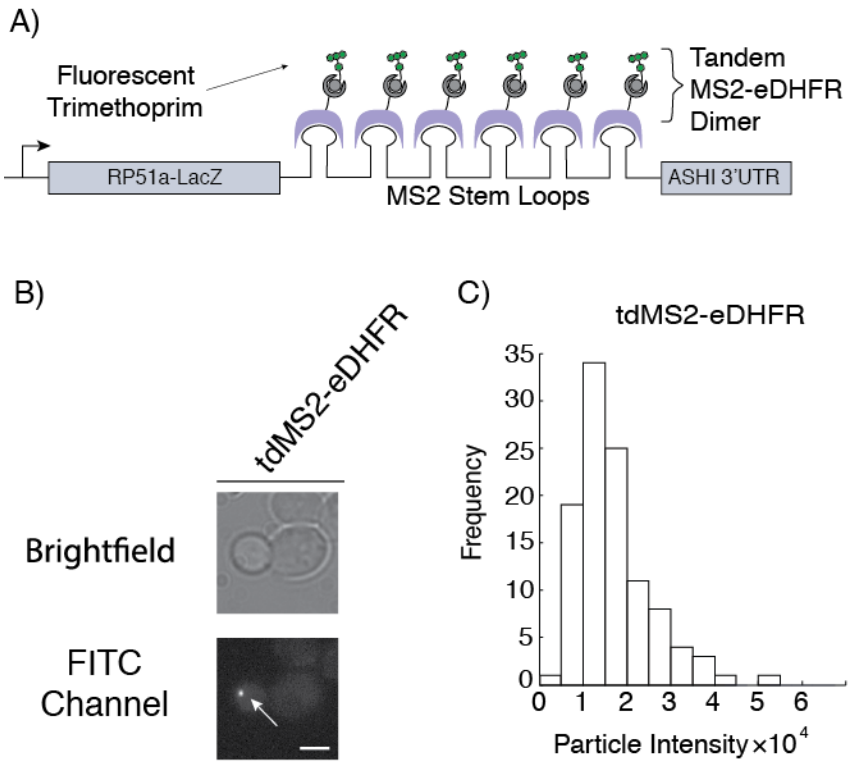
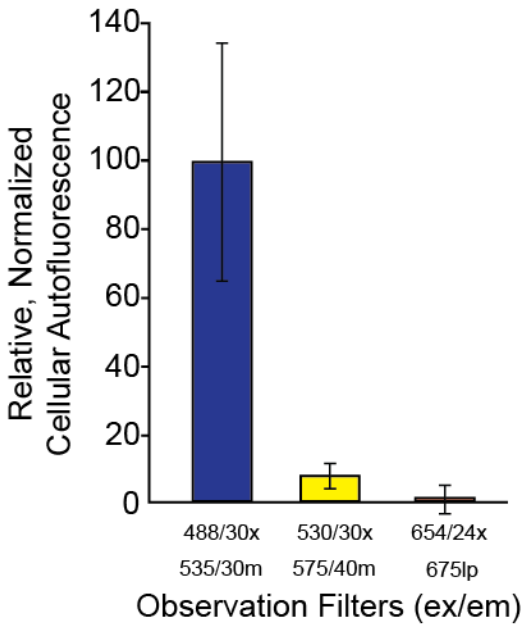


Supplemental Figure 1. (A) Histogram of particle intensity using MS2-eGFP. (B) Histogram of particle intensity using MS2-eGFP.



Supplemental Figure 2. (A) Illustration depicting RNA localization using a tandem MS2 dimer. Each tdMS2-eDHFR protein will bind a stem loop present in the reporter mRNA, resulting in a maximum of 6 copies of eDHFR per mRNA. (B) Micrographs of living yeast cells. mRNAs can be localized to the bud tip using fluorescein-TMP in conjunction with the tdMS2-eDHFR tag. The scale bar represents a length of 3 μm. (C) Histogram of fluorescent particle intensities. The overall distribution of tdMS2-eDHFR is similar to MS2-eDHFR (Supplemental Figure 1).



Supplemental Figure 3. Cellular autofluorescence of live *S. cerevisiae* when imaged at three different wavelengths. The cellular background is reduced ~10-fold when imaging is performed at wavelengths capable of exciting dyes such as hex-TMP (530/30x) versus shorter wavelengths commonly used to excite GFP (488/30x). A further ~10-fold reduction is observed at wavelengths that can be used to excite far-red dyes such as SiRhod-bG (654/24x).

METHODS AND MATERIALS

Unless otherwise noted, Herculase II DNA polymerase (Stratagene) was used for PCR. All plasmids (**Supplemental Table 1**) were confirmed by sequencing. Yeast transformation and growth was carried out using standard techniques and media.[1] Fluorescent TMP derivatives were obtained from ActiveMotif. Fluorescent ligands for the SNAP tag were obtained from New England Biolabs. Ivan Correa, Jr. (New England Biolabs) generously provided bG-SiRhod and SNAP-Cell® 505-Star.

RNA-binding Protein Plasmid Construction:

The eDHFR coding sequence was fused to the C-terminus of the MS2 coat protein by first amplifying the eDHFR gene from plasmid pMONDHFR[2] with PCR primers TJC001 and TJC002 (**Supplemental Table 2**). The resulting PCR product contained the eDHFR gene along with an N-terminal, six amino acid linker (AGSGSG) and NotI and HpaI restriction enzyme recognition sites at the 5' and 3' ends of the gene, respectively. The pGFP-MS2/Leu plasmid[3] (gift of R. Singer, **Supplemental Table 1**) containing the dlFG mutant of the MS2 coat protein along with a N-terminal nuclear localization signal (NLS) and a hemagglutinin (HA) tag was digested with the NotI and HpaI restriction enzymes to remove the GFP coding sequence. The eDHFR fragment was then ligated into this plasmid at these same sites to yield MS2-eDHFR (pAAH0099, **Supplemental Table 1**). MS2-SNAP was created using primers TJC003 and TJC0004 (**Supplemental Table 2**) to amplify the SNAP gene from the $SNAP_f$

Vector (New England Biolabs). The PCR product was digested with NotI/HpaI and ligated into the digested pGFP-MS2/Leu plasmid to yield pAAH0096 (**Supplemental Table 1**). To create the tdMS2-eDHFR plasmid (pAAH0176, **Supplemental Table 1**) containing the gene for eDHFR fused to a tandem dimer of the MS2 protein, three gene blocks (IDT) were ligated together by Gibson Assembly (New England Biolabs), amplified by PCR using *Taq* DNA Polymerase (Promega) and primers ah103_tdMS2_DHFR_F and ah104_tdMS2_DHFR_R (**Supplemental Table 2**). The product was then ligated into plasmid p425 GPD (www.atcc.org) at the SpeI and HindIII restriction sites. The full gene and amino acid sequence of this construct is shown below.

tdMS2-eDHFR Gene Sequence:

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GGGACTAGTATGCCAAAGAAGAAAAGGAAAGTTGGCTACCCCTACGACGTGCCCGACTACGCCATCGAAGG
CCGCCATATGCTAGCCGTTAAAATGGCATCAAATTTCACACAATTTGTACTAGTTGATAACGGCGGAACTG
GCGACGTGACTGTCGCCCCAAGCAACTTCGCTAACGGGATCGCTGAATGGATCAGCTCTAACTCGCGTTCA
CAGGCTTACAAAGTAACCTGTAGCGTTCGTCAGAGCTCTGCGCAGAATCGCAAATACACCATCAAAGTCGA
GGTGCCTAAAGGCGCCTGGCGTTCGTACTTAAATATGGAACTAACCATTCCAATTTTCGCCACGAATTCCG
ACTGCGAGCTTATTGTTAAGGCAATGCAAGGTCTCCTAAAAGATGGAAACCCGATTCCCTCAGCAATCGCA
GCAAACTCCGGCATCTACATCTACGCCATGGCTTCTGCTTCTAACTTTACTCAGTTCGTTCTCGTCGACAA
TGGCGGAACTGGCGACGTGACTGTCGCCCCAAGCAACTTCGCTAACGGGATCGCTGAATGGATCAGCTCTA
ACTCGCGTTCACAGGCTTACAAAGTAACCTGTAGCGTTCGTCAGAGCTCTGCGCAGAATCGCAAATACACC
ATCAAAGTCGAGGTGCCTAAAGGCGCCTGGCGTTCGTACTTAAATATGGAACTAACCATTCCAATTTTCGC
CACGAATTCCGACTGCGAGCTTATTGTTAAGGCAATGCAAGGTCTCCTAAAAGATGGAAACCCGATTCCCT
CAGCAATCGCAGCAAACTCCGGCATCTACGCGGCCGCTGGTTCTGGTTCTGGTATGATCAGTCTGATTGCG
GCGTTAGCGGTAGATCGCGTTATCGGCATGGAAAACGCCATGCCGTGGAACCTGCCTGCCGATCTCGCCTG
GTTTAAACGCAACACCTTAAATAAACCCGTGATTATGGGCCGCCATACCTGGGAATCAATCGGTCGTCCGT
TGCCAGGACGCAAAAATATTATCCTCAGCAGTCAACCGGGTACGGACGATCGCGTAACGTGGGTGAAGTCG
GTGGATGAAGCCATCGCGGCGTGTGGTGACGTACCAGAAATCATGGTGATTGGCGGCGGTCGCGTTTATGA
ACAGTTCTTGCCAAAAGCGCAAAAACTGTATCTGACGCATATCGACGCAGAAGTGGAAGGCGACACCCATT
TCCCGGATTACGAGCCGGATGACTGGGAATCGGTATTCAGCGAATTCCACGATGCTGATGCGCAGAACTCT
CACAGCTATTGCTTTGAGATTCTGGAGCGGCGGTAAAAGCTTGGG
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tdMS2-eDHFR Protein Sequence:

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MPKKKRKVGYPYDVPDYAIEGRHMLAVKMASNFTQFVLVDNGGTGDVTVAPSNFANGIAEWISSNSRSQAY
KVTCSVRQSSAQNRKYTIKVEVPKGAWRSYLNMELTIPIFATNSDCELIVKAMQGLLKDGNPIPSAIAANS
GIYIYAMASASNFTQFVLVDNGGTGDVTVAPSNFANGIAEWISSNSRSQAYKVTCSVRQSSAQNRKYTIKV
EVPKGAWRSYLNMELTIPIFATNSDCELIVKAMQGLLKDGNPIPSAIAANSGIYAAAGSGSGMISLIAALA
VDRVIGMENAMPWNLPADLAWFKRNTLNKPVIMGRHTWESIGRPLPGRKNIILSSQPGTDDRVTWVKSVDE
AIAACGDVPEIMVIGGGRVYEQFLPKAQKLYLTHIDAEVEGDTHFPDYEPDDWESVFSEFHDADAQNSHSY
CFEILERR.
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Reporter RNA Plasmid:

Reporter RNAs bearing multiple MS2 stemloops were expressed by transformation of yeast strains (**Supplemental Table 3**) with pGAL-lacZ-MS2-ASH1/TRP as described elsewhere.[3]

Reporter Induction and *In Vivo* labeling:

Prior to fluorescent labeling, yeast were transformed by plasmids expressing MS2 fusion proteins either in the presence or absence of a second plasmid expressing the reporter RNA. Single colonies of transformed yeast were then grown at 30°C to early mid-log phase in the appropriate synthetic dropout media (25 mL) containing 2% w/v raffinose and 0.02% w/v glucose. To induce RNA expression, a portion of this culture (100 µL) was removed, placed into a sterile 1.5 mL microfuge tube, pelleted by centrifugation, and resuspended in media (100 µL) containing 3% w/v galactose. For labeling of RNAs with trimethoprim, cells were treated with trimethoprim dyes (10µM) for 0.75-3h at 30°C while rotating. Care was taken to avoid excessive light exposure during and after staining. For labeling of RNAs with SNAP dyes, a portion of an overnight culture (100 µL) was removed, placed into a sterile 1.5 mL microfuge tube, and incubated with SNAP dyes (5 µM) for 7 h. Cells were then collected by centrifugation and fresh, galactose-containing media with the SNAP dye (5 µM) was added. Cells were then grown for an additional 3h, washed with fresh media (1 mL), and plated for imaging.

Image Acquisition and Analysis:

Yeast were imaged in glass bottom microwell petri dishes (MatTek)

pretreated with concanavalin A (1 μg/ml, Sigma) for surface adherence. Images were acquired using a Nikon TE-2000 microscope (100X 1.45NA oil immersion objective, 100ms exposure time) and either a CoolSnap CCD (0.017μm^2 per pixel, Photometrics) or EMCCD (0.027μm^2 per pixel, Photometrics) camera. The microscope was controlled using MetaMorph imaging software. Fluorophores were excited using a mercury arc lamp (Exfo). eGFP, fluorescein, and SNAP-Cell® 505-Star were imaged using 488/30x and 535/30m excitation and emission filters, respectively. Hexachlorofluorescein was imaged using 530/30x and 575/40m excitation and emission filters, respectively. SiRhod was imaged using 654/24x and 675lp excitation and emission filters, respectively.

Images were first analyzed by visually determining the proportion of cells in a population possessing a fluorescent particle localized within the budding daughter cell. Integrated intensity values for individual particles within the bud were determined using ImageJ software. These particles were enclosed in a 13×13 pixel box for measurement and background was subtracted using an identically-sized region adjacent to the fluorescent particle.

Supplemental References

1. D. C. Amberg, D. Burke, and J. N. Strathern, *Methods in Yeast Genetics*, CSHL Press, 2005.
2. L. W. Miller, Y. Cai, M. P. Sheetz, and V. W. Cornish, *Nat Meth*, 2005, **2**, 255–257.
3. E. Bertrand, P. Chartrand, M. Schaefer, S. M. Shenoy, R. H. Singer, and R. M. Long, *Mol Cell*, 1998, **2**, 437–445.

Supplemental Table 1. Protein expression plasmids

Designation	RNA-Binding Protein	Fusion Tag
pAAH0096	MS2	SNAP
pAAH0099	MS2	eDHFR
pAAH0176	tdMS2	eDHFR
pGFP-MS2/Leu[a]	MS2	eGFP

[a]Plasmid described in Reference 3.

Supplemental Table 2. Primers used in this work. Restriction enzyme recognition sites are shown in bold.

Primer Designation	Sequence (5' to 3')
TJC001	GA**GCGGCCGC**TGGTTCTGGTTCTATGATCAGTCTGATTGCGGCG
TJC002	CC**GTTAAC**TTACCGCCGCTCCAGAATCTC
TJC003	GA**GCGGCCG**CAGGAGGATCAGGAGGATCAATGGACAAAGACTGCGAAATG
TJC004	GAG**TTAAC**TTAACCCAGCCCAGGCTT
ah103_tdMS2_DHFR_F	GGG**ACTAGT**ATGCCAAAAAAGAAAAGAAAAGTTGG
ah104_tdMS2_DHFR_R	CCC**AAGCTT**TTACCGCCGCTCC

Supplemental Table 3. Strains used in this work: The yeast strain K699[a] was used for all experiments.

Strain Designation	Plasmid(s)	Reporter RNA	Tag Expressed
yAAH0143	pAAH0096	-	MS2-SNAP
yAAH0152	pAAH0096, pGAL-lacZ-MS2-ASH1/TRP	+	MS2-SNAP
yAAH0144	pAAH0099	-	MS2-eDHFR
yAAH0155	pAAH0099, pGAL-lacZ-MS2-ASH1/TRP	+	MS2-eDHFR
yAAH0146	pAAH0176	-	tdMS2-eDHFR
yAAH0157	pAAH0176, pGAL-lacZ-MS2-ASH1/TRP	+	tdMS2-eDHFR
yAAH0142	pGFP-MS2/Leu	-	MS2-eGFP
yAAH0153	pGFP-MS2/Leu, pGAL-lacZ-MS2-ASH1/TRP	+	MS2-eGFP

[a]All yeast used in this study are transformation products of the yeast strain K699 (Mat**a**, *his3-11, leu2-3, ade2-1, trp1-1, ura3, ho; can1-100)*.