Supplementary Methods

DNA constructs and strains

To clone eIF4A fragments into Sall-NotI sites of pACYCDuet-1 (pMB09); and pETduet-1 (Novagen) (pMB11), we PCR-amplified each domain from the mouse eIF4A gene in plasmid pGEX-4AI (kindly donated by C. Proud) with the following sets of primers:

5'-CCCGAGTCGACATGGAGCCGGAAGGCGTCATCGA-3'
5'-CGAGCGGCCGCTCAAGGGTCTCTCATAAATTTCTT-3' (for amplification of F1, residues 1-215) and
5'- CCCGAGTCGACATTCGGATTCTTGTCAAGAAGGAAG-3'
5'- CGAGCGGCCGCTCAAATGAGGTCAGCAACGTTGAG-3' (for amplification of F2, residues 216-406).

Similarly, we PCR-amplified EGFP fragments, Alpha (A) and Beta (B) from pEGFP (Clonetech) with the following sets of primers:

5'- CCCGACCATGGTGAGCAAGGGCGAGGAGCTGTTC-3'
5'-CCCGAGGATCCCTGCTTGTCGGCCATGATATAGAC-3' (For amplification of A fragment, residues 1-158) and
5'- CCCGACCATGGGCAAGAACGG CATCAAGGTGAAC-3'
5'- CCGAGGATCCTTGACAGCTCGTCCATGCCGA-3' (For amplification of B fragment, residues 159-238).

We cloned A and B fragments into Ncol-BamHl sites of pACYCDuet-1 (pMB08) and pETduet-1 (pMB10), respectively. We next constructed a chimeric gene (A-F1) where the C-terminal end of EGFP fragment A is fused to the N-terminus of eIF4A fragment F1 via a 10-aa flexible polypeptide linker (Gly-Ser-Ser-Gly-Ser-Gly-Ser-Gly-Ser-Ser) according to Vasl et al. Briefly, we designed two sets of 5'-phosphorylated oligonucleotides and obtained them from Integrated DNA Technology (Corallville, IA):
1. 5’- pCGAAGATCCAGAGGATCCCTGCTTGTCGGCCATGATATAG-3’
2. 5’- pGGTTCTGGTAGGAGCCGGAAGGTCATCGA-3’,
3. 5’- pCGAAGATCCAGAGGATCCCTTGTACAGCTCGTCCATGCC-3’
4. 5’- pGGTTCTGGTAGCATTCTGTTGCAAGAAGGA-3’

The underlined regions in the oligonucleotides correspond to the 3’ end of the A fragment (Oligo #1); the start of the F1 sequence (Oligo #2); the 3’ end of fragment B (Oligo #3); and the beginning of the F2 fragment (Oligo #4). The rest of the oligonucleotide sequence (in italics) corresponds to the coding sequence for the peptide linker GSSGSS (in oligonucleotides 1 and 3) and GSGS (in oligonucleotides 2 and 4).

We cleaved plasmids pMB08 (carrying fragment A of EGFP) and pMB09 (carrying fragment F1 of eIF4A) with XhoI and EcoN1 restriction enzymes since these sites were upstream from the site where the oligonucleotides annealed. We performed PCR with these linearized plasmids and oligonucleotides 1 and 2 using the Expand Long Template PCR system (Roche Diagnostics) with the following program: 94°C for 3min.; 30 cycles of 94°C for 30s, 61°C for 30s, and 72°C for 10 min, ending with a final 11-min elongation step at 72°C. We used the enzyme DpnI to remove any methylated template DNA from the PCR mix. We next isolated the resulting 5.5 kbp PCR product by gel purification with a Qiagen gel-purification kit and ligated it using 1 U of T4 DNA ligase (New England Biolabs). We transformed the ligated product into XL-10 competent cells (Stratagene). We confirmed the presence of the new chimeric gene, A-F1 (pMB12) by restriction enzyme digests and DNA sequencing. Finally, we followed a similar protocol to create the chimeric B-F2 gene in vector pETDuet-1 (pMB13) using oligonucleotides #3 and #4. To maintain similar levels of expression for both chimeric genes, we integrated the B-F2 fragment into the second MCS of pMB12 (carrying the A-F1 gene) according to the
protocol by Geiser et al. Briefly, we PCR-amplified a B-F2 fragment from pMB13 with the following primers:

5. 5' - GGAGATATACATATGGCACGATCTCAAGAAGCCGATCAAGGTGAAC-3'
6. 5' - CCAGACTCGAGGGTACCGACTCAAATGAGGTCAGCAACGTTGAG-3'

The sequence in italics corresponds to 24-25 bp of flanking vector sequences immediately upstream (Oligo # 5) and downstream (Oligo #6) from the point of insertion in pMB12. We next carried out a PCR reaction containing the recipient vector and the B-F2 fragment which would anneal to the uncut vector via the flanking homologous sequences. The PCR program consisted of a denaturation step at 95°C for 30s followed by 18 cycles of 30s at 95°C, 30s at 55°C and 8-10 min at 68°C using Pfu turbo DNA polymerase. We treated the amplified product with the enzyme DpnI for 3 hours to remove the original methylated template DNA. We transformed XL-10 competent cells with an aliquot of the purified PCR product. We were able to isolate plasmids carrying both protein fragments (A-F1 + B-F2) and confirmed these by DNA sequencing (pMB33). Finally, we cloned a Ncol-NotI fragment containing full-length EGFP from pEGFP (Clonetech) into Ncol-NotI sites of the expression vector pACYCDuet-1 (Novagen) to serve as a positive control for EGFP expression (pMB38).

For expression of the target RNA, we PCR-amplified the eIF4A-interacting aptamer sequence (58 nt-long) from pMB14 (not in this study) with oligonucleotides: 5'-CAGTCTAGAACAGGGGACCGCGCC-3' 5'-GCAAGATCTGTTGTGAGCCTCCTGTGCTCTGT-3' for cloning of the aptamer between Xbal-BgIII sites of pETDuet-1 (pMB23). This cloning strategy produced a non-translatable T7-transcript containing two copies of the desired aptamer sequence in tandem. We used a similar procedure to clone the eIF4A-interacting aptamer into pRSFDuet-1 (Novagen), a vector with a higher copy number
Supplementary Fig. 2). Construct pMB71 consisted of a BamHI-EcoRI fragment containing the partial \(rrnB\) operon from plasmid pKK5-1 \(^{30}\) (kindly provided by P. Moore). We cloned this fragment in pETDuet-1, and then modified the 5S rRNA sequence by inserting the eIF4A aptamer at the 3’ end of the gene (pMB84) via a PCR-directed insertion using the following primers:

1. 5’- CCAGCGGATAGTTAATGATAGCCCAC-3’
2. 5’p-CGAAACGTAGATTCGACAGGAGGCTCACAACTCAAATAAAACGAAA GGCTCAGTCAAAGACTGG-3’
3. 5’-GCGCTCGGCCCTTCCGGCTG-3’
4. 5’p-GCCTCACTCACATGTGGGGCGCGGTCCCCTGTGCTCCTG
CAGTTCCCTACTCTCTC-3’

We used 5’-phosphorylated primers #8 and 10 in combination with primers 7 and 9, respectively, for PCR-amplification using pMB71 as the template. We generated two separate DNA fragments, each with a 5’ phosphorylated end. We next mixed equimolar amounts of the fragments and ligated them using T4 DNA ligase (New England Biolabs). We then re-amplified the ligated product using primers #7 and 9. Next, we digested the DNA fragment containing the modified 5S rRNA gene with the enzymes XbaI and AvrII and cloned this fragment into pETDuet-1 vector. We confirmed pMB84 structure by sequencing. Finally, pMB99 is also a derivative of pETDuet-1 where the coding sequence for the \(\beta\)-galactosidase gene (\(\text{LacZ}\)) from pBAD/His/LacZ \((\text{Invitrogen})\) was cloned upstream of the eIF4A-interacting aptamer to generate an mRNA target with an aptamer tag located at the 3’ end of the gene, immediately after the stop codon.

We used \(E.\ coli\) strains XL10-Gold (Tet\(^r\) \(\Delta(mcrA)183 \Delta(mcrCB-hsdSMR-mrr)173\) endA1 supE44 thi-1 recA1 gyrA96 relA1 lacHte [F’ proAB lacQZΔM15 Tn10 (Tetr) Amy Camr]) and XL10-Gold Kan\(^r\) (Tetr \(\Delta(mcrA)183 \Delta(mcrCB-hsdSMR-mrr)173\) endA1 supE44 thi-1 recA1 gyrA96 relA1 lac Hte [F’ proAB lacQZΔM15 Tn10 (Tetr) Tn5 (Kanr) Amy].
(Stratagene) for cloning purposes. We used BL21(DE)3 (E. coli B F− dcm ompT hsdS(b− m−) gal(λ(DE3)) (Stratagene) for expression of fusion proteins and the target RNA.

**Image Acquisition and Processing**

We took images with exposure times of 150-300 ms using a digital B&W camera (12 bit; 20 mHz) with a 100x magnification objective controlled by IPLab v.3.7 software (Scanalytics, Inc). We used an ND4 filter to reduce cell photodamage. Image processing was performed using ImageJ 1.36 B software (Wayne Rasband, NIH). Fluorescent images obtained through microscopy were read into ImageJ in JPEG format converted into 8-bit type or in IPLab format. We adjusted the threshold level manually for each image. The upper threshold bound was set to the maximum value and the lower threshold boundary was empirically set so that there were no pixels in the background identified as objects. We obtained total cell fluorescence using the option ‘Analyze Particles’. The output contained Area of pixels, Mean, Minimum, and Maximum Grayscale of identified objects. We calculated total fluorescence of the cells by integrating the product of the Mean minus Minimum (grayscale/pixel) by Area (of pixels). This calculation method subtracted background. We calculated kinetics of total fluorescence changes in a single cell in a similar way, this time by applying a rectangular segment tool to the cell of interest. For quantification of fluorescence distribution along the cell, we measured fluorescence profiles along the long axis of the bacterial cell. We measured each cell 3 to 4 times, and averaged the results. Background fluorescence around each cell was quantified similarly and subtracted from the cell fluorescence profile.

**Real competitive PCR**

We performed reverse transcription of total RNA isolated from cell culture for one hour at 42°C with 1 μg of random hexanucleotides and an ImProm II reverse transcriptase (Promega) in 25 μL total volume. We designed primers and competitors using Sequenom’s Assay Designer software and obtained them from Integrated DNA Technology (Coralville, IA). We performed amplification of cDNA using PCR primers at 100 nM, competitors at varying concentrations, MgCl₂ at 2.75 mM, and 200 μM dNTP
using 0.1 U HotStar Taq DNA polymerase (Qiagen) in five μL with the following PCR conditions: 95°C hot start for 15 min, followed by 45 cycles of 95°C for 30 seconds, 56°C for one minute, then 72°C for 1 minute, with a final hold of 72°C of seven minutes. We treated PCR products with 0.04 U shrimp alkaline phosphatase (Sequenom), which inactivates unused dNTPs from the amplification cycles, for 20 minutes at 37°C followed by heat inactivation at 85°C for five minutes. For the extension cycle, we added extension primer with 1.2 μM final concentration and 0.6 U of ThermoSequenase (Sequenom) to a total reaction of nine μL with the termination mix containing specific dideoxynucleotides and deoxynucleotides for each reaction at 50 μM for each base. The extension conditions include a 94°C hold for two minutes with 75 cycles of the following: 94°C for five seconds, 52°C for five seconds, and 72°C for five seconds.

**MALDI TOF MS**

Prior to MALDI TOF MS analysis, we removed salts from the reactions using SpectroCLEAN resin and 16 μL of water. Then we performed analysis using the MassARRAY system (Sequenom) by dispensing ≈ 10 nL of final product onto a 384-plate format MALDI TOF MS SpectroCHIP using a SpectroPOINT nanodispenser (Sequenom). We analyzed mass spectrometric data using TITAN³ software set at the default values.

**References**

