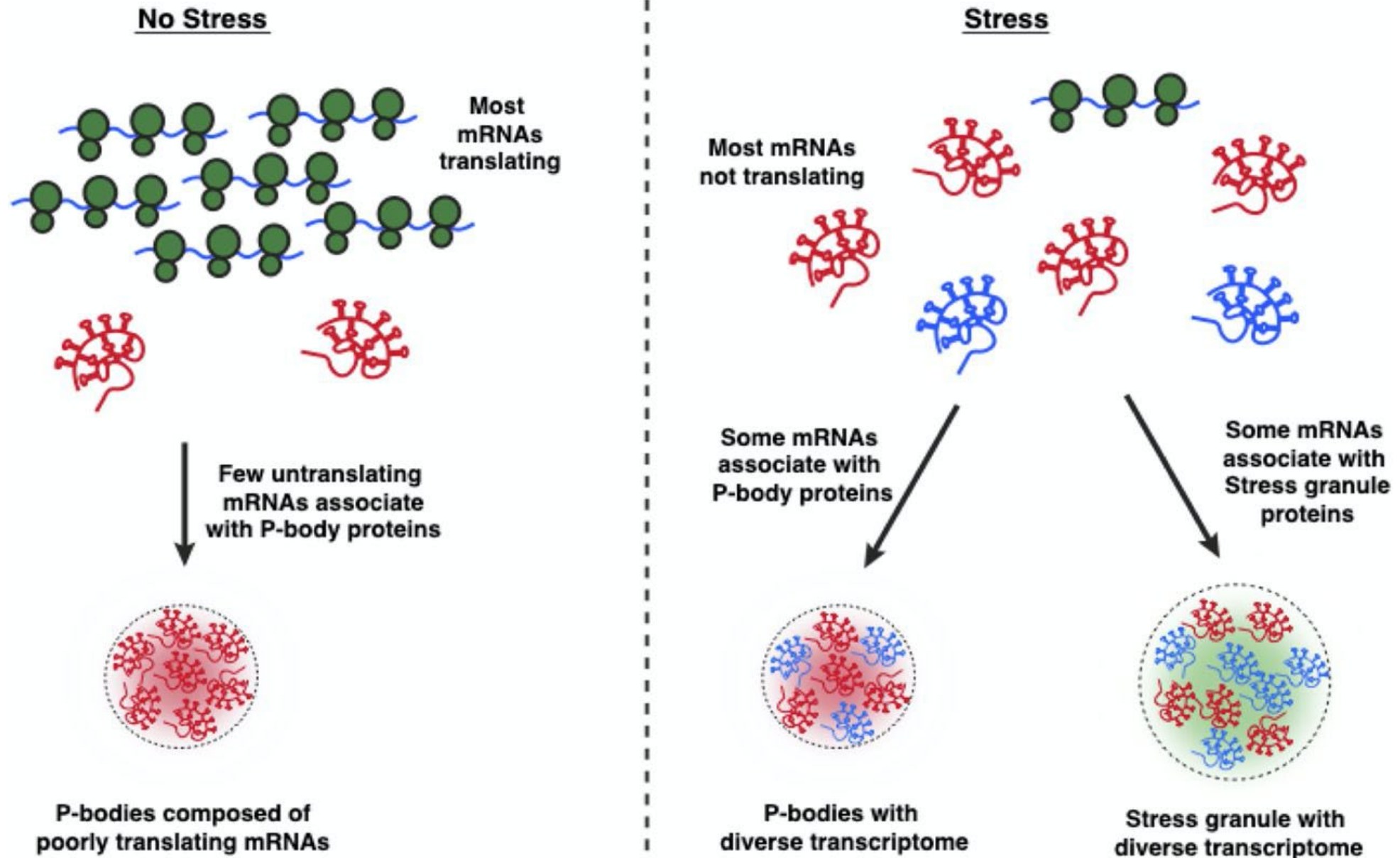


Imaging for stress granules: debate for mRNA translation repression

Technical Journal Club

Tingting Liu
19-01-2021

Membrane-less organelles under stress conditions



Whether does translation of mRNAs in stress granules is suppressed or not?

1. Translation of mRNAs in stress granules may provide further signaling coding during cellular stress.
2. What's the dynamic characteristics of mRNA translation in stress granules, in particular comparing with cytosolic mRNA translation under physiological and stress conditions?

mRNAs species in stress granules

KDM5B mRNAs

(Moon et al., 2019, Nature cell biology)

5'TOP mRNAs--constitutes of ~20% of transcripts

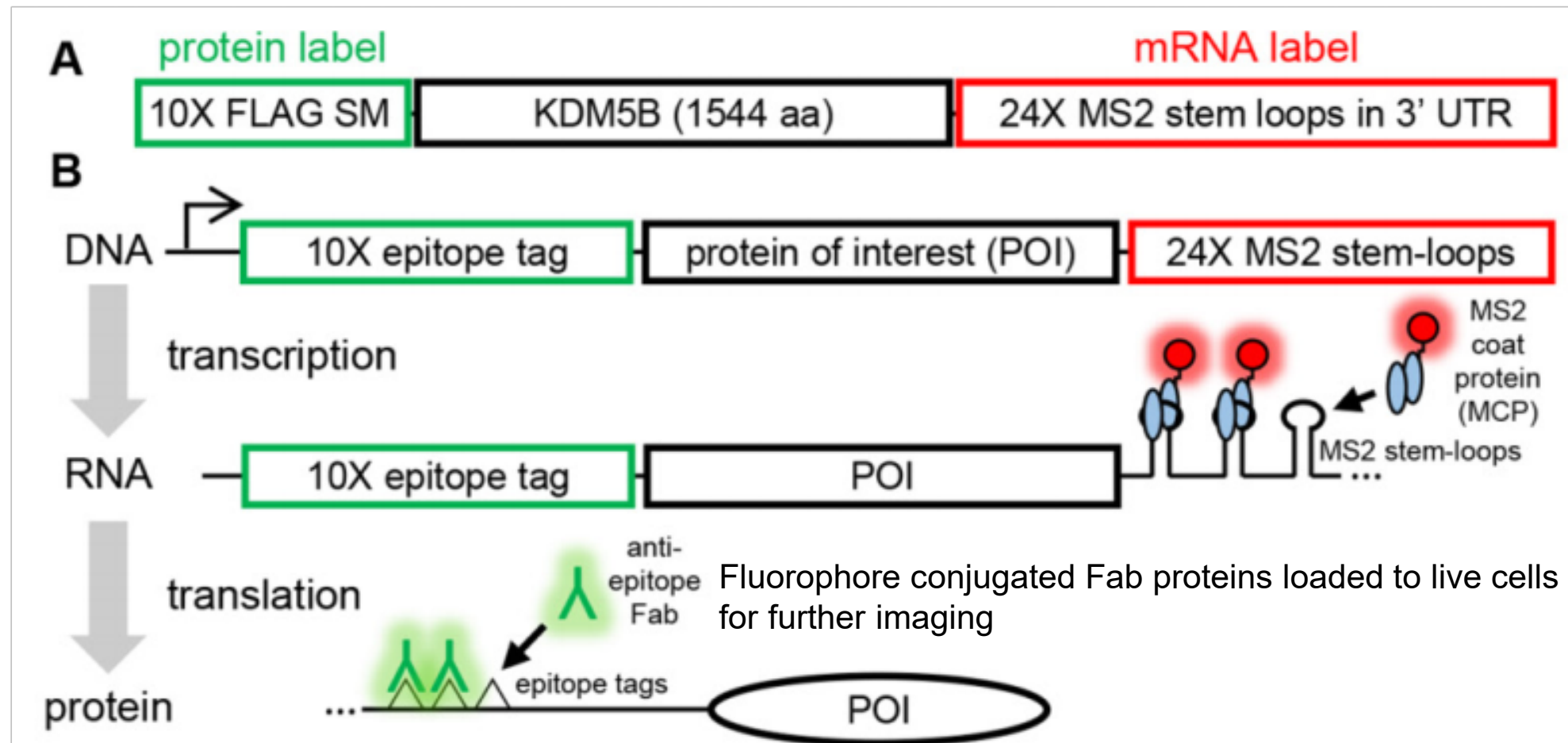
(Wilbertz et al., 2019, Molecular cell)

ATF4 mRNAs--constitutes of ~50% of transcripts

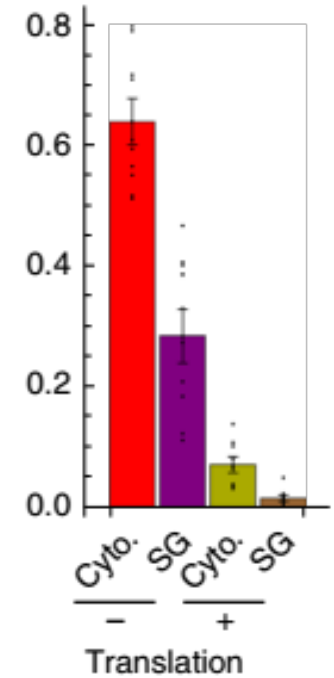
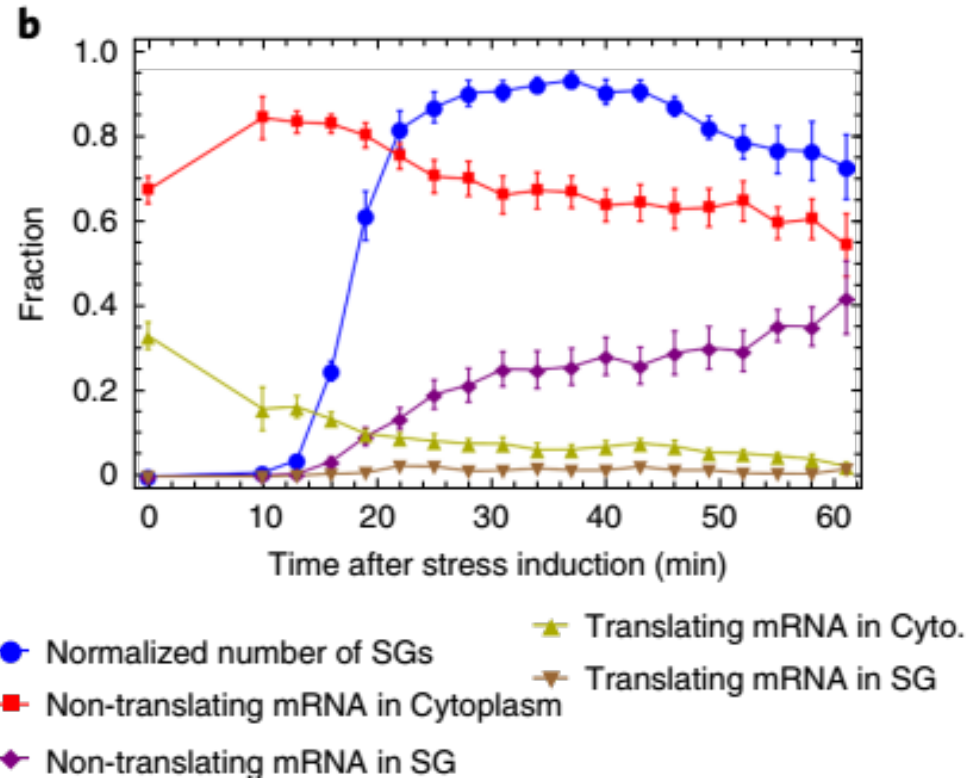
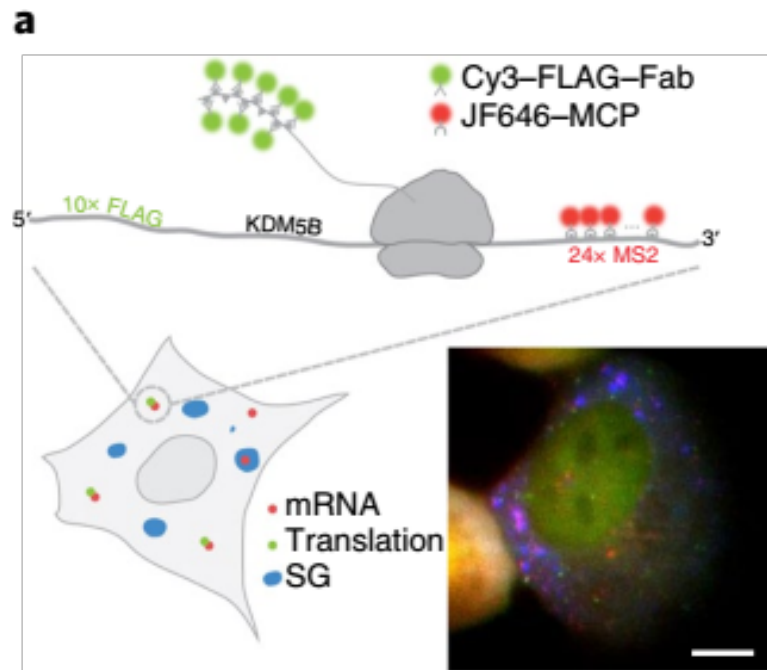
(Mateju et al., 2020, Cell)

Multicolour single-molecule tracking of mRNA interactions with RNP granules

Stephanie L. Moon ^{1,2,5}, Tatsuya Morisaki ^{3,5}, Anthony Khong^{1,2}, Kenneth Lyon³, Roy Parker ^{1,2*} and Timothy J. Stasevich ^{3,4*}



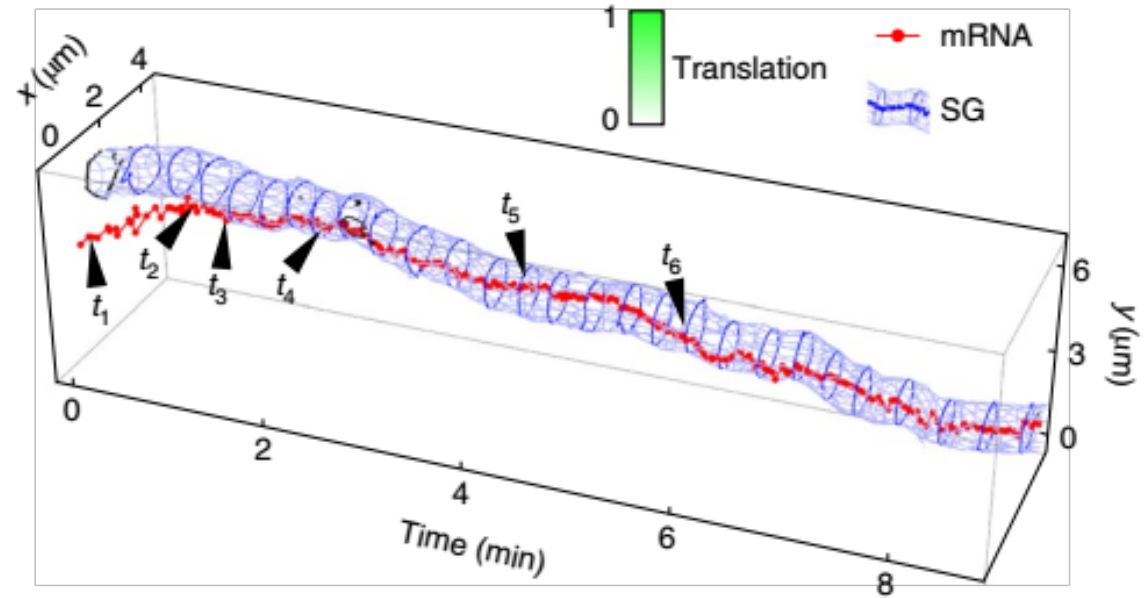
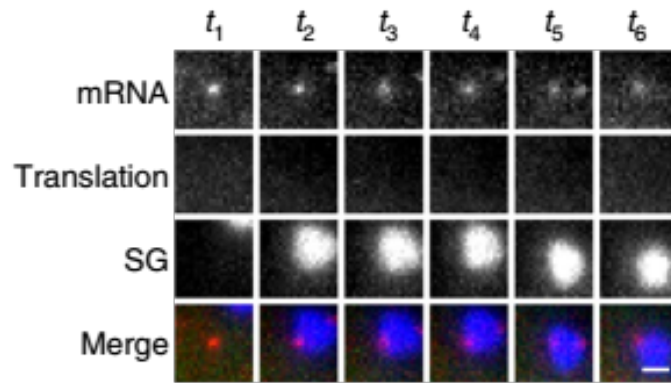
mRNA translation is suppressed under stress conditions



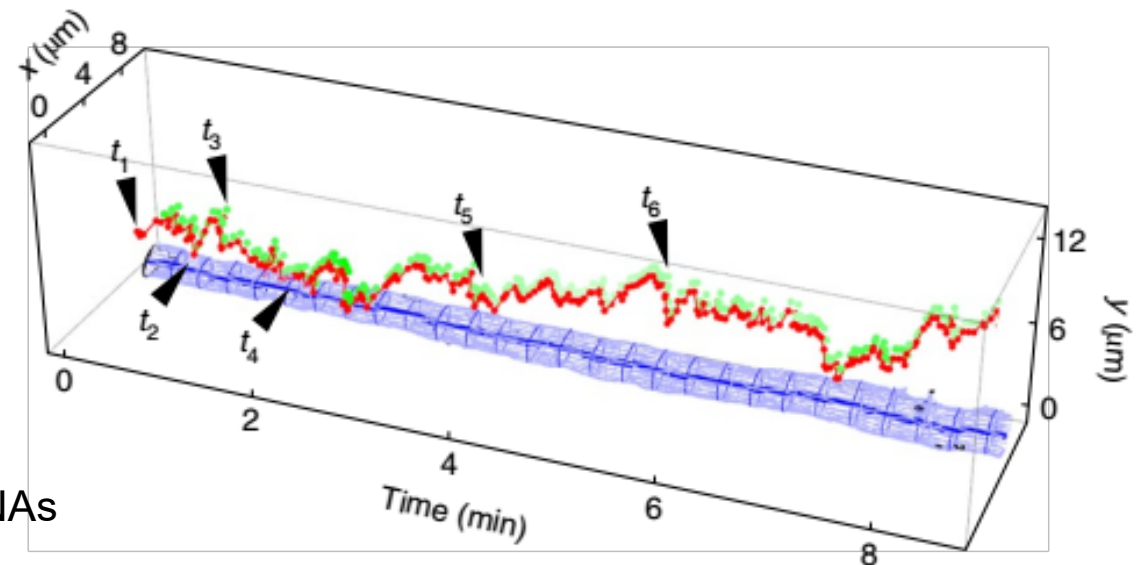
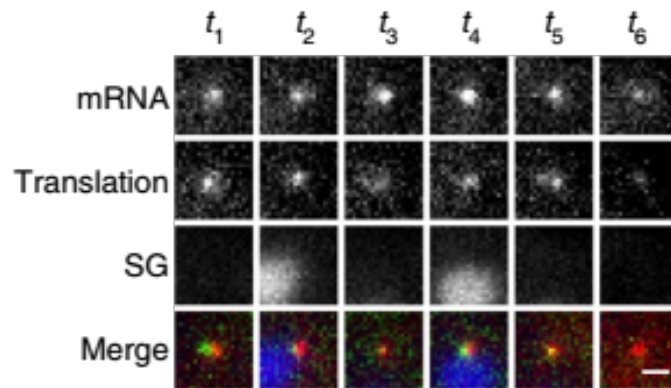
Stress induction: Arsenite treatment

mRNA translation is suppressed under stress conditions

c

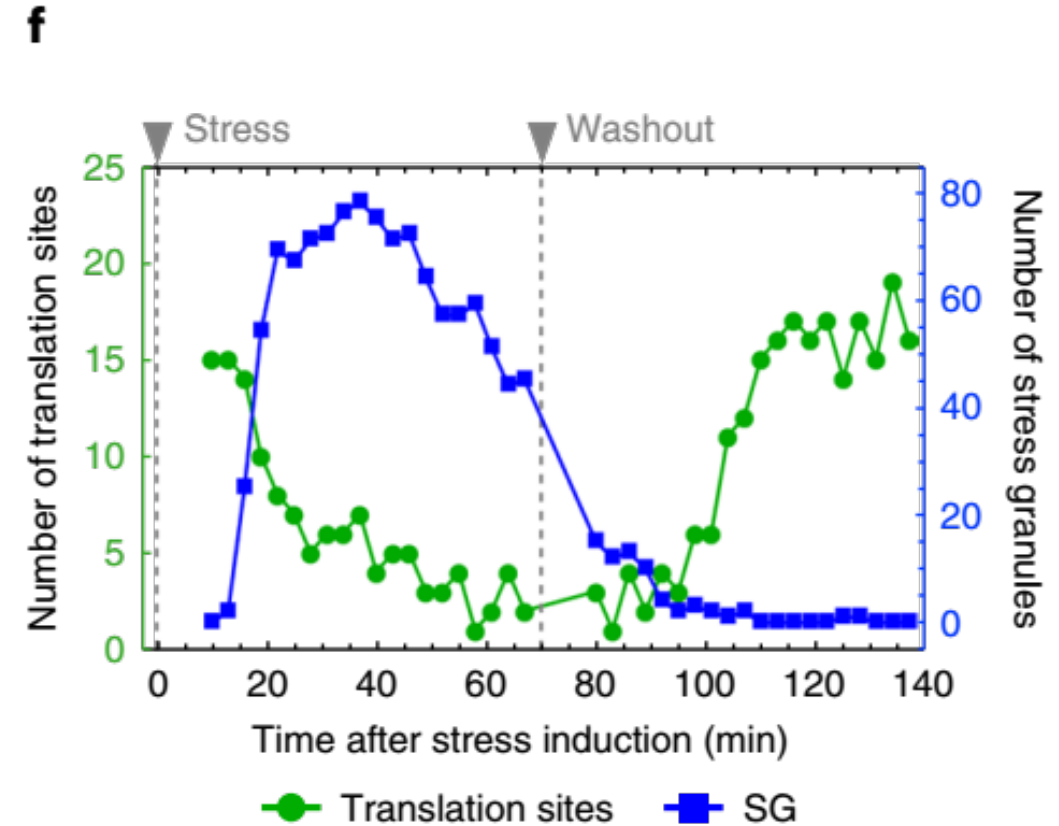
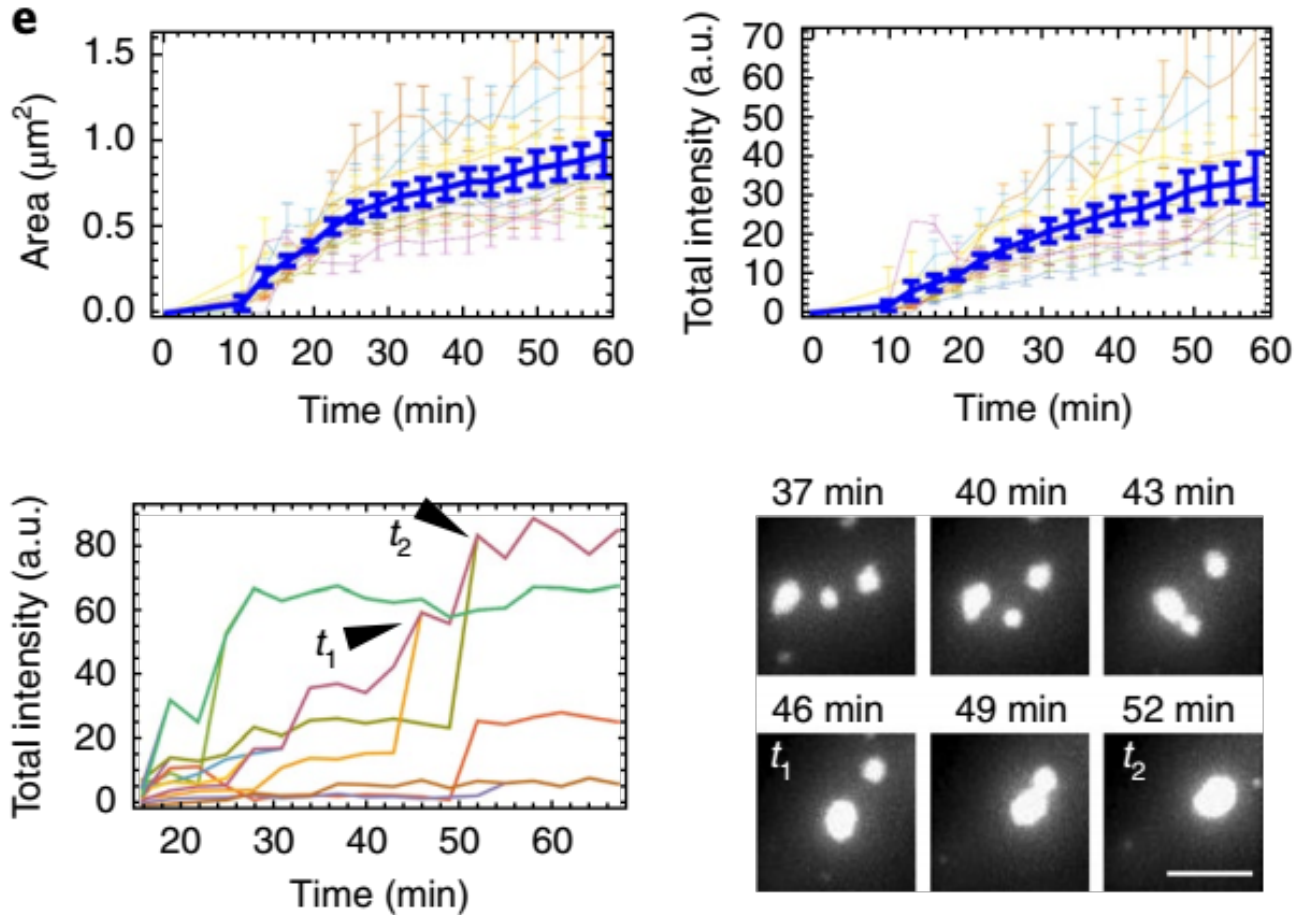


d



1 translation case from 334 tracked mRNAs

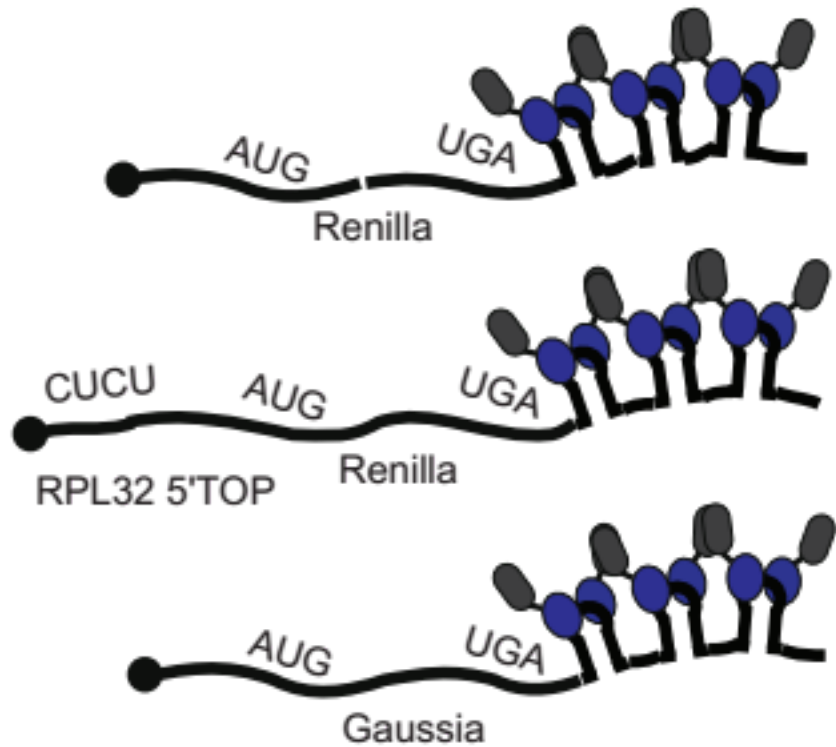
Resume of mRNA translation following SG disassembly



Growth of SGs following arsenite stress

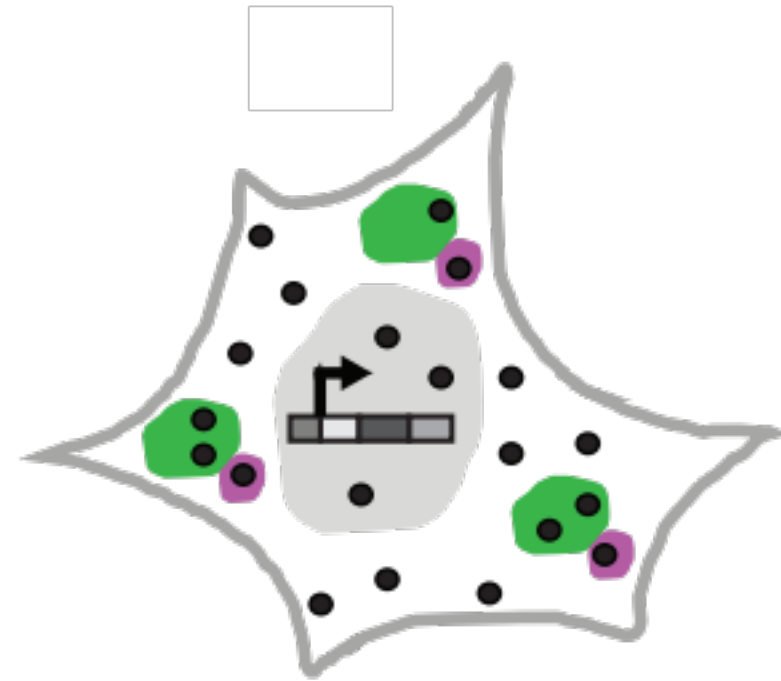
Single-Molecule Imaging of mRNA Localization and Regulation during the Integrated Stress Response

Johannes H. Wilbertz,^{1,2} Franka Voigt,¹ Ivana Horvathova,^{1,2} Gregory Roth,¹ Yinxiu Zhan,^{1,2} and Jeffrey A. Chao^{1,3,*}



PB: TagRFP-T-DDX6
SG: G3BP1-GFP
mRNA: NLS-MCP-Halo

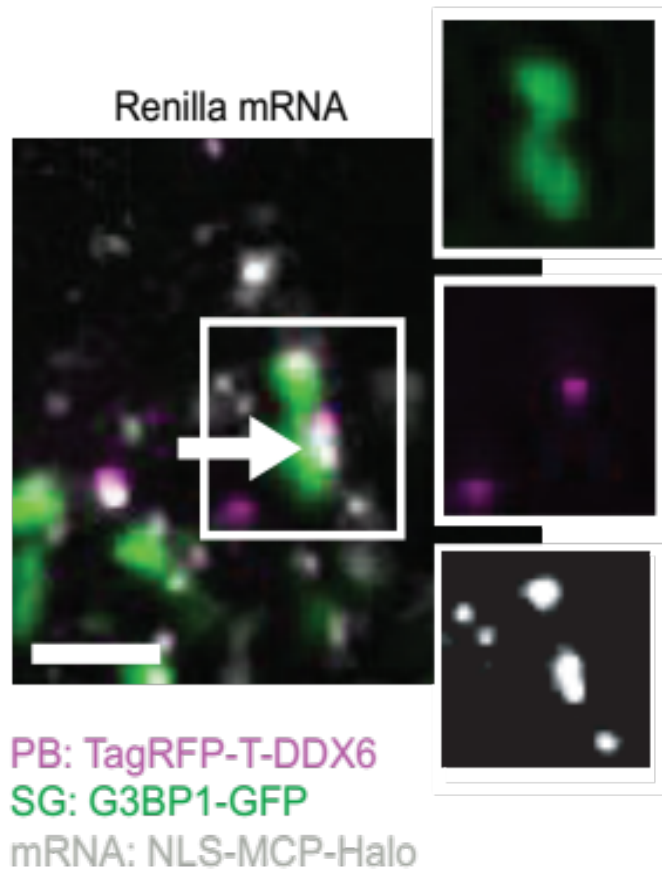
Genomic integration & FACS



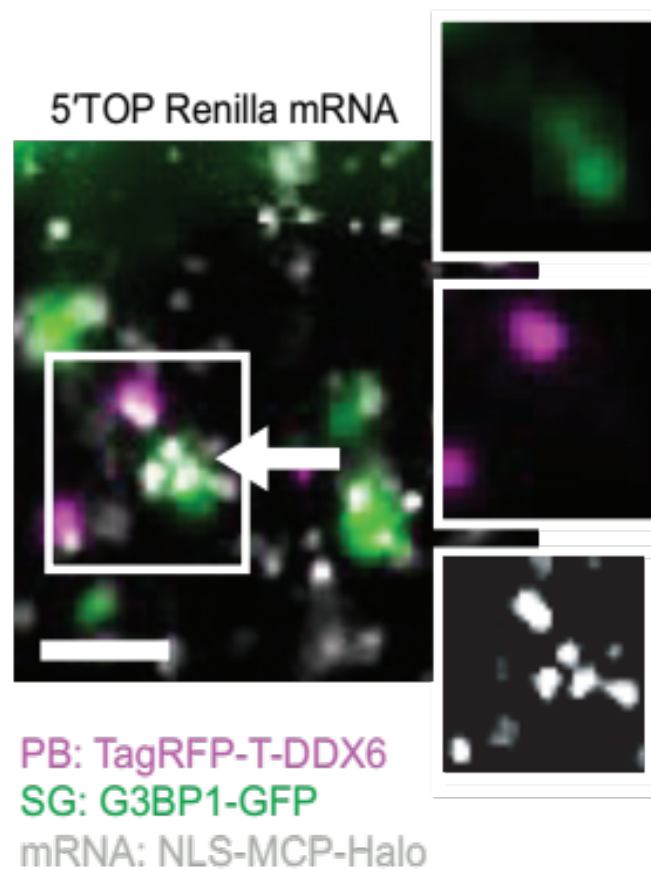
5'-TOP, 5' terminal oligo pyrimidine *cis*-acting element;
Renilla, 5'-TOP Renilla, Gaussia (secreted form): reporter mRNA;
SG, stress granule; PB, processing bodies.

Three-color live-cell imaging identifies distinct mRNA localization to PBs and SGs

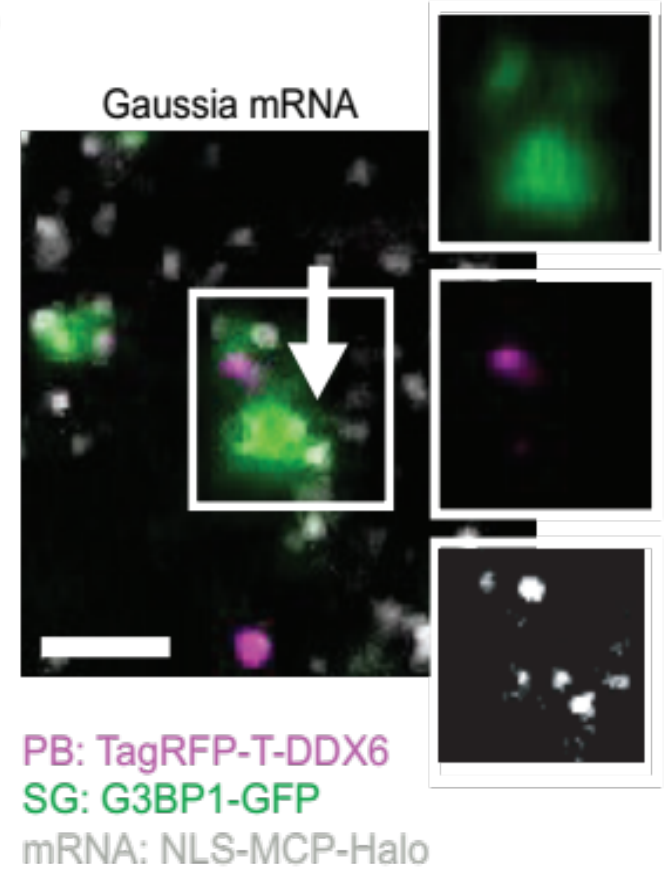
B



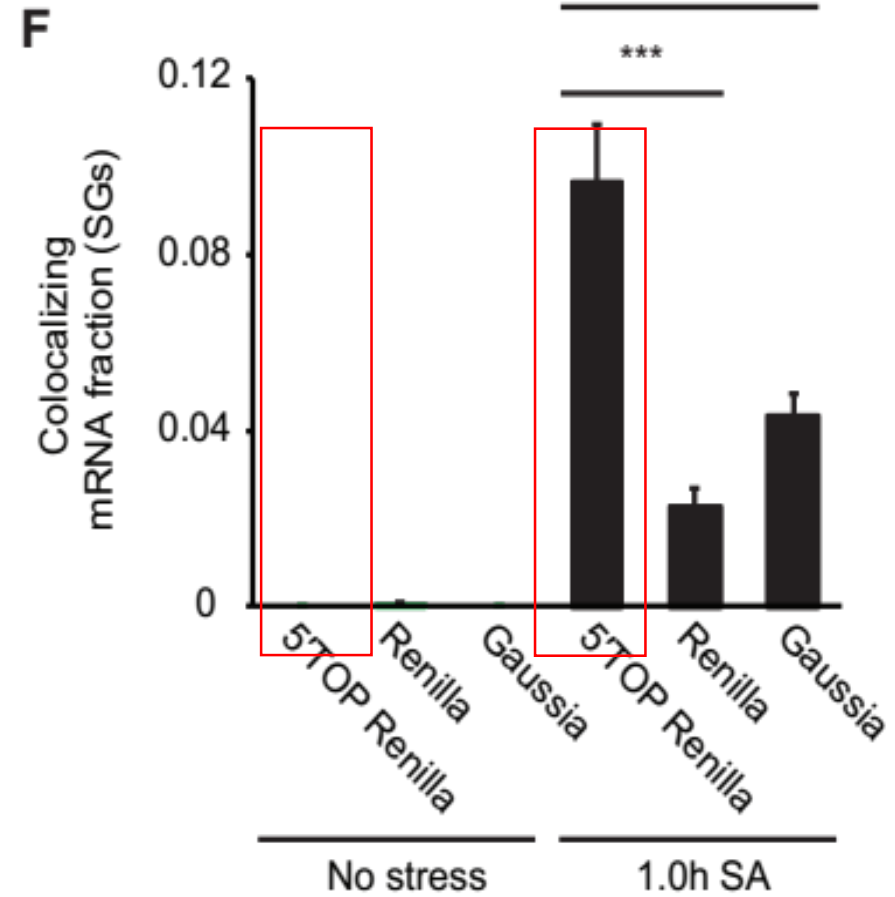
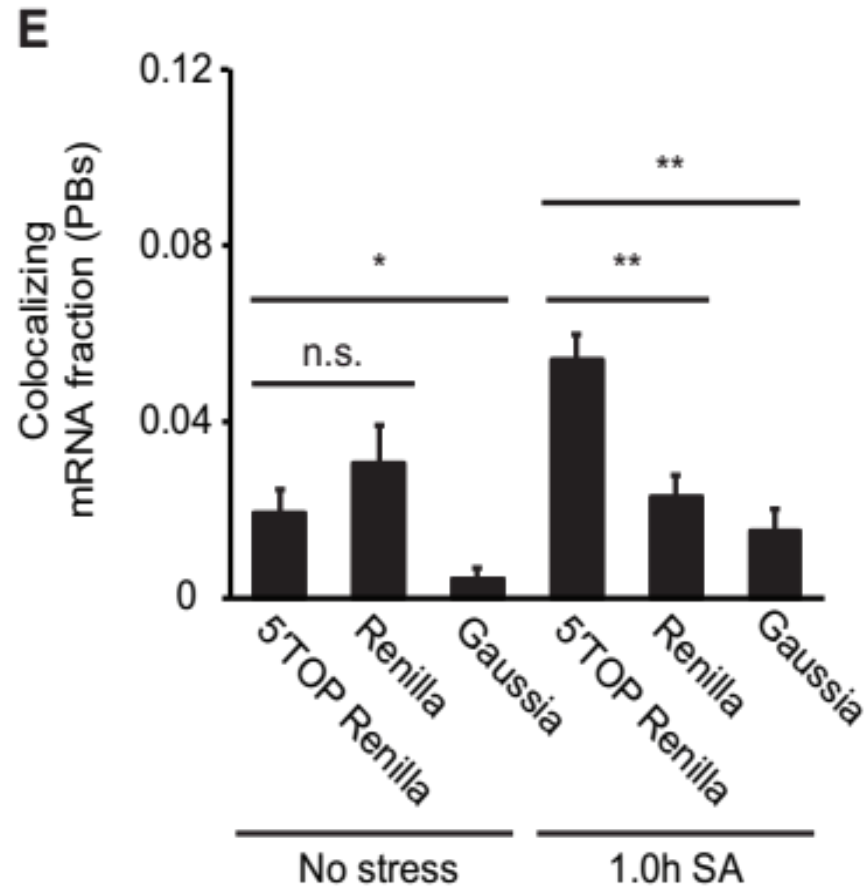
C



D

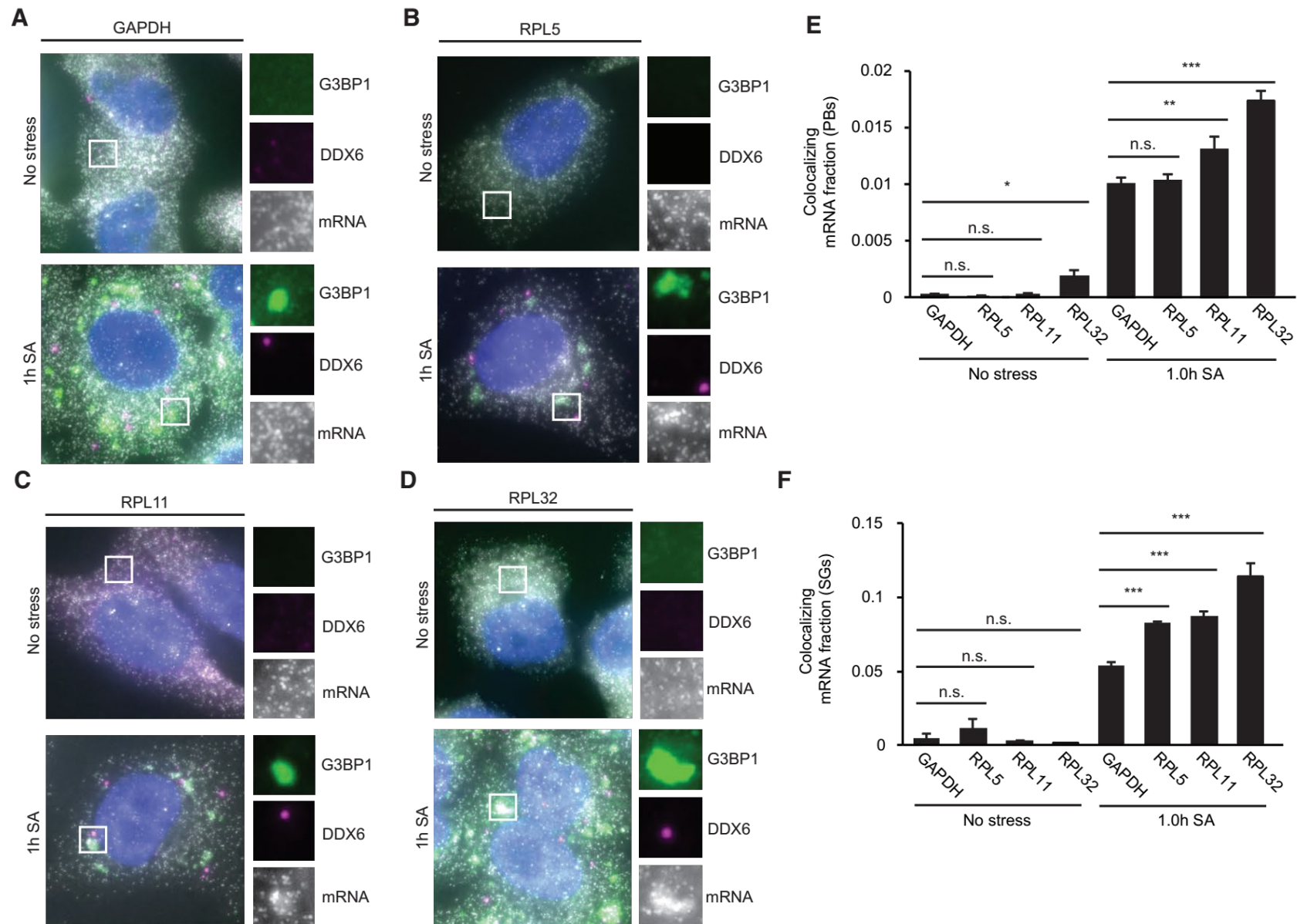


Three-color live-cell imaging identifies distinct mRNA localization to PBs and SGs

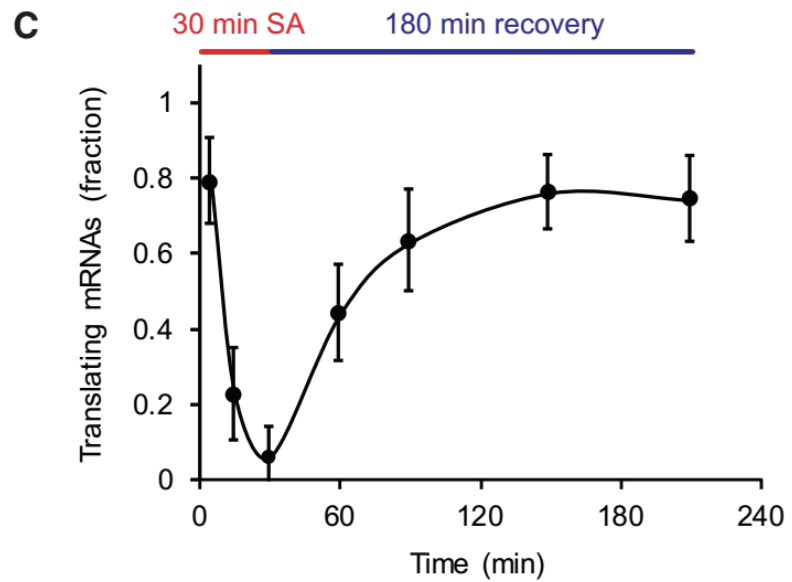
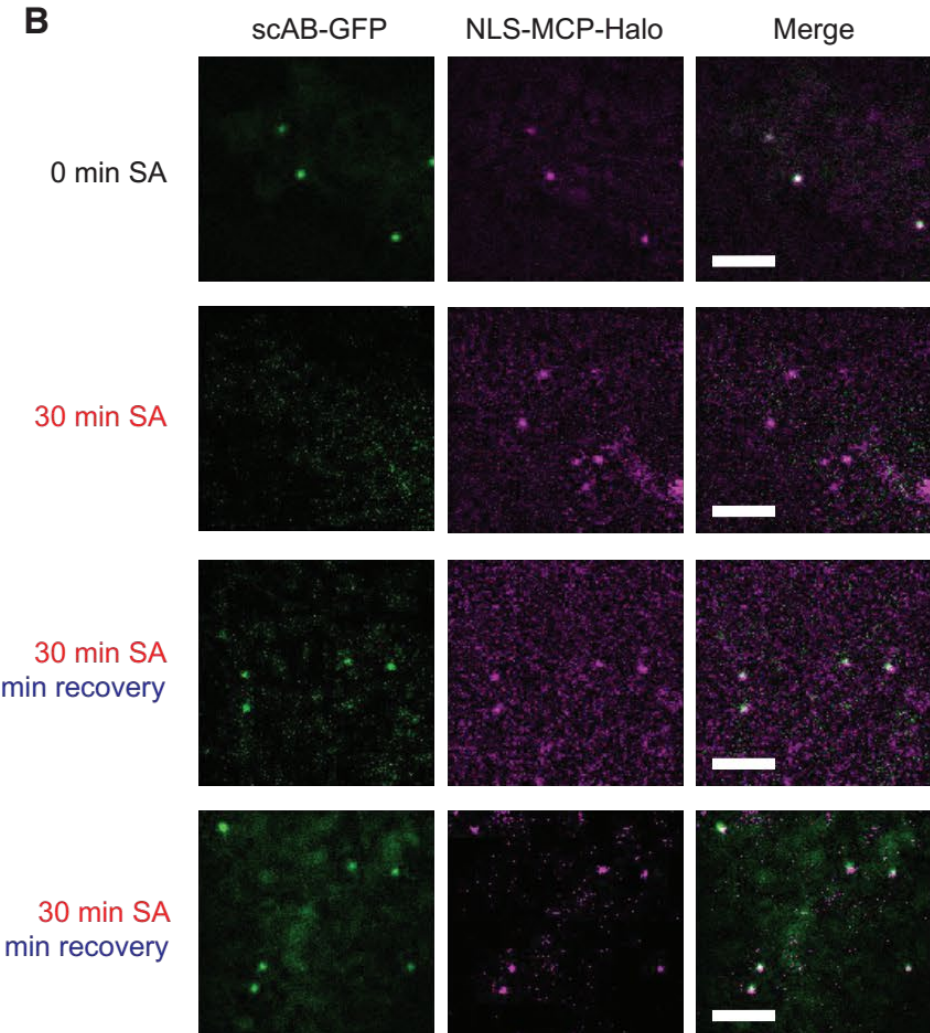
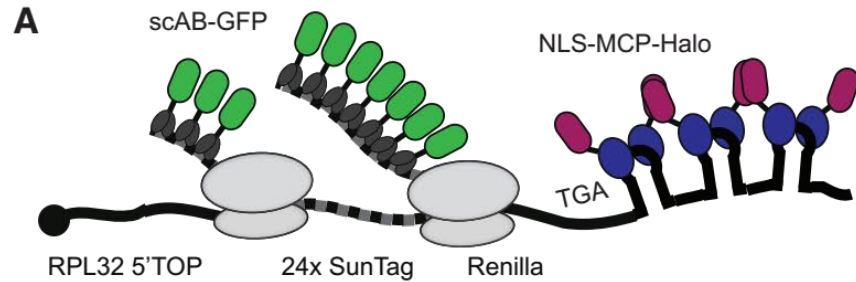


SA, sodium arsenite

RNA smFISH against endogenous RPL5, RPL11, and RPL32 confirms 5'-TOP-element-dependent mRNA localization to SGs and PBs



5' TOP mRNAs resume translation during recovery from stress

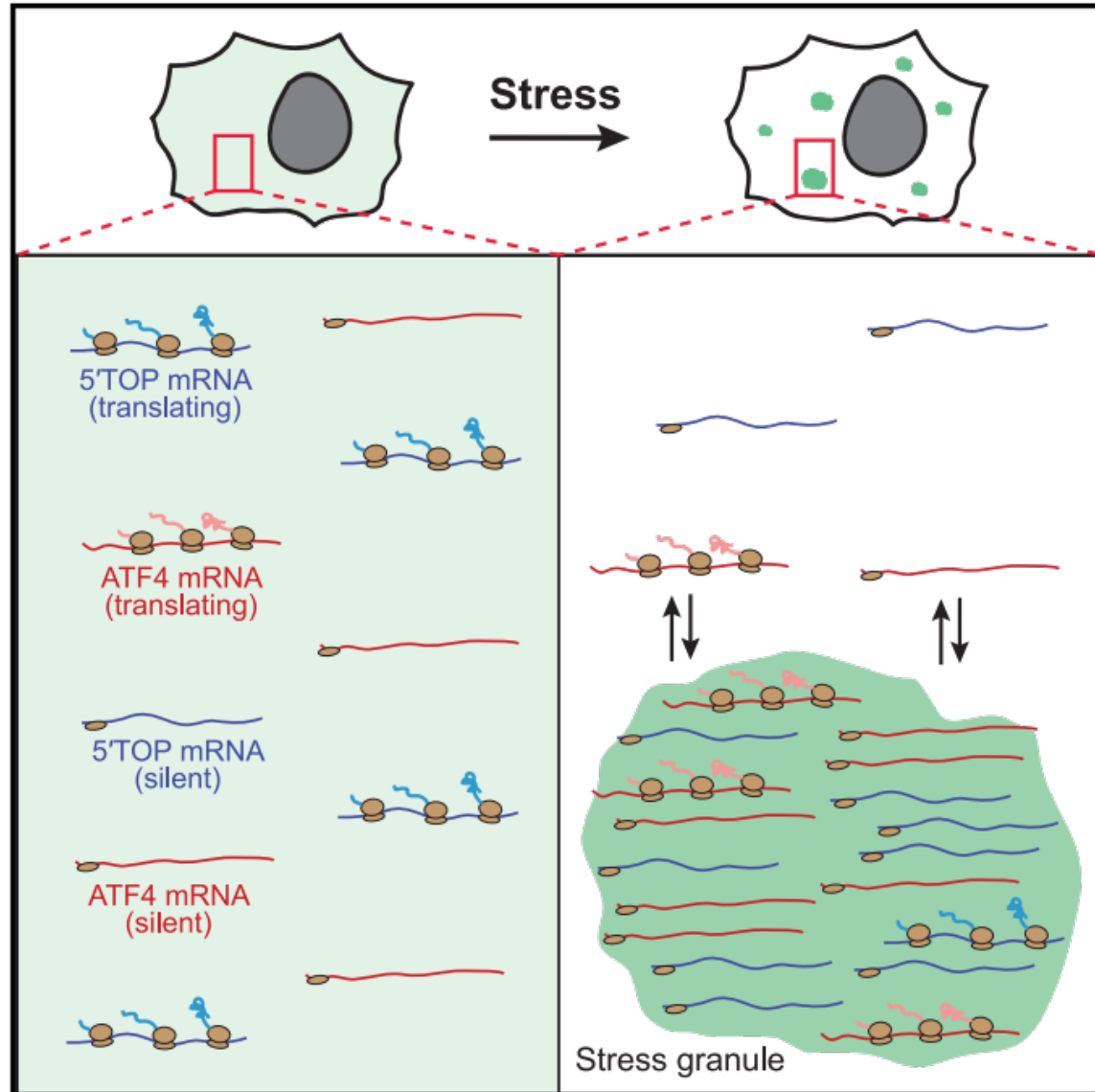


Single-Molecule Imaging Reveals Translation of mRNAs Localized to Stress Granules

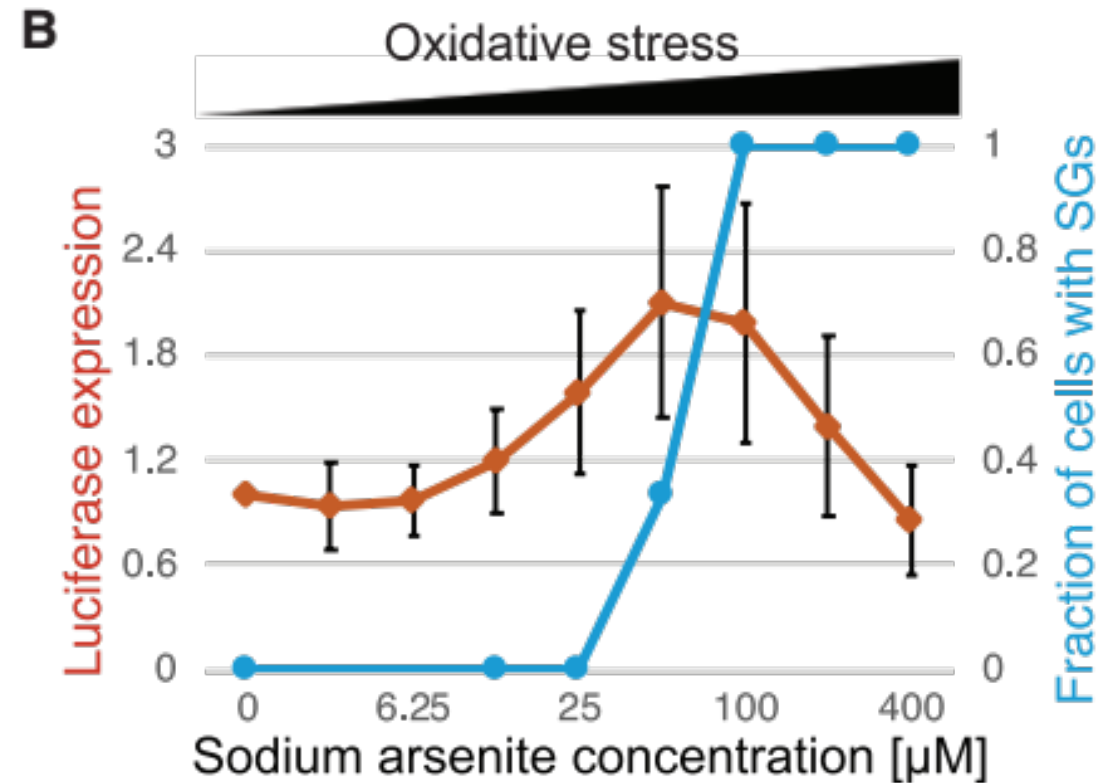
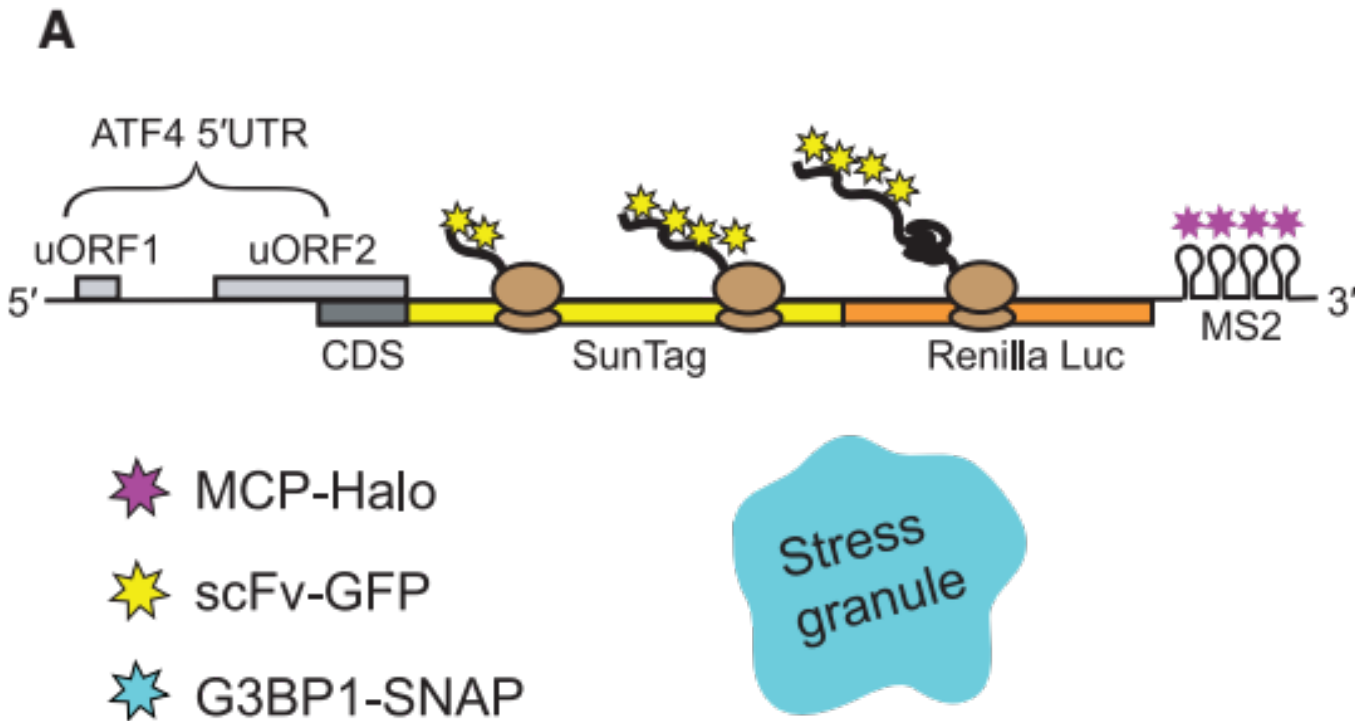
Authors

Daniel Mateju, Bastian Eichenberger, Franka Voigt, Jan Eglinger, Gregory Roth, Jeffrey A. Chao

Cell, 2020

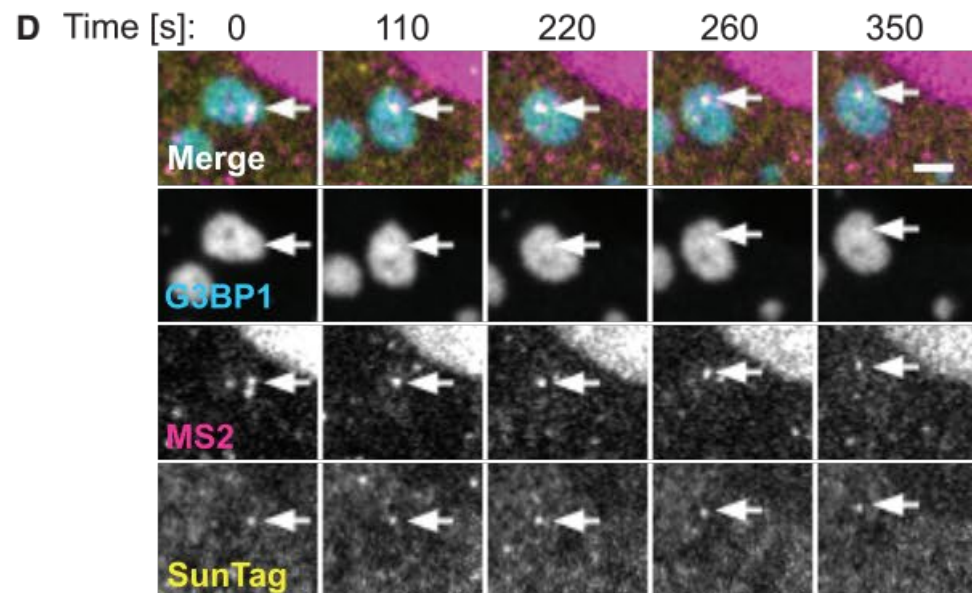
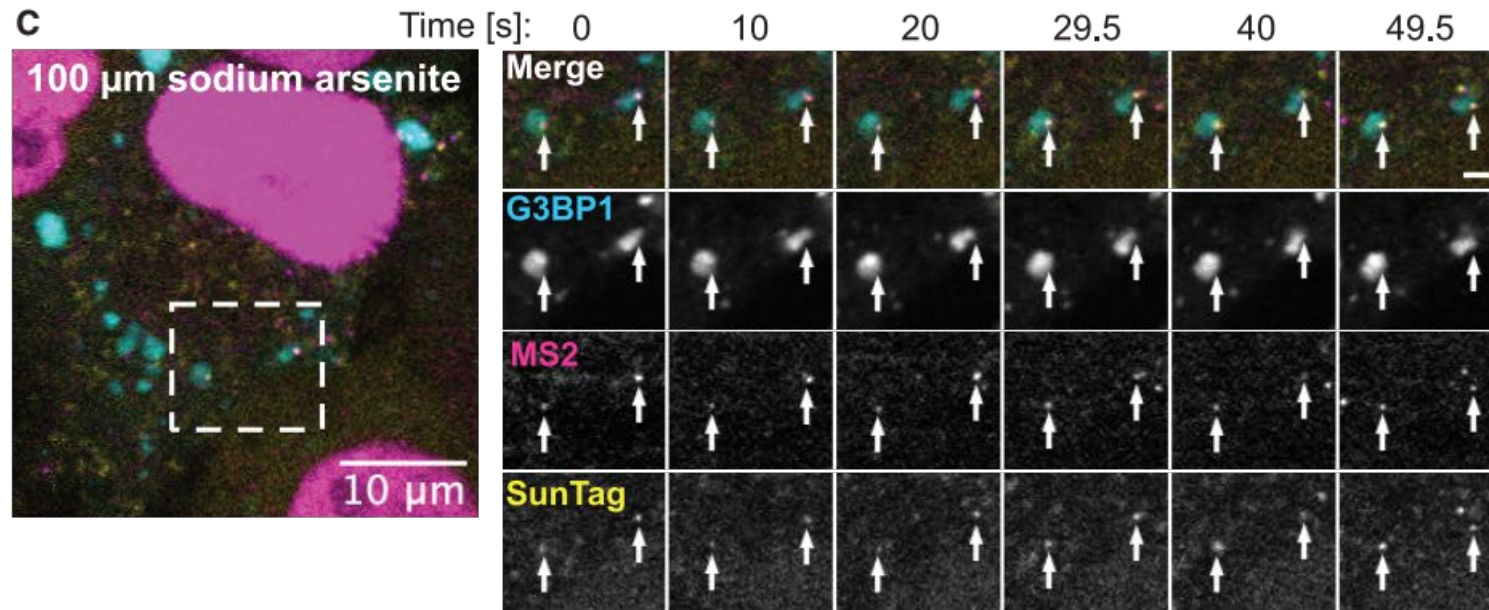


Single-Molecule Imaging Reveals mRNA Translation Associated with SGs



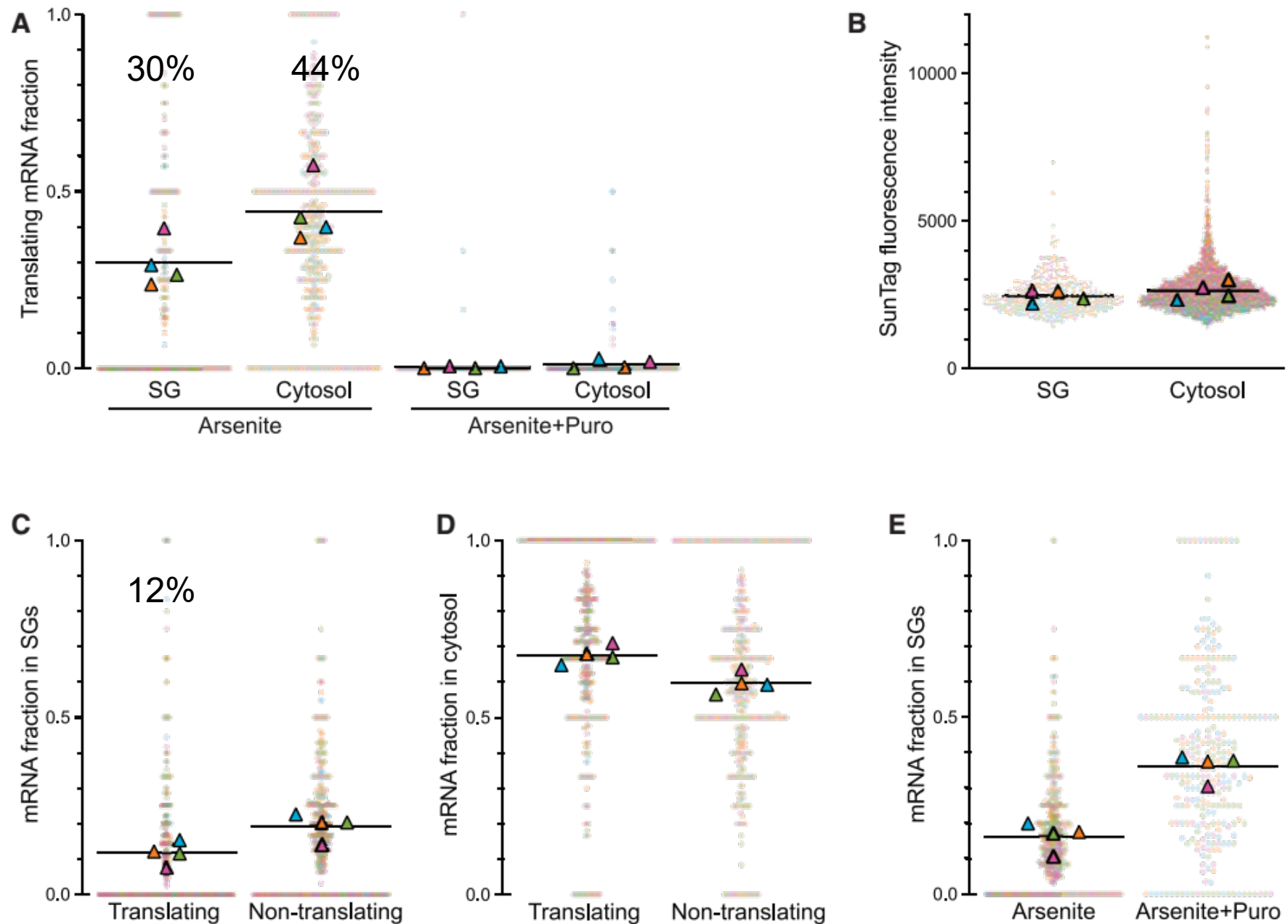
Individual reporter mRNAs, MS2 stem loops;
Complementary assays for mRNA translation, SunTag array in frame with Renilla luciferase;
Hela cell line stably expressing scFc-GFP and MCP-Halo;

Single-Molecule Imaging Reveals mRNA Translation Associated with SGs

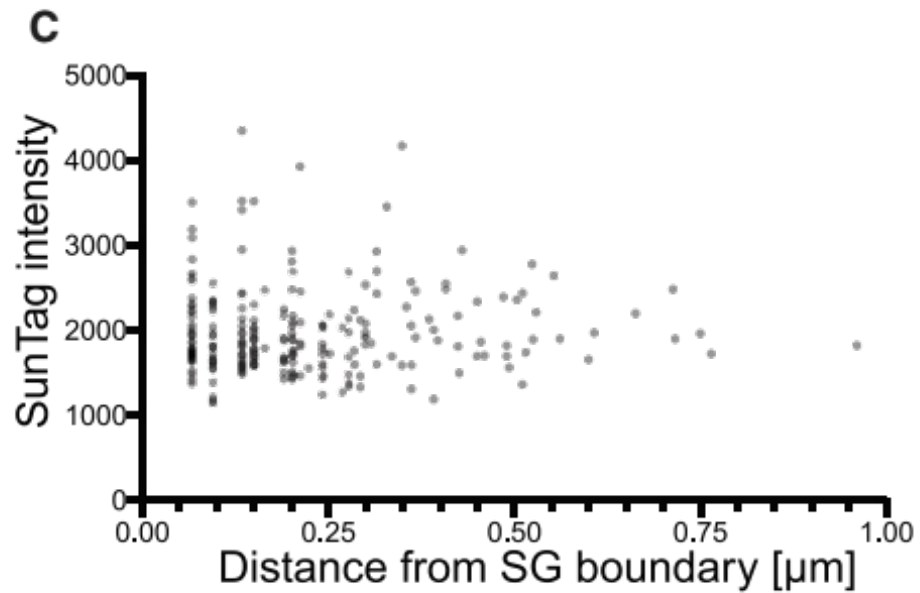
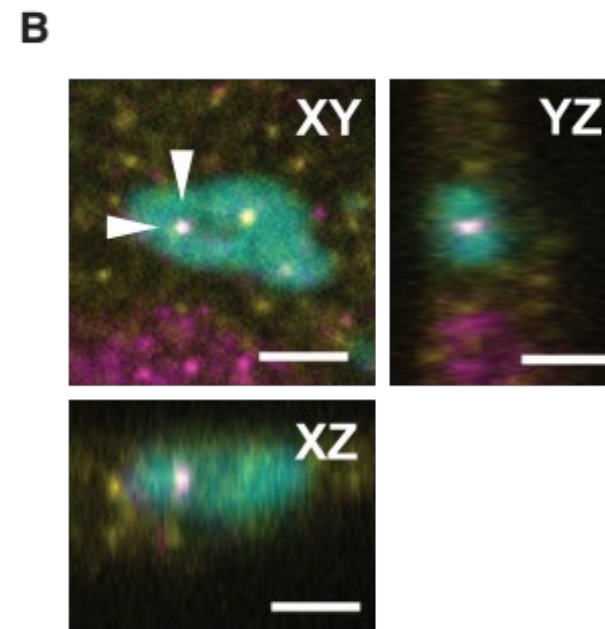
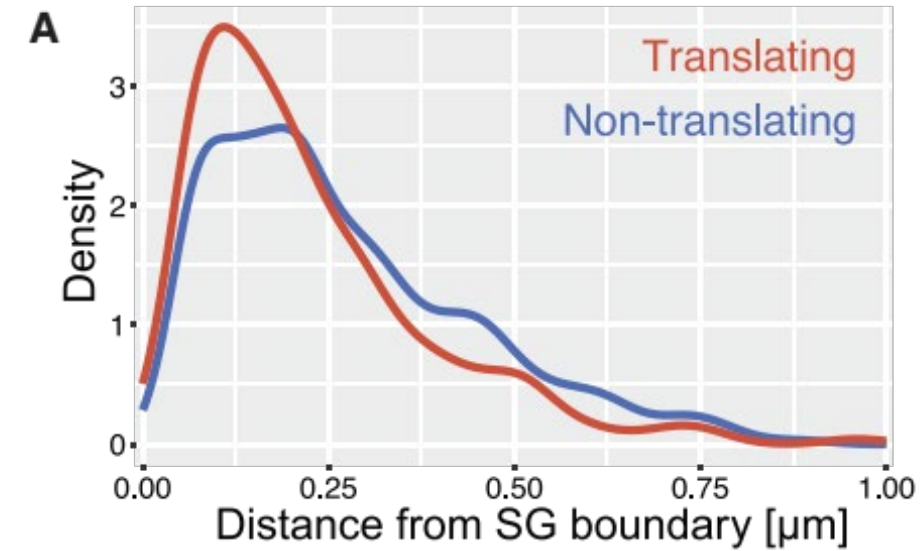


Translation signal lasts over 5 min

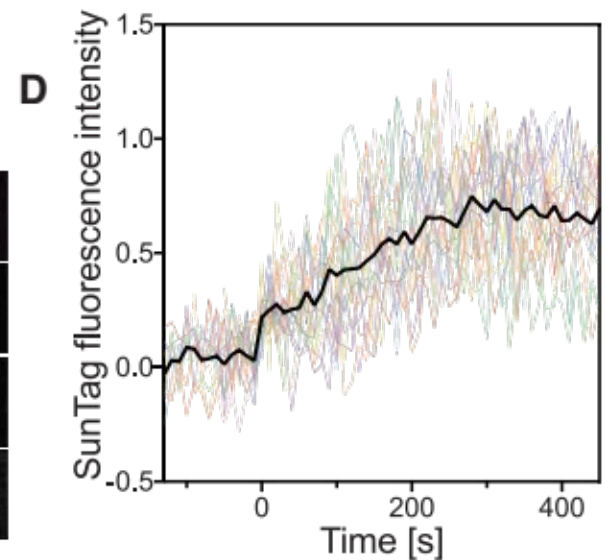
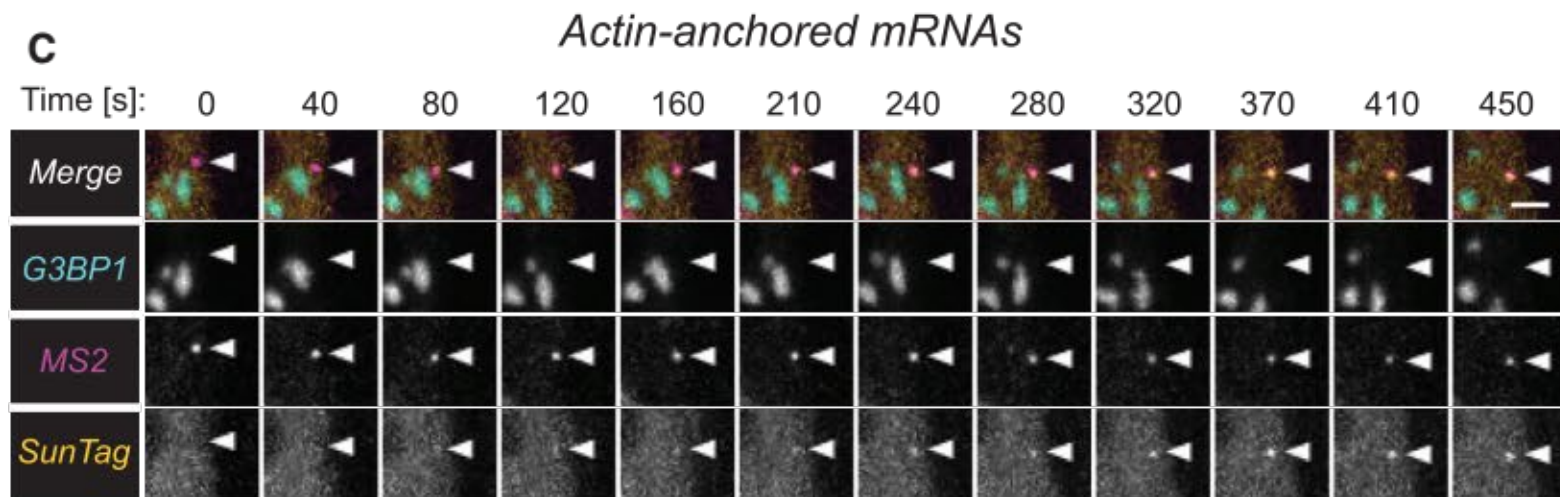
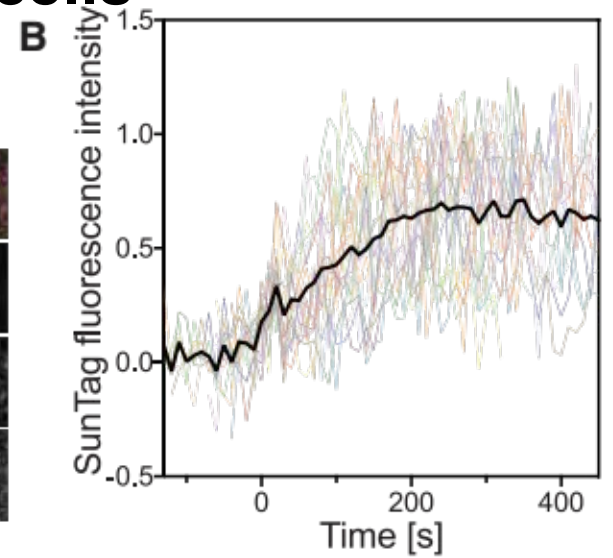
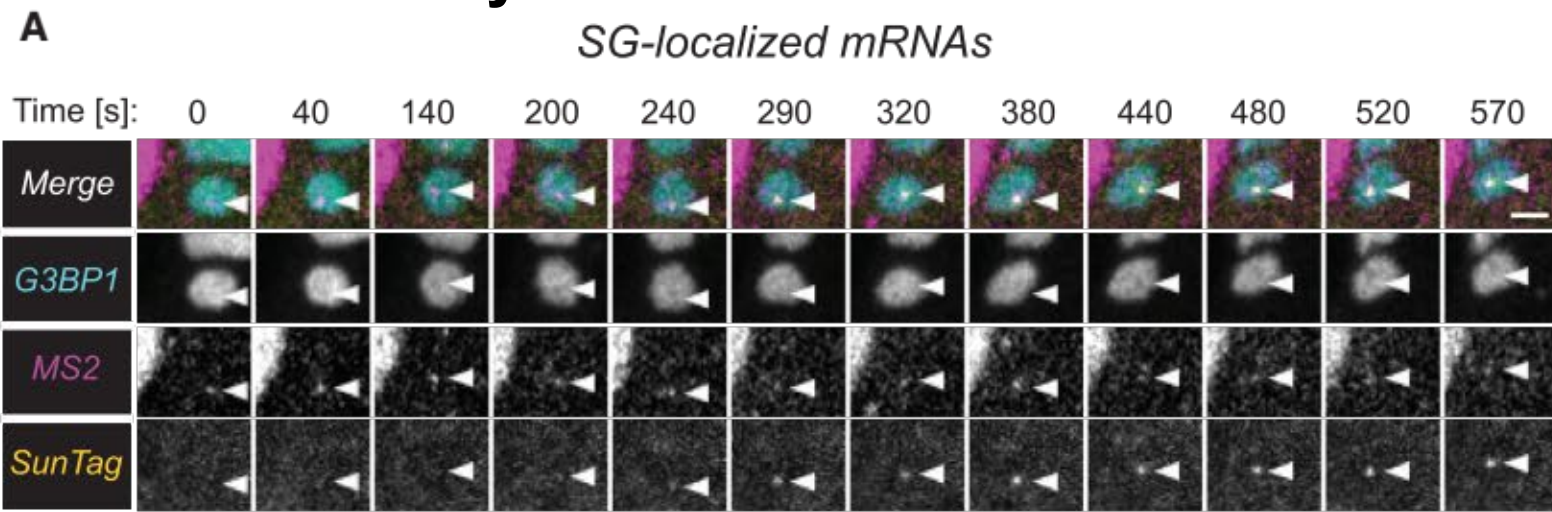
SG-associated translation is not a rare event



Position of mRNAs in SGs is similar for translating and repressed transcripts

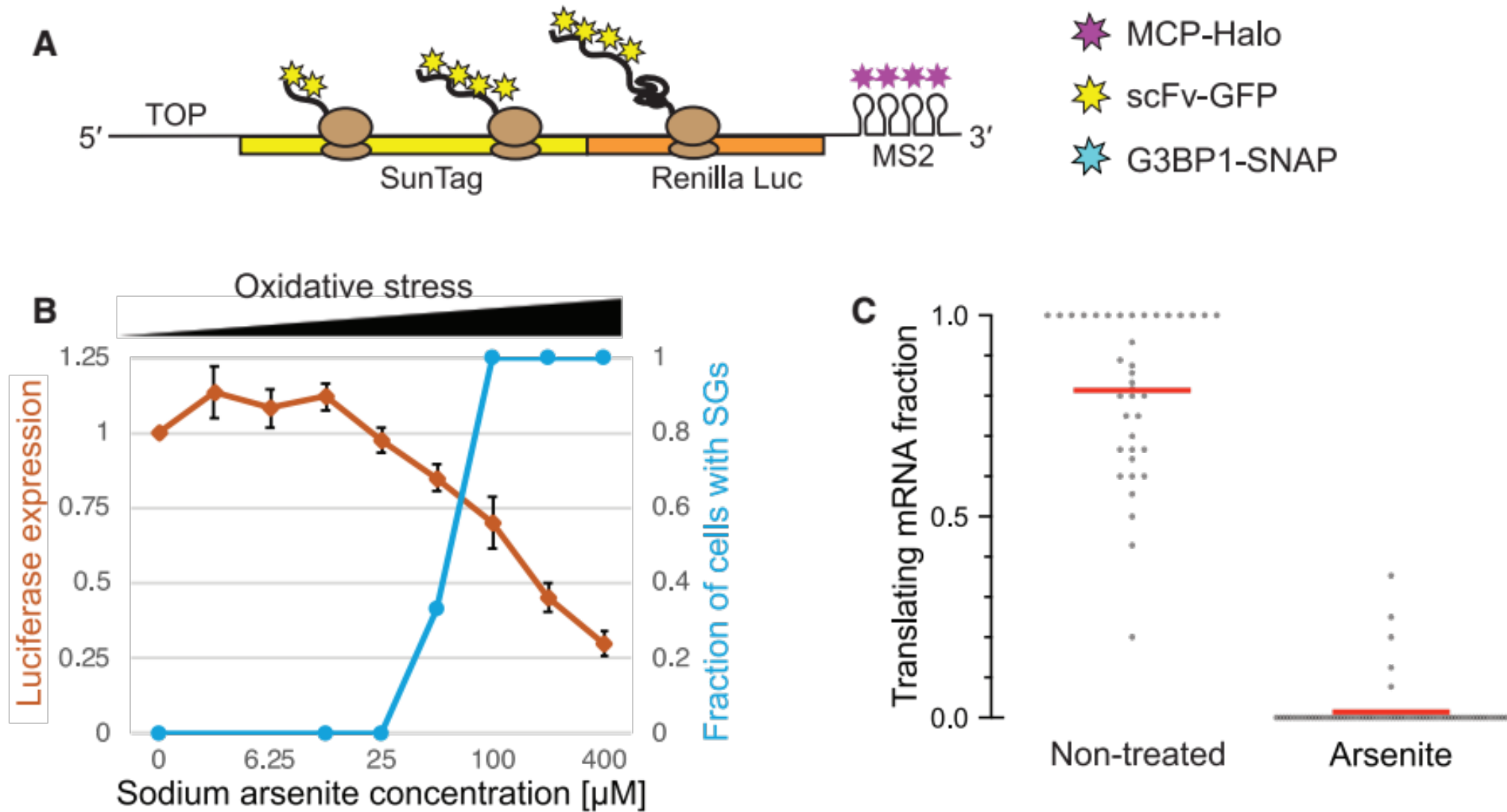


Translation of SG-associated mRNAs is similar to translation in the cytosol of stressed and unstressed cells



Initiation and elongation state of mRNA translation

SG-associated translation is suppressed for 5' TOP mRNAs



Summary

- mRNA translation state varies in different mRNA species.
- Localization to SGs does not prevent mRNA translation as exemplified by ATF4 mTNAs.
- The continued development of single- molecule imaging techniques and fluorescent biosensors coupled with the identification of separation-of-function mutants or chemical biology tools plays a key role toward understanding the function of biomolecular condensates.
- Functional machinery of these membrane-less organelles is an intriguing scientific question that still remains unknown.